Prognostic factors and late effects of treatment in localised high grade extremity osteosarcoma
Bramer, J.A.M.

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Curriculum Vitae

Jos Bramer was born on November the 20th 1962 in Tilburg. After a very happy youth in the south of the Netherlands, he studied medicine at the University of Amsterdam. Despite severe distraction by several jobs, sea-scouting, a short period in Africa, and other things, he acquired his MD in 1991. After some years as general surgery registrar he specialized in orthopaedic surgery in the Reinier de Graaf Gasthuis (Delft), the Academic Medical Centre (Amsterdam) and the SlotervaartZiekenhuis (Amsterdam).

Following this, he worked in the Flevo Hospital (Almere) as consultant orthopaedic surgeon for 1 year. In 2003 he was awarded a grant from the Dutch Cancer Foundation (KWF) for a clinical fellowship in oncology for 2 years. These were spent at the oncologic orthopaedic departments of the Universitair Medisch Centre St Radboud (Nijmegen), the Leiden University Medical Centre, the Royal Orthopaedic Hospital (Birmingham), the Istituto Ortopedici Rizzoli (Bologna) and the Academic Medical Centre (Amsterdam).

Interesting shorter working visits were paid at the University Hospital of Muenster and the Memorial Sloan Kettering Cancer Centre (New York). Later, in 2008, another fellowship was done in the spinal unit of the Royal Orthopaedic Hospital, focusing on spinal tumors and fractures. From 2005 Jos is working in his current job as consultant orthopaedic surgeon at the Academic Medical Centre specializing in orthopaedic oncology as well as oncology and fractures of the spine. Other passions are sailing, playing tennis, and enjoying life with family and friends. Jos is happily married to Anouk Lucas and is very proud of his 2 sons Bauke and Max and his daughter Eva.
Prognostic Factors and Late Effects of Treatment in Localised High Grade Extremity Osteosarcoma
Prognostic Factors and Late Effects of Treatment in Localised High Grade Extremity Osteosarcoma

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
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Prof. Dr. D.C. van den Boom
ten overstaan van een door het college voor promoties ingestelde commissie, in het openbaar te verdedigen in de Aula der Universiteit
op donderdag 17 juni 2010, te 10.00 uur

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Jozef Albertus Maria Bramer

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Faculteit der Geneeskunde
Voor mijn ouders:
Jan Bramer
en Mieke Bramer - Gilissen
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ADDENDUM: Overview of prognostic factors, reported in the literature from 1992 to 2006

Bibliography
General Introduction and Outline of the Thesis
1.1 A short history of survival in osteosarcoma

An often cited remark is that of a famous surgeon in the beginning of the last century who summarized a scientific meeting on bone sarcomas in the 1920’s by acknowledging: “If you do not operate, they die; if you do operate, they die just the same. Gentlemen, this meeting should be concluded with prayers”(1).

High grade osteosarcoma is the most common malignant, primary, non-hematopoietic, bone tumour. It still is a rare disease with an incidence of around 3 per million(2), with a peak in the second decade of life. The incidence in children under 18 years of age is found to be 6.9 per million in the Dutch population(3). Males are about 1.5 times more affected than females. Before the 1970’s, treatment of high grade osteosarcoma was limited to surgery. In the majority of extremity osteosarcoma patients, amputation was carried out. Survival was only 15-20%(4-6).

In the early 1970’s, adjuvant, postoperative, chemotherapy treatment was introduced. In combination with surgery, this treatment resulted in survival rates of about 50%. In the late seventies, neoadjuvant; pre-operative chemotherapy was added to this. Standard treatment nowadays consists of “sandwich therapy” with pre-operative induction chemotherapy, followed by resection of the tumor, after which post-operative chemotherapy is given. Chemotherapy usually concerns combinations of doxorubicin, cisplatin, high dose methotrexate, and ifosfamide. The aim of surgery is resection of the tumor with adequate margins. Most patients in Europe and America are treated within one of the multicentered trials like those of the European Osteosarcoma Intergroup (EOI)(7;8), the Cooperative Osteosarcoma Study Group (COSS), the Italian Sarcoma Group / Scandinavian Sarcoma Group or lately the European and American Osteosarcoma Study (EurAmos) , and EUROBOSS (European Bone Over 40 Sarcoma Study)(9;10).

With these changing therapeutical modalities, survival in patients with high grade osteosarcoma has improved from 15-20% to 60-70%(7;11-15), and has even been reported to be 93% in one population(16).

1.2 Changing challenges in osteosarcoma treatment

The success of chemotherapy, with increasing survival and the possibility of downsizing the tumour with pre-operative chemotherapy, has led to a change from predominantly ablative surgery to limb saving surgery in about 90% of patients nowadays(7;12;13). The development of imaging techniques and reconstruction materials has contributed to this change as well. First Computer Tomography (CT), and especially later the Magnetic Resonance Imaging (MRI) techniques have proven
to be very helpful in planning surgical procedures. This way, adequate margins could be achieved with closer resections, leading to less mutilation and better possibilities for reconstruction(17-19). Biomedical engineering has led to the development of more successful implants for reconstruction of defects after resection. Tumour prosthesis have shown good functional results and reasonable long term prosthetic survival(20-26). Because the majority of osteosarcoma patients are children, and the majority of tumours are located in the meta- and epiphyseal region, the growth plate often has to be sacrificed. Especially in young children this poses the problem of progressive leg length discrepancy. One solution for this is rotationplasty, of which the functional results were reported to superior, or at least comparable, to those of amputation and endoprosthetic replacement(27-32). Cosmetically, rotationplasty is less desirable compared to these other possibilities, although differences in quality of life or self esteem could not be objectified(33-36). Later, extendable, “growing” prostheses for reconstruction of long bone defects in children were successfully implanted as well(37-41). A promising new development is the non invasive growing prosthesis, which can be lengthened from outside the body, without the need for re-operations(42-44). Tumour prostheses, however, are still associated with late loosening, infects, and peri-prosthetic fractures and other complications(45-47), with a reported revision rate of 42%(20). Allografts possibly allow better fixation of soft tissue and partly fuse with the patient’s remaining bone, but infections and allograft fracture pose problems(48;49). Preferably, a defect in a long bone would be replaced by biological material, if possible living bone. Reconstruction of bone defects with a vascularised fibula autograft or callus distraction results, if successful, in living bone. This allows the patient a more normal and more physically active life. The risk of infection is lower, and if a fracture occurs, it has a healing potential(50;51). Combinations of allografts with prosthesis or with a vascularised fibula graft are other possibilities for reconstruction. Both have their advantages and disadvantages(19;52-57). On rare occasions, amputation or rotationplasty still is the best option. The challenge is to choose the right surgical strategy for each individual patient.

Another challenge in modern osteosarcoma treatment is the management of long term effects of treatment. Because of the current survival rate of 60 to 70%, there is a growing number of adults, who were treated for osteosarcoma in their youth(58). They have to live with the consequences of this treatment(59-62). Living with an amputation, rotationplasty, or with a large endoprosthesis, can compromise quality of life. Long term effects of chemotherapy can occur on the basis of cardiotoxicity(63), ototoxicity(64;65), infertility, renal failure(66;67), or psychosocial disturbance(68). Chemotherapy can be also the cause of secondary malignancies(69-71).
1.3 The importance of individual prognostication and prognostic factors

With the diversity of reconstructive possibilities, chemotherapy, and early and late consequences of treatment, decision making in osteosarcoma patients has become multifactorial. The first priority is always to achieve the best possible oncological result for each patient. Within the oncological optimal frame, the best possible functional result should be aimed at. Therapeutic choices have become very much individualized. The choice of reconstruction method from the variety of possibilities obviously does have implications for the patient. Some (especially biological) reconstruction methods, have excellent long term results but require a long (up to 2 year) rehabilitation time(53-55), whereas others, such as endo-prosthetic replacements, allow early mobilization but can lead to problems on the long run(19;35;45) (see table 1.1).

In spite of the dramatic improvement of survival in osteosarcoma patients, up to 40% of them still die of the disease. To counsel patients and their parents adequately, it would be helpful if an accurate estimation of survival chances could be made, if possible early in the course of treatment. Choice of chemotherapy and of surgical approach could be tailored to the patient. Furthermore, if reaction on chemotherapy could be predicted early, the chemotherapy regimen could be changed accordingly.

Individual prognostication in osteosarcoma remains difficult. Many prognostic factors have been reported, but only few seem to be independently predictive in a multivariate analysis. The literature is abundant, but very divers. Most consistently reported as valid factor is chemotherapy response(11-13;15;72). A number of literature reviews were published on specific factors(73-76), but reviews covering a “complete set” of factors for predicting survival are rare. In 1997 Saeter gave an narrative overview about most known predictive factors in which stage at diagnose was considered the most important, followed by chemotherapy response, tumor volume, age, sex, and possibly p-glycoprotein expression(77).

In 1994, Davis et al published a systematic review of the literature until 1992(72), analyzing age, sex, tumor location, tumor size and necrosis after chemotherapy. The authors concluded that chemotherapy response was the only proven independent factor predicting survival.

This response however, can only be established after neo-adjuvant chemotherapy and resection are carried out. Ideally, a prognostic factor would be assessable before, or early in the course of treatment. Furthermore, factors should be easy to assess, and practical in clinical use.
1.4 Outline of the thesis and research questions

Aim of the thesis was to establish practical and reliable prognostic factors predicting survival in patients with extremity osteosarcoma. It was chosen to focus on patients without metastatic disease at the time of diagnosis, because treatment in non-metastatic high grade osteosarcoma nowadays is very uniform, and mostly embedded in one of the abovementioned research protocols. This is not so for patients with metastatic disease at the time the osteosarcoma is first diagnosed. Some of those patients are still treated with a curative prospective; some are only palliated, with treatment, directed at quality of life, and sometimes no treatment at all is given. This makes comparing prognostic factors in this group virtually impossible.

In chapter 2 a systematic review is presented on prognostic factors in non metastatic osteosarcoma. The aim was to identify new independent predictive factors, reported in the recent literature. Furthermore an attempt was made to perform a meta-analysis, in order to try and establish pooled estimates of the risk ratio of specific predictive factors.

Chapter 3 discusses the treatment and outcome of the patients, treated in the Emma Children’s Hospital at the Academic Medical Centre Amsterdam. Aim is to compare the oncological results with those in literature and to evaluate known prognostic factors in this population. Furthermore the late effects of treatment are studied in patients surviving more than 5 years after the end of treatment. Second malignancies, organ or psychosocial dysfunction and cognitive problems have been reported. Incidence of these late complications is established in our patient group alongside the burden on patients and the relation with the treatment.

In Chapter 4 the value of Colour Doppler Ultrasound (CDUS) for predicting chemotherapy response and survival in paediatric osteosarcoma is evaluated. CDUS
is widely available, non-invasive, and easy to assess. Our hypothesis was that CDUS could predict chemotherapy response before resection of the tumour is carried out, and survival in high grade extremity osteosarcoma.

Although pre-chemotherapy alkaline phosphatase serum level was already recognised as a predictive factor, and chemotherapy response as the most consistent one, the value of post-chemotherapy alkaline phosphatase was never before established. In Chapter 5 the value of Pre- and post-chemotherapy alkaline phosphatase levels as prognostic indicators in adults with localised osteosarcoma is established in the larger population of the Royal Orthopaedic Hospital, Birmingham, United Kingdom. The hypothesis was that post-chemotherapy alkaline phosphatise level could predict chemotherapy response and survival in adults with localised high grade osteosarcoma.

Chapter 6 deals with the relevance of pathological fractures in bony sarcomas. The literature is contradictive about this. It is suggested that the occurrence of a pathological fracture worsens the prognosis because of tumour spread in the fracture hematoma. To clarify this more, a study was done in the patient populations which were treated for high grade chondrosarcoma, osteosarcoma, and Ewing´s sarcoma in the Royal Orthopaedic Hospital, Birmingham, United Kingdom. The hypothesis was that the presence of pathological fracture influenced and predicted survival and local recurrence rate in patients with high grade chondrosarcoma, osteosarcoma, and Ewing´s sarcoma.

Chapter 7 summarizes the thesis. In chapter 8 (general discussion and future perspectives) an attempt is made to put all of the above in perspective with modern osteosarcoma treatment. Chapter 9 presents our recommendations for clinical practice and future research. Chapter 10, finally, gives summary and recommendations in Dutch.
Reference List


Prognostic Factors in localized Extremity Osteosarcoma; a Systematic Review

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European Journal of Surgical Oncology (2009) 35:1030-6
2.1 Introduction

2.1.1 Survival in osteosarcoma and the importance of prognostication

Since the 1970’s, survival in patients with high grade osteosarcoma has improved from around 15% to 60-70%(2), and has even been reported to be 93% in one population(3). This improvement is generally attributed to the development of adjuvant and neo-adjuvant chemotherapy(4-8). The possibility of downgrading tumors before operation facilitated the evolution of reconstruction methods after tumor resection. Whereas up to the late seventies 80% of patients with an extremity osteosarcoma ended up with an amputation, nowadays limb saving surgery is possible in 90% of patients(9). Decision making has become multifactorial with this. Some (especially biological) reconstruction methods, have excellent long term results but require a long (up to 2 year) rehabilitation time, whereas others, such as endo-prosthetic replacements, allow early mobilization but have problems in the long term(9). A reasonably accurate estimate of survival chance for patients early in treatment would be helpful in counseling patients and their parents and in therapeutic decision making. Choice and possibly change of chemotherapy and of surgical approach could be tailored to the patient.

2.1.2 Prognostic factors

Chemotherapy response has always been the most important, and most consistently reported, predictor for survival(1;2;5;6;8;10;11). Prognostication in individual patients remains a problem. Many prognostic factors in osteosarcoma have been reported. The studies however vary significantly in methodology and quality. Several reviews have been done addressing specific factors(12-15), but their conclusions are cautious because of heterogeneity of the included studies. Attempts to review the complete range of relevant factors are sparse. In 1997 Saeter gave a narrative overview about most known factors(16). Stage at diagnose was considered to be the most important predictive factor, followed by chemotherapy response, tumor volume, old age, sex, and possibly p-glycoprotein expression.

In 1994 Davis et al published a systematic review giving an overview of the literature until 1992(1). Studies were included concerning patients with non-metastatic, high-grade, osteosarcoma of extremities, treated with chemotherapy and surgery. A critical appraisal was done on the methodological quality of included studies. Prognostic factors were analyzed only if they were at least considered in 4 of the included studies. Eventually 8 papers were included in this review. Analyzed factors were age, sex, tumor location, tumor size and necrosis after chemotherapy. Their...
conclusion was that chemotherapy response was the only proven independent factor predicting survival.

2.1.3 Aim of the study
The objective of our current systematic review was to elaborate on the work of Davis and colleagues, to try and identify new independent predictive factors, and to investigate whether meta-analysis of the results of different studies was possible, in order to establish pooled estimates of the effect of specific predictive factors.

2.2 Methods

2.2.1 Search strategy and study selection
MEDLINE and Embase were searched for eligible studies published in English, French or German between January 1992 and August 2006. We applied the following search strategy: [“osteosarcoma” OR “osteogenic sarcoma”] AND [“prognosis” OR “survival”]. Reports were selected, specifically addressing factors predicting survival in osteosarcoma patients. Inclusion was limited to patients with non-metastatic, high grade, primary osteosarcoma of an extremity.

2.2.2 Quality assessment and analysis
For all included studies we assessed methodological quality(17) (Table 2.1) and abstracted data. Data were recorded on a standardized form. Information was collected on patient characteristics, prognostic factors, adjusted relative risks for death (event free survival or overall survival), and timing of follow-up measurements. Study selection, assessment of methodological quality and data extraction were done by two reviewers independently. Disagreements were resolved by discussion with a 3rd reviewer.

Studies fulfilling all these quality criteria were selected for further meta-analysis. Studies for which the participants, prognostic factors, outcome measures, timing of follow-up measurements and adjustments for confounders were considered to be sufficiently similar were combined. We pooled adjusted relative risks of each prognostic factor by the use of a random effects model. To assess statistical heterogeneity we used the Chi-square test (p-value < 0.10) and heterogeneity was quantified by the I² statistic. In case of statistical heterogeneity, we explored sources of heterogeneity by meta-regression analysis. Meta-analysis was done by the use of Review Manager (RevMan [Computer program]. Version 4.2 for Windows. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2003) and SPSS version 11.5 (SPSS, Chicago, IL). Studies that were clinically heterogeneous
or did not present the data in sufficient detail to enable statistical pooling were qualitatively summarized. The level of evidence of studies was determined according to Harbour at al(18).

### 2.3 Results

#### 2.3.1 Search results and inclusion of papers

The search resulted in 1777 “hits”, of which 93 were included. Sufficient follow-up (≥90% completeness, ≥3 years) was absent in 60% of these studies. No multivariate analysis had been performed in 41%, no representative patient sample had been addressed in 35%. Ninety-four percent of rejected papers had more than one methodological flaw. Thirteen papers (6%) met all quality criteria mentioned in Table 2.1, and were included in the meta-analysis.

Although the majority of papers could not be included in our detailed analysis, many potentially interesting prognostic factors were found. An overview of these is given in the addendum at the end of this thesis, and is briefly considered in the discussion section. Among the included 13 papers, 2 concerned a double and 1 a five-fold publication of the same patient group (Glasser(8;19), Bielack(6) and Bieling(20), Bacci(5;21-23) and Ferrari(24)). Of those publications, the most recent were used, which in all cases also was the most complete one. In the study of Bielack et al(6), patients with primary metastatic disease, and patients with axial osteosarcomas were included as well, but because the group with extremity osteosarcoma was analyzed separately, and primary metastasis was analyzed as a factor in multivariate analysis, we felt this study could still be included.

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<table>
<thead>
<tr>
<th>Table 2.1 - Methodological criteria for inclusion in the meta-analysis*</th>
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<tbody>
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<td>1 Study participation</td>
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<td>2 Study attrition</td>
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<td>3 Prognostic factor measurement</td>
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<td>4 Outcome measurement</td>
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<td>5 Confounding measurement and account</td>
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<td>6 Analysis</td>
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*adopted from Hayden et al(17)
2.3.2 Characteristic of included studies and prognostic factors

Seven studies were included in the review (2;5;8;10;11;25). All were retrospective, with a low chance of bias (level of evidence of 2+ or 2++) (18). Sample size varied from 81 to 1702 patients. Most authors addressed event free survival (EFS), except for Smeland (25) (metastasis free survival), and Akatsuka (10) and Bielack (6), who addressed both EFS and overall survival. Because in the latter study an unclear margin after resection was regarded as an event, this factor could only be analyzed for overall survival.

Table 2.2 - Overview of prognostic factors, tested in the 7 included studies by Uni- or Multivariate analysis

<table>
<thead>
<tr>
<th></th>
<th>Akatsuka(10) (n=81; EFS+OS)</th>
<th>Bacci(5) (n=789; EFS)</th>
<th>Baldini(11) (n=92; EFS)</th>
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<td>MVA</td>
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<td>Local recurrence</td>
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uva = univariate analysis, - = not tested, MFS = Metastatic free survival, mva = multivariate analysis, * = cut off point in years between brackets, ns = not significant, EFS = Event free survival, s = significant, OS = Overall survival
Each of the studies evaluated a different combination of prognostic factors. All authors evaluated age as a factor in the univariate analysis, but only 4 did this in a multivariate analysis, using different cut-off points. Tumor location was analyzed by all authors, but 4 types of categorization were used. Chemotherapy response was analyzed in a more similar way. Weeden et al(2) analyzed the effect of local recurrence as a fixed co-variate, a time-dependant co-variate and in a landmark analysis. Because histological response was only taken into the analysis with local recurrence as a time-dependent variable, we used this analysis in the review. Surgical margin was evaluated in multivariate analysis by Bacci(5), who used the Enneking

<table>
<thead>
<tr>
<th></th>
<th>Bielack(6)</th>
<th>Glasser(8)</th>
<th>Smeland(25)</th>
<th>Weeden(2)</th>
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<tr>
<td></td>
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<td>(n=216; EFS)</td>
<td>(n=113; MFS)</td>
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<td>s</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>s</td>
<td>s</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>P-glycoprotein</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>ns</td>
</tr>
<tr>
<td>Erb2-expression</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Pathological fracture</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Serum methotrexate level</td>
<td>ns</td>
<td>ns</td>
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<td>ns</td>
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<tr>
<td>Chemotherapy protocol</td>
<td>s</td>
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<td>s</td>
</tr>
<tr>
<td>Delay of therapy</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Treatment duration</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>Chemotherapy response</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>Timing of surgery</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Surgery type</td>
<td>S</td>
<td>s</td>
<td>ns</td>
<td>s</td>
</tr>
<tr>
<td>Surgical margin</td>
<td>s</td>
<td>s</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Local recurrence</td>
<td>-</td>
<td>-</td>
<td>ns</td>
<td>-</td>
</tr>
</tbody>
</table>
classification(26), and by Bielack(6), who defined a clear margin if all tumor tissue was macroscopically completely resected.

In most cases multivariate analysis was done exclusively on factors that were found to be significant in the univariate analysis (table 2.2). Moreover, most authors only reported the effect estimates of factors that were significant in the multivariate analysis.

Table 2.3 presents the relative risks for death of the various prognostic factors and their p-values. Factors that were independently predictive for a worse outcome were poor response to chemotherapy(2;5;6;8;11), larger tumors (size or volume)(5;25), incomplete tumor excision(5;6), ablative surgery(2;10), age under 14 years(5), male gender(25), high alkaline phosphatase(5), local recurrence(2), p-Glycoprotein expression(11), and absent Erb2 expression(10).

2.3.3 Pooling of study results

Figure 2.1 presents the pooled results for factors that were reported more than once. Poor chemotherapy response (pooled RR = 2.37; 95% confidence interval [95% CI] 2.07 – 2.70), large tumor volume (pooled RR = 1.36; 95% CI 1.18 – 1.58) and ablative surgery (pooled RR = 2.18; 95% CI 1.58 – 3.00) were predictors of a bad outcome. For absence of an adequate surgical margin there was vast heterogeneity

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Akutsaka(10) EFS</th>
<th>Bacci(5) EFS</th>
<th>Baldini(11) EFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR Poor chemotherapy response</td>
<td>1.92 (0.14)</td>
<td>2(&lt;0.01)</td>
<td>2.25 (0.04)</td>
</tr>
<tr>
<td>RR age &lt; 14</td>
<td>-</td>
<td>1.3 (0.044)</td>
<td>-</td>
</tr>
<tr>
<td>RR Male gender</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RR Osteoblastic subtype</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RR “large” tumour</td>
<td>-</td>
<td>1.4 (0.01)</td>
<td>-</td>
</tr>
<tr>
<td>RR proximal location</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RR high alkaline phosphatase</td>
<td>-</td>
<td>2.1 (&lt;0.01)</td>
<td>-</td>
</tr>
<tr>
<td>RR Ablative surgery</td>
<td>3.39 (&lt;0.01)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RR non radical excision</td>
<td>-</td>
<td>1.3 (0.044)</td>
<td>-</td>
</tr>
<tr>
<td>RR local recurrence</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RR p-glycoprotein expression</td>
<td>-</td>
<td>-</td>
<td>3.37 (&lt;0.01)</td>
</tr>
<tr>
<td>RR No Erb-2 expression</td>
<td>2.24 (0.03)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

EFS = evidence free survival, RR = relative risk, OS = overall survival, - = not tested, MFS = metastatic free survival
Because of this no pooling was performed. The RRs were 1.3 (95%-CI 0.99 – 1.70) and 3.6 (95%-CI 2.33 – 5.57).

### 2.4 Discussion

#### 2.4.1 Scope of the review

Our aim was to systematically study the literature after 1992 to find out whether new factors had proven to be of predictive value for survival in osteosarcoma, and whether new studies could elicit more about the value of known factors. Contrary to Davis(1), we chose not to limit ourselves to factors reported at least four times. It was decided to include only papers specifically addressing prognostic factors. By doing so, possibly valuable studies were missed, reporting about prognostic factors as secondary product of research. This literature however is diverse, and the studies are not designed to properly investigate prognostic factors.

Another choice was to exclude papers without multivariate analysis. Almost none of the published papers provided raw data, and if they did usually it concerned only very small patient samples. Because of this, only multivariate analysis gives the opportunity to see whether studied factors independently predict outcome, and only in this way an attempt of pooling of study results could be done. The decision to
limit papers to those about non-metastatic, high grade, extremity osteosarcomas was taken because this group of patients, which is the majority of osteosarcoma patients, is treated very uniformly, making comparisons valid. In patients with axial or metastatic osteosarcoma, treatment varies widely, making comparisons impossible.

FIGURE 2.1 Pooled relative risk of death or recurrence for poor chemotherapy response (A), large tumor size (Bielack) or volume (Bacci, Smeland) (B), and ablative surgery (C).
Papers were still considered for inclusion if the groups with axial or metastatic disease were analysed separately or in multivariate analysis. With these limitations we tried to optimize the chance of being able to pool study results.

2.4.2 Overview of prognostic factors in the literature

In the literature since 1992, some factors were only sporadically reported, but some were consistently reported by several authors to have a predictive value. Known factors, such as more proximal location and larger tumors were regarded as a poor sign, but were inconsistently reported(2;5;6;8;10;19;25;27-37). A uniform definition differentiating between large and small tumors does not exist. Chemotherapy response, although usually seen as the most reliable factor, is not found to be predictive by all authors(10;25;38-41). The newly reported factors are mostly “indirect” factors, like protein expression in serum or genetic imbalances of tumor tissue. Most promising among these seem to be the high expression of p-glycoprotein(11;42-47), expression of the human epidermal growth factor receptor 2 (HER2)(10;48-51), expression of vascular endothelial growth factor (VEGF)(38;52-54), and loss of heterogeneity of the Rb-gene(55).

It is possible that some of these factors may be of true predictive value, but it is important to keep in mind that the majority of these studies had methodological flaws. Because of this, simply counting the papers with a supposed predictive value is dangerous. Valid conclusions can not yet be drawn from this part of the literature.

2.4.3 Discussion of the 7 included studies

The 7 studies that could be included in the current review were all of high methodological quality. Still, some peculiarities struck us studying them. Akutsaka et al(10) report increased expression of the transmembrane glycoprotein ErbB2 to be the only independent predictor for better survival in their patients. Multivariate analysis however, also shows limb saving surgery to be a significant independent predictor for better event free survival, but not for overall survival. Tumor size is not taken into the multivariate analysis and could bias these results as the authors state in their discussion.

In Baldini’s study(11), p-glycoprotein expression is associated with a decrease in event free survival. Strangely enough in this study, chemotherapy response is significantly related to event free survival in the multivariate analysis, but not in the univariate analysis. The authors conclude that both p-glycoprotein expression and chemotherapy response are independent prognostic factors.
Weeden et al.(2) analyzed the effect of local recurrence as fixed co-variate, time-dependent co-variate and in a landmark analysis. As the authors explain, the influence of local recurrence will probably be underestimated if local recurrence is being analyzed as a fixed co-variate, because patients who have a bad prognosis and die early in the course of their disease might not have had the time to develop local recurrence. Landmark analysis, as the authors performed, eliminates this bias. However, the authors include histological response to chemotherapy only in an analysis with local recurrence as a time-dependent co-variate, not in their landmark analysis, and still conclude histological response and local recurrence to be independent predictive variables.

In the group of patients with an extremity osteosarcoma Bielack(6) finds inadequate surgical margins, poor chemotherapy response, proximal tumor location, and tumor size independent prognostic factors. However, as inadequate surgical margin is considered an event, the most complete analysis, including this factor, is on overall survival only. Although the authors do not describe this in their conclusions, tumor size seems to lose its independent predictive value in this last multivariate analysis.

A major concern is the heterogeneity of the studies and of the reported data. The difference in cut off points for various factors may make the pooled results less reliable. Although for chemotherapy response, tumor size and type of surgery the statistical heterogeneity in our included studies was acceptable, in general this methodological heterogeneity might hamper pooling of study results. Different authors report on different sets of factors in their multivariate analysis making pooling of results less valuable. Because of the relatively small sample sizes in the included studies, even very powerful prognostic factors may not have become significant(56) and may have been left unreported. If the non-significant results could have been pooled, more precise estimates of the effect might have been possible. On the other hand, because usually the actual figures about prognostic factors only were published if they appeared to be significant, the pooled relative risks that we calculated from these publications might be overestimated (outcome bias).

### 2.4.4 What can be concluded?

Notwithstanding numerous publications on prognostication in osteosarcoma, predicting survival chance for individual patients remains very difficult. Osteosarcoma is a rare disease with an incidence of around 3 per million(57). For evaluation of prognostic factors, long term results are needed. For these reasons, reports about prognostic factors are almost entirely based on historic patient cohorts. Early reports are mostly about small series from single institutions. Nowadays, bone tumor treatment is more and more concentrated in specialized centers, and in multicenter
co operations. Recent literature therefore can report about larger patient populations, leading to more powerful inferences.

Reliable meta-analysis of prognostic factors in osteosarcoma is still hampered by the heterogeneity of study designs and reporting.

From the available information in the literature one may assume that chemotherapy response is an independent prognostic factor, a poor response increasing the risk for dying of the disease possibly approximately 2.4 times. Other factors that are presumably independently predicting a worse outcome are large tumors, inadequate excision margin, ablative surgery, age under 14 years, male gender, high alkaline phosphatase, local recurrence, p-Glycoprotein expression, and absent Erb2 expression. Pooled relative risks could not be calculated for these factors.

There is an urgent need for methodologically high quality studies with more uniform study design and more uniform reporting. Results of all factors studied should be reported, whether significant or not. It would be most useful if raw data could be made available eg in collaborative databases. Cooperative studies that are being conducted at the moment, such as the EurAmos initiative, will hopefully improve the situation to eventually enable combining of data and possibly make individual prognostication more accurate.

Conflict of interest: None declared.
Reference List


47. Park YB, Kim HS, Oh JH, Lee SH. The co-expression of p53 protein and P-glycoprotein is correlated to a poor prognosis in osteosarcoma. *International Orthopaedics* 2001;24:307-10.


Prognostic Factors and Late Effects of Treatment in Pediatric Non-metastatic Osteosarcoma

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M.J.J. Veth¹
C. vd Bos²
J. Bras³
G.R. Schaap¹
C.N. van Dijk¹
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Submitted
3.1 Introduction

With the current modalities of treatment, survival in high grade osteosarcoma is reported to be 60-70%, occasionally even higher(1-4). The development of imaging techniques and the possibility of downgrading the tumor before resection has made it possible to perform limb saving procedures with adequate margins in about 90% of patients. Reconstruction techniques have taken a big flight and there are several options, from large endoprosthetic replacements to complete biological reconstructions. Each of these, however, has its own pro’s and con’s. Rehabilitation can take more than a year after a biological reconstruction. Long term problems can be challenging, especially after large endoprosthetic replacements(5). As a result of the increased survival, there is a growing number of long term survivors of high grade osteosarcoma(6). They survived their malignant disease, but possibly face adverse effects of the intensive treatment. Chemotherapy and resection / reconstruction surgery can lead to invalidating or even life threatening long term disease(7;8). Although various prognostic factors have been reported in the literature, prediction of survival chance and occurrence of long term adverse events is hardly possible for the individual patient(9;10). Optimizing this would be helpful in counseling patients and their parents and it could facilitate the choice of chemotherapeutic and surgical treatment. Furthermore, knowledge about long term treatment related events can improve alertness and possibly allow timely adequate reaction. The aim of this study was to evaluate survival, prognostic factors, and the occurrence of late adverse effects of treatment in the patients, treated in our institute: the Emma Children’s Hospital/Academic Medical Center (EKZ/AMC), Amsterdam, the Netherlands.

3.2 Patients and methods

3.2.1 Survival and prognostic factors

A retrospective survey was conducted, including all consecutive patients, treated in our institution between January 1985 and January 2006 for high grade, non-metastatic, extremity osteosarcoma, and who were less then 18 years of age at the time of diagnosis. Only those patients were included who had received standard treatment, consisting of neo-adjuvant, pre-operative, chemotherapy, followed by resection of the tumor, and post-operative chemotherapy. Chemotherapy was given according to protocols of the European Organization for Research and Treatment of Cancer (EORTC)(3;11), and consisted of combinations of cisplatin, doxorubicin, ifosfamide and methotrexate. Of 81 patients, treated in the studied period for high grade extremity osteosarcoma, 11 were excluded; 10 because they had metastatic disease at diagnose, 1 because he did not receive chemotherapy pre-operatively,
and developed multiple metastases during post-operative chemotherapy which was discontinued. Of the 70 included patients, 3 died of a cause other than osteosarcoma. Two of them died of severe cardiomyopathy, one 2 months after completion of therapy, one 18 years later. In the third patient the cause of death was myelodysplastic syndrome, 11 years after treatment. In the analysis of prognostic factors these patients were censored at the time of their death. The study population consisted of 38 females and 32 males. The mean age at diagnosis was 12.4 years, the median 12.5 years (5.0 – 18.0). Possible prognostic factors, identified from the literature, were analyzed in a uni- and multivariate analysis. These concerned age at diagnosis (> or ≤ 14 years), gender, size of the tumor (> or ≤ 1/3 of bone length), proximity of the tumor (proximal humerus and proximal femur were defined as proximal, others as distal), osteosarcoma subtype, type of surgery (ablative or limb saving), surgical margin (wide or radical margins were defined as adequate, others as inadequate), chemotherapy response (good if there was less than 10% viable tumor in the resection specimen), and local recurrence. At the time of the analysis, 45 patients were alive with a mean follow-up time of 150 months, median 163 months (10 – 252 months). Two of these patients were lost to follow-up (both living abroad), 10 and 16 months after diagnosis. Thus completeness of follow-up at 3 years was 96%.

3.2.2 Late adverse effects of treatment

Of the surviving patients, available for follow-up, 40 were alive 5 years after the end of treatment. These patients were invited to visit the outpatient clinic, especially established to evaluate long term effects after treatment of childhood cancer. According to a standardized protocol a full medical investigation was performed including history, physical examination, radiological and laboratory investigations. Patients were also seen by a psychologist or specialized nurse. Adverse effects of treatment were recorded and graded according to the Common terminology Criteria for Adverse Events version 3.0 (CTCAEv3.0, available at http://ctep.cancer.gov/forms/CTCAEv3.pdf). These criteria describe the severity for each event (grade 1: mild; grade 2: moderate; grade 3: severe; grade 4: life threatening or disabling; grade 5: adverse event–related death). In this paper we limited the analysis of adverse events to those categories of the CTCAE, which are most likely to be directly related to the cancer treatment, and which are the most objective ones (eg cardiac, metabolic, secondary tumors, bone marrow related, endocrine, musculoskeletal, lymphatic, neurological, reproductive, auditory). For grading of auditory damage, the system of Brock et al was used(12), because this takes hearing loss in speech frequencies into account, thus giving a better estimate of functional hearing loss. In the musculoskeletal category, rotationplasty was scored the same as amputation.
(grade 4). Only those late events were considered, that were still present at the time of the last follow-up. For this reason we considered it more appropriate to talk about “effects” in stead of “events”. Total burden of adverse effects in individual patients was established in the same fashion, formerly used by Geenen et al (Low burden: 1 or more grade 1 event; Medium burden: 1 or more grade 2 and/or 1 grade 3 event; High burden: 2 or more grade 3 effects, or 1 grade 4 event and at the most 1 grade 3 event; Severe burden: more grade 3/4 effects or a grade 5 event)(8).

3.2.3 Statistical analysis

Nominal variables were compared between groups with an $\chi^2$ test, continuous variables with a t-test (level of significance 0.05). Survival was estimated with a Kaplan-Meier analysis. Comparison of survival between groups was done with a log rank test. If subgroups consisted of less then 10 patients, comparison was refrained of, because this was regarded statistically inadequate. Factors which appeared relevant in the univariate analysis were tested in a multivariate analysis with the Cox regression model.

3.3 Results

3.3.1 Patient, tumor, and treatment characteristics

Table 3.1 shows patient and tumor characteristics, figure 3.1 the distribution of tumor locations. The majority of patients (48 out of 70 patients) were younger than 14 years, with a median age of 12.5 years. There were slightly more females (38) than males (32). The mean duration of symptoms before diagnose was 11.4 weeks, with a maximum of 56 weeks. Thirty-six patients had complains during 4 to 12 weeks before diagnose, 25 patients had had complains for more than 3 months. The majority of tumors were located distally, mostly around the knee. The mean tumor length was 11.6 centimeter, the ratio of tumor length and bone length was larger than 1/3 in 23 patients, no adequate information about the tumor length could be acquired in 9 patients. Sixty-three of 70 tumors were conventional type osteosarcomas, most of which of the osteoblastic subtype. In only 2 of the patients the osteosarcoma was intra-compartmental (stage II-a), all other patients had extra-compartmental growing tumor (stage II-b). Three of the patients presented with a pathological fracture, in 2 patients a pathological fracture occurred during treatment.

All patients were treated with neo-adjuvant chemotherapy, followed by surgery, and adjuvant chemotherapy. Surgery was ablative in 53 of 70 patients and concerned amputation or exarticulation of the limb in 24, and rotationplasty in 29 patients. In the other 17 patients, resection was followed by reconstruction with an auto- or
Table 3.1 Patient and Tumor Characteristics

<table>
<thead>
<tr>
<th>Age at diagnose</th>
<th>Gender</th>
<th>nr</th>
<th>Duration of symptoms</th>
<th>weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Female</td>
<td>38</td>
<td>Median</td>
<td>8.0</td>
</tr>
<tr>
<td>(range 5.0 – 18.0)</td>
<td>Male</td>
<td>32</td>
<td>(range 1.0 – 56.0)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proximity</th>
<th>Subtype</th>
<th>nr</th>
<th>Tumor length cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>Conventional</td>
<td>63</td>
<td>Median 10.0</td>
</tr>
<tr>
<td>Distal</td>
<td>Osteoblastic</td>
<td>30</td>
<td>(range 4.0 – 36.0)</td>
</tr>
<tr>
<td></td>
<td>Chondroblastic</td>
<td>3</td>
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</tr>
<tr>
<td></td>
<td>Fibroblastic</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not specified</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>High grade surface</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Small cell</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Telangiectatic</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surgery type</th>
<th>Surgical margin</th>
<th>nr</th>
<th>Chemotherapy response</th>
<th>nr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limb saving</td>
<td>Adequate</td>
<td>54</td>
<td>Good</td>
<td>26</td>
</tr>
<tr>
<td>Rotationplasty</td>
<td>Inadequate</td>
<td>12</td>
<td>Poor</td>
<td>44</td>
</tr>
<tr>
<td>Amputation</td>
<td>Unknown</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
allograft or with an endoprosthesis. Resection margins were mostly adequate, but in 12 of the 70 patients the closest margin was marginal (11 patients) or intralesional (1 patient). In the resection specimen, tumor necrosis was more than 90% in only 26 of 70 patients.

3.3.2 Survival and prognostic factors

Forty-five patients were alive at the date of last follow-up. Two of them were alive after recurrent disease (after resection of lung metastases), one was alive with disease (lung metastases), and 42 were without evidence of disease. The overall survival probability was 75% at 5 years and 66% at 10 years. Analysis of prognostic factors is shown in table 3.2 (univariate) and table 3.3 (multivariate). Proximity of the tumor and osteosarcoma subtype were not analyzed because the subgroups consisted of less than 10 patients. Large tumor size and poor chemotherapy response were significant

<table>
<thead>
<tr>
<th>Table 3.2 Univariate analysis of possible prognostic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prognostic factor</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>All patients</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>≤ 14</td>
</tr>
<tr>
<td>&gt; 14</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>female</td>
</tr>
<tr>
<td>male</td>
</tr>
<tr>
<td>Tumor size(^1)</td>
</tr>
<tr>
<td>&lt;=1/3</td>
</tr>
<tr>
<td>&gt;1/3</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Surgery type</td>
</tr>
<tr>
<td>Limb saving</td>
</tr>
<tr>
<td>Ablative</td>
</tr>
<tr>
<td>Surgical margin</td>
</tr>
<tr>
<td>Adequate(^2)</td>
</tr>
<tr>
<td>Inadequate(^3)</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Chemotherapy response</td>
</tr>
<tr>
<td>Good(^4)</td>
</tr>
<tr>
<td>Poor(^5)</td>
</tr>
</tbody>
</table>

\(^1\)ratio of tumor length and bone length; \(^2\)wide or radical; \(^3\)marginal or intra-lesional; \(^4\)<10% viable tumor in resection specimen; \(^5\)>10% viable tumor in resection specimen
predictors for worse survival in the univariate analysis; chemotherapy response just lost significance in the multivariate analysis, but showed a very strong trend. In the 22 patients that died of the osteosarcoma, the mean survival time was 38.5 months from the date of diagnose till death (median 30.5 months, from 11 – 100 months). None of the analyzed prognostic factors correlated with survival time in the deceased.

### 3.3.3 Late adverse effects of treatment

Table 3.4 shows the adverse effects that were found in the 40 long term survivors who were screened at the clinic. Only one patient had no adverse effects. Thirty-nine patients (98%) had one or more adverse effect (see figure 3.2). Musculoskeletal problems comprise 40 out of 78 late effects (52%). Thirty-six of the 70 reported late effects (46%) were mild or moderate (grade 1 or 2), 40 (51%) were severe or disabling/life threatening (grade 3 or 4).

Two patients had a fatal adverse late effect (grade 5). One died of myelodysplastic syndrome, 11 years after the end of treatment. The second patient died of congestive heart failure, 18 years after the end of treatment. Other cardiac late effects mainly concerned left ventricular systolic dysfunction, which was mild in 5 patients (left ventricular shortening fraction between 24 and 30%), and moderate in 2 patients (left ventricular shortening fraction between 15 and 24%). One patient had asymptomatic valvular heart disease.

Metabolic adverse effects consisted of decreased glomerular filtration rate in 6 of the 7 involved patients and were mild (grade 1; between 50 and 75% of the lower limit of normal). One patient had a moderately (grade 2) decreased sodium and magnesium level. No secondary tumors or endocrine adverse effects were found.

Twenty-nine grade 4 effects (life threatening or disabling) occurred in 28 patients (70%)(see figure 3.3). Almost all of these grade 4 effects were of the musculoskeletal category, and in most cases concerned the long term result of ablative surgery; in 16 of the surviving patients a rotationplasty had been done, in 12 amputation of the extremity. Other musculoskeletal adverse effects were end result of arthrodesis in 5 patients, joint function impairment after resection and reconstruction with a spacer.
in 2 patients, after reconstruction with a prosthesis in 1, late re-operations impairing 
function in 3 patients, and edema in 1 patient.

Lymphatic problems were sporadic and mild. Neurological late effects occurred 
in 2 patients and were severe (grade 3) in one, suffering from seizures, and life 
threatening / disabling (grade 4) in the other, who had severe depression resulting 
in suicide attempts. Sexual/Reproductive function was moderately impaired (grade 
2) in 3 patients, who had diminished fertility, and severely in 1 patient (grade 3) in 
whom a symptomatic gynaecomastia required an operation.

Auditory problems were seen in 35% of patients and mainly were mild or moderate 
(grade 1 or 2) but severe in 2 patients who had a significant hearing loss (>=40 
decibel) in the speech frequency area.

The total burden of effects is shown in table 3.5. Twenty-three patients suffered 
from 2 or more adverse effects. Only 1 out of 40 patients had a low burden score. 
As many as 29 patients had a severe or high burden. The high burden score in these

<table>
<thead>
<tr>
<th>Category (CTCAE version 3.0)</th>
<th>Effects (number)</th>
<th>Patients (%)</th>
<th>Grade (median)</th>
<th>Grade (minimum)</th>
<th>Grade (maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>8</td>
<td>8(20)</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Metabolic (including kidney)</td>
<td>8</td>
<td>7(18)</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Secondary tumors</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1</td>
<td>1(3)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Endocrine</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>40</td>
<td>38(95)</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Lymphatic</td>
<td>1</td>
<td>1(3)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neurological</td>
<td>2</td>
<td>2(5)</td>
<td>3.5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Sexual/reproductive</td>
<td>4</td>
<td>4(10)</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Auditory</td>
<td>14</td>
<td>14(35)</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total of adverse effects</td>
<td>78</td>
<td>39(98)</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Median nr of effects per patient</td>
<td>2</td>
<td>39</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Minimum nr of effects per patient</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maximum nr of effects per patients</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Distribution of grades      |                  |              |                |                |                |
| Grade 1                     | 21               | 15(38)       | -              | -               | -               |
| Grade 2                     | 15               | 11(28)       | -              | -               | -               |
| Grade 3                     | 11               | 10(25)       | -              | -               | -               |
| Grade 4                     | 29               | 28(70)       | -              | -               | -               |
| Grade 5                     | 2                | 2(5)         | -              | -               | -               |
29 patients was mainly caused by the musculoskeletal outcome. Twenty-five of these 29 patients (86%) had a grade 3 or 4 musculoskeletal effect which was responsible for the high burden score. One patient had several effects, among which a grade 4 musculoskeletal effect and a fatal cardiac effect, 1 patient had only 1 effect which was fatal. Two patients had several grade 1 and 2 effects accumulating to a high burden score.

**FIGURE 3.2** Distribution of number of effects per patient

**FIGURE 3.3** Distribution of grades among the different categories of adverse effects

---

<table>
<thead>
<tr>
<th>nr of effects</th>
<th>nr grade 1 effects</th>
<th>nr grade 2 effects</th>
<th>nr grade 3 effects</th>
<th>nr grade 4 effects</th>
<th>nr grade 5 effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table 3.5 Total Burden of adverse effects

<table>
<thead>
<tr>
<th>Burden score</th>
<th>Nr of patients</th>
<th>Nr of grade 5 effects</th>
<th>Nr of grade 4 effects</th>
<th>Nr of grade 3 effects</th>
<th>Nr of grade 2 effects</th>
<th>Nr of grade 1 effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe (n=6)</td>
<td>1 → 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 → 1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>&gt;2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 → 0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2 → 0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>High (n=23)</td>
<td>12 → 0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3 → 0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2 → 0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>&gt;2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 → 0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>&gt;2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4 → 0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1 → 0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Medium (n=9)</td>
<td>3 → 0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2 → 0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1 → 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt;2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 → 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt;2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1 → 0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Low (n=1)</td>
<td>1 → 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt;1</td>
<td>&gt;1</td>
</tr>
</tbody>
</table>

(Low burden: 1 or more grade 1 event; Medium burden: 1 or more grade 2 and/or 1 grade 3 event; High burden: 2 or more grade 3 effects, or 1 grade 4 event and at the most 1 grade 3 event; Severe burden: more grade 3/4 effects or a grade 5 event)(8).

In table 3.6 the cumulative chemotherapy doses and the adverse effects are shown for patients treated with or without ifosfamide. In both groups, musculoskeletal adverse effects were the most common ones. Cardiac disease was seen more in the group in which ifosfamide was included in the regimen, the patient with fatal cardiomyopathy had been treated in this group. The only late effect in the bone marrow was in the patient with the fatal myelodysplastic syndrome 11 years after treatment, who had been treated with an extensive regimen including cisplatin, doxorubicin, ifosfamide, methotrexate, etoposide, cytarabin and idarubicin. Hearing problems were about equally spread among the two treatment groups.
Survival, prognostic factors, and late adverse effects of osteosarcoma treatment in children, treated in our institute were studied. Five year survival was 75%, higher than in other published series (1-4). Tumor size and chemotherapy response were found to independently predict survival. All but one surviving patients had one or more adverse effect, twice this was fatal. The majority of adverse effects concerned the musculoskeletal system.

To allow a proper comparison of prognostic factors and late adverse effects between groups, and rule out bias by treatment variations, patients who presented with metastasis were excluded. These patients are often treated in a non standardized manner, curative or sometimes only palliative. Patients with axial osteosarcoma were excluded as well, because this behaves differently, and also is treated variably (13;14).

### Table 3.6 Chemotherapy and late adverse effects in 40 patients surviving >5 years after treatment

<table>
<thead>
<tr>
<th>Chemotherapy regimen</th>
<th>Cis+doxo(^1,2) N=19</th>
<th>Cis+doxo+ifos(^3) N=21</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cumulative dose(^4)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cisplatin</td>
<td>653</td>
<td>312</td>
<td></td>
</tr>
<tr>
<td>doxorubicin</td>
<td>424</td>
<td>415</td>
<td></td>
</tr>
<tr>
<td>ifosfamide</td>
<td>-</td>
<td>16300</td>
<td></td>
</tr>
<tr>
<td><strong>Follow-up time(^5)</strong></td>
<td>147(77-220)</td>
<td>190(66-252)</td>
<td>0.003</td>
</tr>
<tr>
<td>Ptnts with Adverse effects</td>
<td>19(100%)</td>
<td>20(95%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Cardiac</td>
<td>1(5%)</td>
<td>7(33%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Metabolic(incl kidney)</td>
<td>5(26%)</td>
<td>2(10%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Secondary tumors</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>0</td>
<td>1(5%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Endocrine</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>19(100%)</td>
<td>19(90%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Lymphatic</td>
<td>0</td>
<td>1(5%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Neurological</td>
<td>0</td>
<td>2(10%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Sexual/reproductive</td>
<td>1(5%)</td>
<td>3(14%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Auditory</td>
<td>7(37%)</td>
<td>7(33%)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

\(^1\) In 3 patients cisplatin was discontinued and changed to carboplatin because of severe ototoxicity, \(^2\) In 2 patients methotrexate was added, in 1 patient bleomycin cyclofosfamide and actinomycin was added, \(^3\) In 3 patients metothrexate was added, in 1 etoposide, in 1 etoposide, cytarabin and idamycin, \(^4\) Mean, in mg/kg body weight, \(^5\) Mean in months (minimum – maximum)

### 3.4 Discussion

Survival, prognostic factors, and late adverse effects of osteosarcoma treatment in children, treated in our institute were studied. Five year survival was 75%, higher than in other published series (1-4). Tumor size and chemotherapy response were found to independently predict survival. All but one surviving patients had one or more adverse effect, twice this was fatal. The majority of adverse effects concerned the musculoskeletal system.

To allow a proper comparison of prognostic factors and late adverse effects between groups, and rule out bias by treatment variations, patients who presented with metastasis were excluded. These patients are often treated in a non standardized manner, curative or sometimes only palliative. Patients with axial osteosarcoma were excluded as well, because this behaves differently, and also is treated variably (13;14). Because both chemotherapy and surgery are considered essential in the treatment of
osteosarcoma(15-17), only patients were included who completed the full treatment. These decisions might explain the favorable survival rate of 75% that we found.

Some prognostic factors (proximity and subtype of the tumor, and pathological fracture) could not be analyzed because the subgroups were too small to compare. Age, gender, surgery type and surgical margin were not of prognostic value. For age and gender this is in accordance with most other studies. Bielack finds a correlation of age with survival in a univariate, but not in the multivariate analysis(18). Grimer et al showed that patients over 40 years of age have a worse survival, but for primary extremity osteosarcoma patients, the difference was small(19). A recently published cooperative study of 2680 patients, treated in 10 large tumor centers, showed increasing age to increase risk of death of the disease of 7% per decade(17). Probably, age can only been found of significance in large populations, and is of limited prognostic value in children.

Ablative surgery was found by several authors to correlate with worse survival. In the cooperative study of Pakos(17), this was a strong indicator. Usually this is explained by the bias effect of these patients having larger tumors, where resection is deemed impossible. Our ablatively treated patients did not show this worse survival rate. In the studied period, the use of growing prostheses or biological reconstructions was still uncommon in our center. The abovementioned selection bias was probably not present because of the often chosen solution of rotationplasty, for other reasons than tumor size.

Adequate surgical margin after resection of the tumor did not show correlation with survival in our patients and has been inconsistently reported to do so(9;10;20). This variance doubtlessly is due to the differences in definition of an adequate margin. Tumor size was predictive for survival in our population. A problem with this is that adequate information is often missing(17). Size is difficult to establish accurately, and varies when measured on plain X-ray, CT-scan, or MRI. It seemed reasonable not to use a more precise parameter than relative length of the tumor, according to other authors(18). The relative risk for dying of the disease in our patients was more than threefold for patients with a large tumor. This confirms the results of others(18;21). Chemotherapy response still is the most consistently reported independent predictive factor for survival in osteosarcoma(1;9;10;20). In our study, poor response was associated with an almost threefold risk of dying of the disease, although this just lost its significance in the multivariate analysis, probably due to the small sample size.

The burden of adverse effects was considerable. The most common type involved the musculoskeletal system. This is the inevitable result of treatment, in which adequate tumor resection is obligatory. Many patients had a grade 4 musculoskeletal adverse effect. This can be explained by the large proportion of patients in which
rotationplasty was performed. In the CTCAE classification, absence of a limb is defined as a grade 4 event. Technically, rotationplasty should be counted as a grade 4 effect. Functional studies, however, show equal results of rotationplasty compared to endoprosthetic replacement, and better than after amputation(22;23). In modern oncologic orthopedics, over 90% of patients can be treated with limb saving surgery(5). This will favorably effect long term outcome and decrease burden of long term effects.

The second most frequently found type of adverse long term effect was auditory. This is not surprising; All of the used chemotherapy regimen contained cisplatin, which is known for its ototoxicity(24-27). Although mostly mild or moderate, some patients suffer severe hearing loss in speech frequencies.

Cardiac effects occurred in 20 % of patients, and were also mostly mild or moderate, apart from in the 1 patient who died of cardiomyopathy, 18 years after treatment. The possibility of late cardiac toxicity has been reported and, although uncommon, is known to have a high mortality(28). This is usually attributed to the use of anthracyclines. Alkylating agents seem to add to this effect(8). Toxicity should be minimized where possible, by lowering the cumulative dose, or by the use of cardio protective agents. Long term follow-up and alertness are important, allowing early detection and treatment(29-31).

The high proportion of chronic disease in the survivors in our study population is in accordance with publications which show that bone tumor patients are at higher risk, compared to patients treated for other cancers(8;32-35). Mostly chronic morbidity in these patients is cardiovascular, musculoskeletal, and/or neurological. Therapies that increase the risk are surgery, alkylating agents and/or anthracyclines, and radiotherapy(32;33;36-38). Because osteosarcoma patients receive at least two out of these, they are at high risk. The Childhood Cancer Survivor Study (CCSS) published self assessed scores for physical disability in long term survivors; 71.8% of survivors of lower extremity bone sarcoma reported some disability, and as much as 25.6% mentioned severe impairment in daily life activities(37;39). Osteosarcoma patients scored worse than patients with other types of cancer.

In conclusion, survival was good in this patient group. Chemotherapy response and tumor size were of prognostic value. Late adverse effects were mainly musculoskeletal. Predicting survival still is hardly possible for the individual patient. There is a need for large scale prospective evaluation of prognostic factors, and for the development of more accurate ones. Attention should also be directed at adverse effects in survivors. Better prognostication, for survival and for adverse effects, should lead to a more individually planned treatment strategy improving both oncologic outcome and long term health status of survivors.
Reference List


Color Doppler Ultrasound predicts Chemotherapy Response, but not Survival in Pediatric Osteosarcoma

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4.1 Introduction

Histological response to pre-operative chemotherapy is an important prognostic factor in high-grade osteosarcoma. It has an important role in therapeutic management(1). Regrettably, it can only be assessed after a full chemotherapy regimen followed by resection. It would be advantageous to be able to predict histological response at an earlier stage in treatment. Because the majority of osteosarcomas occur in children, the method used should preferably be non-invasive, easy to plan, and not too demanding for the patient. In 1995 vd Woude et al showed Color Doppler Ultrasound to be reliable in predicting response after a full course of chemotherapy(2). The equipment used in their study however, is not widely commercially available. The aim of our study was to establish whether not only histological response to chemotherapy, but also survival in children with osteosarcoma, could be predicted with CDUS using widely available ultrasound equipment.

4.2 Patients and methods

Study design was prospective. 23 Consecutive children were included, in whom a high-grade osteosarcoma of one of the extremities was diagnosed, and confirmed by biopsy. The Medical Ethical Committee of the hospital approved the study. Informed consent by proxy was obtained before patients were included. Two patients were excluded from the study, because their parents found it inconvenient for them to travel to the hospital for the 2nd ultrasound scan. Of the remaining 21 patients, 12 were female and 9 male, with a mean age of 12.4 years at diagnosis (range 6 years 8 months to 17 years 3 months). The tumor was located in the distal femur in 14 patients, in the proximal tibia in 4, the proximal fibula in 1, the proximal humerus in 1, and the distal humerus in 1 patient. Nineteen Tumors were staged IIB, according to Enneking(3), 2 Patients had metastasis at diagnosis (stage III). Eighteen tumors were of the conventional type, 1 was a high-grade surface osteosarcoma, 1 a small cell osteosarcoma, and 1 a telangiectatic osteosarcoma. Treatment of the patients was not influenced by the study. The clinical team was not aware of the ultrasound studies results.

All patients were initially treated with 3 cycles of chemotherapy according to the protocol of the European Organization for Research and Treatment of Cancer(4) with combinations of cisplatin, adriamycin, doxorubicin and methotrexate. All patients but one were treated surgically after chemotherapy: 8 underwent amputation; 7 patients had rotationplasty according to van Nes-Borggreve(5); 5 patients had limb salvage surgery performed (local resection, followed by reconstruction). In one patient there was significant tumor growth and development
of metastases during chemotherapy. No operation was performed, only palliative treatment was given. This patient was not analyzed in respect to chemotherapy response.

After resection the tumor specimens were evaluated by a pathologist, specialized in bone tumor pathology (HB). He was also unaware of CDUS results. Response to chemotherapy was assessed according to the protocol of the European Osteosarcoma Intergroup. A good response is defined as less then 10% viable tumor in the specimen (Huvos III and IV)(6;7).

Ultrasound studies were done before and after chemotherapy. The first ultrasound study was done before the start of chemotherapy, just after the diagnosis was established. The second ultrasound study was performed 1 to 2 weeks after the completion of the full chemotherapy regimen (3 cycles), and just before resection was carried out.

Ultrasonography was performed with a high resolution 7.5 MHz linear array transducer (Acuson XP 128, Acuson corp., Mountain View, California, and Aloka SDD-1700, Aloka Co., Tokyo, Japan). All ultrasound studies were performed by one of two operators (FMG, MM). Ultrasound studies in a patient before and after chemotherapy were always performed by the same operator.

The blood flow pattern in the soft tissue component of the tumor was evaluated using Color Doppler imaging. The whole outer surface of the tumor was examined to identify the vessel with the highest flow. Then, angle correction was performed and the Peak Systolic Velocity (PSV) was measured.

Secondly spectral analysis of the feeding artery proximal to the tumor localization was done. The contra lateral corresponding artery was used as a control. The subclavian artery was used for tumors in the humerus, the common femoral artery for femoral tumors, the popliteal artery for tumors in the lower leg.

Figure 4.1 shows the flow pattern of the femoral artery of a patient with an osteosarcoma in the distal femur before and after chemotherapy. The Resistive Index (RI) was calculated from the Peak Systolic Velocity (PSV) and End Diastolic Velocity (EDV) in the artery (RI = PSV – EDV / PSV). In accordance to the method of vd Woude et al(8), the Quotients of Resistive Index in the artery of the extremity with the tumor and the healthy leg were calculated (QRI). Both parameters were measured and compared before and after chemotherapy. In a pilot study, carried out before the actual study, a PSV decrease of 20% or more seemed to be the level at which good chemotherapy response occurred. Based upon this, a good response with CDUS was defined as being a decrease in the PSV of at least 20% in the soft tissue component of the tumor, as well as an increase in the QRI of the feeding artery (any increase). CDUS results were compared with histological response and with survival.
FIGURE 4.1 Example of the flow pattern of the femoral artery in a patient with an osteosarcoma of the distal femur. In Figure 4.1-A the pattern before chemotherapy is showing a loss of the normal trifasic pattern, with an end diastolic velocity $> 0$. In Figure 4.1-B the pattern after chemotherapy is normalized to a trifasic one, with end diastolic velocity $= 0$. The Resistive Index (RI) was calculated from the Peak Systolic Velocity (PSV) and End Diastolic Velocity (EDV): $RI = \frac{PSV - EDV}{PSV}$. 
To evaluate differences in PSV change and QRI change between the different patient groups (chemotherapy responders versus non-responders and survivors versus non-survivors) an unpaired t-test was used with level of significance 0.05.

4.3 Results

Response to chemotherapy was scored as Huvos II-III in 2 patients, so they could not properly be scored as good or poor chemotherapy responders. In both of these patients the CDUS response was good. In order to take a critical approach to our results it was decided to consider them as poor chemotherapy responders.

Qualitative CDUS response is compared with histological response in table 4.1. There was a good CDUS response in 7 patients, 5 of which had a good histological response. A poor CDUS response was seen in 14 patients, 1 of them was the patient with progressive disease during chemotherapy, mentioned in the Patients and Methods section. No surgery was performed on this patient. Of the other 13 patients, 12 also showed a poor histological response (in 6 patients chemotherapy was scored as Huvos I, in 6 Huvos II). In 4 patients the CDUS parameters were contradictory. In 3 of these patients there was a >20% decrease in PSV, but no increase in QRI. Two of these 3 patients showed a poor chemotherapy response, one a good response. In one patient the QRI increased, but there was no decrease of the PSV. This patient also showed a poor chemotherapy response.

According to these results the specificity of CDUS for predicting chemotherapy response was 86% and the sensitivity 83%. The predictive value of a good CDUS response for a correspondingly good chemotherapy response was 71% and the predictive value of a poor CDUS response for a poor chemotherapy response 92%.

Had both the above-mentioned patients with Huvos II-III been considered to be good chemotherapy responders, the specificity would have been 88% and the sensitivity 100%. Likewise, the predictive value for good response would have been 100% and for poor response 92%.

Table 4.2 shows quantitative CDUS response compared with response to chemotherapy. There was no difference in mean QRI between good and poor chemotherapy responders before chemotherapy.

The mean change in QRI was 15.17% in the 6 patients with a good chemotherapy response (standard deviation 12.73) and 3.85% in the 14 patients with a poor chemotherapy response (standard deviation 7.87).

This difference was statistically significant in a Students-T test (p=0.030).
Table 4.1 - Qualitative CDUS versus Chemotherapy Response

<table>
<thead>
<tr>
<th></th>
<th>Good CT response</th>
<th>Poor CT response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>PSV decrease &gt;20%</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>PSV decrease &lt;20%</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Increase QRI</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>No increase QRI</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Good CDUS response</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Poor CDUS response</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

CDUS = Color Doppler Ultrasound; CT = Chemotherapy; PSV = Peak Systolic Velocity; QRI = Quotient of Resistive Index; Good CDUS response is defined as both a decrease of >20% in PSV, and an increase of QRI; Good chemotherapy response is defined as less than 10% viable tumor in the resection specimen (6,7).

Table 4.2 - Quantitative CDUS versus Chemotherapy Response

<table>
<thead>
<tr>
<th></th>
<th>Good CT response</th>
<th>Poor CT response</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>6</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Mean PSV before CT (m/s)</td>
<td>0.47 (0.23)</td>
<td>0.60 (0.36)</td>
<td>0.27</td>
</tr>
<tr>
<td>Mean PSV after CT (m/s)</td>
<td>0.23 (0.18)</td>
<td>0.45 (0.35)</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean PSV change (%)</td>
<td>-52.18 (21.11)</td>
<td>24.96 (140.92)</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean QRI before CT (%)</td>
<td>81.83 (11.07)</td>
<td>88.07 (8.21)</td>
<td>0.26</td>
</tr>
<tr>
<td>Mean QRI after CT (%)</td>
<td>96.83 (6.21)</td>
<td>90.43 (9.11)</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean QRI change (%)</td>
<td>15.17 (12.73)</td>
<td>2.57 (7.87)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Comparison of mean PSV and QRI, before and after chemotherapy, between patients groups with good or poor chemotherapy response. CDUS = Color Doppler Ultrasound; CT = Chemotherapy; PSV = Peak Systolic Velocity; QRI = Quotient of Resistive Index; Standard deviation between brackets; Good chemotherapy response is defined as less than 10% viable tumor in the resection specimen (6,7).

Table 4.3 - Qualitative CDUS versus Survival

<table>
<thead>
<tr>
<th></th>
<th>N.E.D.</th>
<th>L.W.D./D.O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>PSV decrease &gt;20%</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>PSV decrease &lt;20%</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Increase QRI</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>No increase QRI</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Good CDUS response</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Poor CDUS response</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

Comparison of CDUS response, between patients groups with different clinical outcome. CDUS = Color Doppler Ultrasound; PSV = Peak Systolic Velocity; QRI = Quotient of Resistive Index; N.E.D. = no evidence of disease; L.W.D. = living with disease; D.O.D. = deceased of disease. Good CDUS response is defined as both a decrease of >20% in PSV, and an increase of QRI.
Table 4.3 shows qualitative CDUS response, compared to survival. The median follow-up in survivors was 63 months (30-84 months). In the non-responder group, 5 patients were disease-free, 8 had died of metastatic disease (1 of whom also suffered local recurrence). 1 Patient was alive with metastatic disease, 36 months after diagnosis. From these figures it can be calculated that specificity of CDUS for predicting survival was 75%, and sensitivity 44%. The predictive value of a good CDUS response for survival was 57%. The predictive value of a poor CDUS response for an adverse clinical course was 64%.

Table 4.4 - Quantitative CDUS versus Survival

<table>
<thead>
<tr>
<th></th>
<th>N.E.D.</th>
<th>L.W.D./D.O.D.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>9</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Mean PSV before CT (m/s)</td>
<td>0.55 (0.36)</td>
<td>0.48 (0.30)</td>
<td>0.48</td>
</tr>
<tr>
<td>Mean PSV after CT (m/s)</td>
<td>0.32 (0.20)</td>
<td>0.49 (0.40)</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean PSV change (%)</td>
<td>-36.39 (30.48)</td>
<td>36.37 (152.69)</td>
<td>0.10</td>
</tr>
<tr>
<td>Mean QRI before CT (%)</td>
<td>86.89 (8.18)</td>
<td>86.58 (10.57)</td>
<td>0.37</td>
</tr>
<tr>
<td>Mean QRI after CT (%)</td>
<td>95.44 (6.58)</td>
<td>89.17 (9.52)</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean QRI change (%)</td>
<td>8.67 (9.14)</td>
<td>2.83 (13.05)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Comparison of mean PSV and QRI, before and after chemotherapy, between patients groups with different clinical outcome. CDUS = Color Doppler Ultrasound; CT = Chemotherapy; PSV = Peak Systolic Velocity; QRI = Quotient of Resistive Index; Standard deviation between brackets N.E.D. = no evidence of disease; L.W.D. = living with disease; D.O.D. = deceased of disease.

Table 4.4 shows quantitative CDUS compared to survival. There was no difference in mean QRI or PSV between survivors and non-survivors before the start of chemotherapy. The mean QRI change in the patients without evidence of disease was 8.67% (standard deviation 9.14). In the patients who developed metastatic disease the mean QRI change was 2.83 (standard deviation 13.05). The mean change in PSV was -36.39% in patients without evidence of disease (standard deviation 30.48) and 36.37 in patients with metastatic disease (standard deviation 152.69). These differences were not statistically significant (p-value in Students-T test were 0.23 and 0.10 respectively).

Table 4.5 compares chemotherapy response to survival. Calculated from these figures the specificity of chemotherapy response for predicting survival was 73%, and sensitivity 33%. The predictive value of a good chemotherapy response for survival was 50%. The predictive value of a poor chemotherapy response for an adverse clinical course was 57%. This seems comparable with the predictive value of CDUS for survival.
4.4 Discussion

Tumor necrosis after pre-operative chemotherapy remains the most important prognostic factor in osteosarcoma, although other factors, such as serum alkaline phosphatase level and tumor volume, gain importance (1). Cell necrosis after chemotherapy is taken into account when further therapeutic strategy is planned. However, this is only possible after chemotherapy, surgical resection of the tumor, and pathologic evaluation. Earlier, pre-surgical, prediction of histological response would have obvious advantages. Timing and type of operation could be adjusted, as could further chemotherapeutic treatment.

Clinical and conventional radiological methods, including conventional MRI, have proven not to be reliable in predicting chemotherapy response (4;9;10). Angiography can be used to assess changes in tumor vascularity, but this method is invasive, and not very accurate in predicting chemotherapy response as well (11). The value of skeletscintigraphy remains controversial. Whole body and 3-phase scintigraphy do not seem of use in predicting response to chemotherapy (8;12-15). Reports about dynamic Thalium-scintigraphy are promising (16-18), as are more recent reports about Positron Emission Tomography (19-21). Published experiences with dynamic MRI are also promising. Although no prediction of response can be done during chemotherapy, response after completion of chemotherapy seems to be accurate (2;22-27).

A disadvantage of all the above-mentioned methods is that small amounts of remaining viable tumor cannot be detected. Moreover these procedures are time-consuming, not entirely non-invasive and need patient compliance. Therefore they are not particularly suitable for children. For children a simple and short procedure, non-invasive, would be desirable.

Color Doppler ultrasound (CDUS) is such a non-invasive, short procedure. It can accurately assess tumor vascularity as demonstrated before by vd Woude et al. In his study however, Color Doppler Flow Imaging equipment was used where flow in the imaged tissue is measured directly, and non-dependent of angle correction. This equipment is not commercially widely available.

### Table 4.5 - Chemotherapy Response versus Survival

<table>
<thead>
<tr>
<th></th>
<th>N.E.D.</th>
<th>L.W.D./D.O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Good chemotherapy Response</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Poor chemotherapy Response</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

Comparison of chemotherapy response between patient groups with different clinical outcome. N.E.D. = no evidence of disease; L.W.D. = living with disease; D.O.D. = deceased of disease. Good chemotherapy response is defined as less than 10% viable tumor in the resection specimen (6;7)
The ultrasound system used in our study is available now in most modern hospitals. The procedure is simple and lasts at the most 20 minutes. Children are not required to lie still all the time. No injections or other invasive actions are necessary. Because of the short duration of the procedure and the availability of the equipment, logistic planning is easy. These are clear advantages of the CDUS method compared to dynamic MRI. A conventional MRI will have to be carried out pre-operatively as well, to establish extent of the tumor and relation to surrounding tissues after chemotherapy. It will however be much shorter and less invasive than a dynamic MRI would be. Apart from this it will be less costly.

A disadvantage of the ultrasound method is that inter-observer variability could occur in the assessment of the PSV of the soft tissue component of the tumor, because the region with the highest flow has to be established visually by the observer. A similar inaccuracy could however also occur with MRI, because only a limited number of slices can be acquired and a region of interest has to be chosen. For the second parameter, the Resistive Index of the feeding artery, the risk of variability is less. The feeding artery usually can be identified easily. Because an Index is calculated the measurements are less dependent on an exact angle correction. The spectrum can be recorded and is therefore easier reproducible. Furthermore a comparison with the normal contra lateral artery is possible.

Our results show that a negative response in ultrasound parameters, predicts a poor response to chemotherapy. The prediction of a positive response is less accurate. CDUS appeared of no use in predicting survival. This could be explained by the fact that CDUS, similar to other methods, does not detect small amounts of remaining viable tumor cells, because these have no effect on vascularity. One should also bear in mind that only the response of the primary tumor is assessed, not that of possibly already present micro metastases. Another factor that makes CDUS less accurate in predicting survival or chemotherapy response, is the fact that the resistive index (RI) of the main feeding artery is influenced not only by the tumor perfusion, but also by changes in the activity of the tumor bearing extremity, and possibly by other factors as well.

CDUS can predict chemotherapy response only after full preoperative chemotherapy. Thus preoperative treatment will not be influenced by the CDUS results. However, knowledge about response to chemotherapy will be useful in planning the operation (both in timing and planning the extent of the resection). For instance, in the case of a tumor, close to the neurovascular bundle, that can only just be resected, the knowledge that chemotherapy response will be good could make the surgeon decide to postpone surgery and give another cycle of chemotherapy. This way the resection might become easier and safer. Also in the case of a good chemotherapy response, the decision to undertake limb saving surgery would be made easier. On the other
hand, if chemotherapy response is expected to be poor, one would be less inclined to take risks by performing limb saving surgery. Our conclusion is that CDUS is a relatively simple procedure, predicting chemotherapy response before surgery is carried out. The method is suitable for children with an extremity osteosarcoma. It could be a useful tool for therapeutic considerations pre-operatively, especially when there is a negative CDUS response.
Reference List


Pre- and Post-Chemotherapy Alkaline Phosphatase as Prognostic Indicators in Adults with Localised Osteosarcoma

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² Academic Medical Center, Amsterdam, the Netherlands

5.1 Introduction
Although survival in high grade osteosarcoma has improved considerably since the introduction of chemotherapy, 30 to 40% of patients still die of the disease (1-5). Prognostication in individual cases remains a problem (2;6). It would be helpful if objective instruments were available for predicting the chance of survival, especially early in treatment, preferably even before surgery. Alkaline phosphatase is easy to determine at any stage of the disease, and has been shown by some authors to have a predictive value for survival (7-11), or chemotherapy response (12). Others however did not find a correlation of alkaline phosphatase for either (13). Most authors report only on alkaline phosphatase level before chemotherapy, or after surgery.
The aim of this study was to determine the value of alkaline phosphatase for predicting chemotherapy response and survival in adults with osteosarcoma. The alkaline phosphatase levels were assessed before chemotherapy, after chemotherapy but before surgery, and the change in the level of alkaline phosphatase after chemotherapy was recorded. Attention was given to osteosarcoma subtype to study both influence on alkaline phosphatase levels and on outcome.

5.2 Patients and methods
A retrospective study was performed using a prospectively recorded database. All patients were included who were treated at a specialist Orthopaedic Oncologic Centre between 1983 and 1999 for a primary, high grade, non metastatic osteosarcoma. Only patients 18 years of age or older at the time of diagnosis were studied, to exclude the influence of growth on alkaline phosphatase levels. Patients with pathological fractures were excluded, as these might confound results due to non tumour related alkaline phosphatase production as well. We included only those patients who had received standard treatment. This consisted of pre-operative chemotherapy, followed by resection of the tumour, and post-operative chemotherapy. Chemotherapy was administered according to protocol of the European Organisation for Research and Treatment of Cancer (EORTC) current at the time (4;14).
A total of 448 patients were treated for primary, non metastatic, high grade osteosarcoma at our centre over the studied period. Of them, 261 were under the age of 18, leaving 187 (42%) adult patients. Of these patients 18 had a pathological fracture and were exluded. A further 37 patients were excluded for several reasons: 7 died of unrelated causes (2 of suicide, 1 of myocardial infarction, 2 of pulmonary embolism and 2 of septicaemia) and 30 did not receive standard treatment (2 only palliative care, 2 no surgery because of irresectability of the tumour, 3 no preoperative chemotherapy because of immediate necessity of resection, 2 refused postoperative...
chemotherapy, 8 did not have chemotherapy because of their age and 1 because of pregnancy, in 7 chemotherapy was not completed due to chemotherapy related complications, in 4 no postoperative chemotherapy was administered without a mentioned reason, 1 was misdiagnosed as benign and had a resection prior to chemotherapy).

Case records and computer records were reviewed for measured values of serum alkaline phosphatase at diagnosis, before the start of chemotherapy (pre-ct AP), and after chemotherapy, but before surgery (post-ct AP). The alkaline phosphatase values were divided into 3 categories: Normal (below the upper normal limit), High (raised, but less then twice the upper limit), and Very High (raised more than twice the upper limit). In 28 of the 132 eligible cases, no alkaline phosphatase values in the periods of interest could be found. In the remaining 104 cases pre-ct AP was available in 89, post-ct AP in 86, and both values in 71 cases.

Patient, tumour and treatment characteristics were studied. The 4 groups of “AP-availability” (none, pre, post or both AP values available), were compared for age, sex, site, surgical margin, local recurrence and survival. Survival, local recurrence, and chemotherapy response were analysed and compared to pre-ct AP, post-ct AP and to the event of normalisation of AP after chemotherapy. Chemotherapy response was defined according to the protocol of the European Osteosarcoma Intergroup as good if less then 10% of viable tumour was found in the resection specimen(15). Correlation of the different AP levels with number of good or poor chemotherapy responders was established as well as correlation with the mean necrosis after chemotherapy. Tumours of pelvis, proximal humerus and proximal femur were considered to be “proximal”, the others “distal”. Radical and wide margins were considered to be “adequate”, marginal or intralesional margins “inadequate”. Because osteosarcoma subtype possibly could be of influence on Alkaline Phosphatase values, pre-ct AP, post-ct AP and normalisation of AP, as well as local recurrence and survival, were compared for the different subtypes according to the WHO Classification of Tumours(16). For the analysis of the normalisation of AP, patients were divided in 3 groups. 1: those where AP did normalize, 2: where it did not, 3: those where AP level at diagnosis was not raised were called “not applicable”, because obviously in these patients AP could not normalise.

**Statistical analysis**

Comparability of the groups of different “AP-availability” was assessed with the Chi-Square test for nominal variables and with the ANOVA post hoc analysis for continuous variables. Survival and local recurrence were determined by Kaplan Meier survival analysis and compared between groups with a log rank test (level of significance p=0.05). Chemotherapy response (good or poor) was compared
between groups with a Chi-square test. Tumour necrosis after chemotherapy (%) was compared between groups using ANOVA with a Bonferroni post hoc analysis (level of significance p=0.0167),

5.3 Results

5.3.1 Comparability of the groups

The group of 132 eligible patients consisted of 95 males and 37 females with a median age of 21 years (range 18-57) at diagnosis. The tumour site was predominantly around the knee, also a considerable number in the proximal humerus, and just a few in the pelvis or distal lower leg (figure 5.1).

![Figure 5.1: Tumour site in the 132 eligible patients.](image)

Surgery was ablative in 24% of patients and limb saving in 76%. The achieved surgical margins were wide or radical in 71%, marginal in 28%, and intrallesional in 1%. Age, sex, site, type of surgery and surgical margins were comparable among the different AP availability groups. Osteosarcoma subtype was conventional in the majority of cases (122 patients, 92%), telangiectatic in 8 patients (6%), high grade periosteal in 1, and small cell type in 1 patient. The 122 conventional type osteosarcomas consisted of 47 osteoblastic, 29 chondroblastic, 18 fibroblastic, and 28 not further specified ones. The subtypes were equally distributed among the groups of AP-availability (p=0.96 in chi square test).
Four patients were lost to follow-up before 5 years after diagnosis, they were censored. The 5-year and 10-year survival (Kaplan-Meier) were respectively 56% and 52%, and local recurrence occurred in 14% of cases. No differences in survival or local recurrence could be found between the groups with pre-ct AP, post-ct AP or both AP values available. The group in which no AP at all was available showed more local recurrence and had a worse survival (table 5.1).

### 5.3.2 Pre-ct AP values and the relation to Local Recurrence, Survival and Chemotherapy Response

Of the 89 patients in whom pre-chemotherapy AP values were available, this was Normal in 48, High in 22 and Very High in 19 patients. No statistical difference was found between these groups in the local recurrence rate, which was 13%, 5% and 5% respectively (p=0.53). 10-Year survival rate was similar in patients with Normal and High pre-ct AP (64% and 76%) but significantly lower when pre-ct AP was Very High (37%, p=0.005) (figure 5.2).

No statistically significant correlation was found between pre-ct AP and chemotherapy response (good versus poor) (p=0.26 in chi square test). In the group with a Normal pre-ct AP the mean necrosis after chemotherapy was 71%. When pre-ct AP was High the mean necrosis was 68%, and when pre-ct AP was Very High the mean necrosis was 59%. This difference did not reach the level of significance in the ANOVA analysis (p=0.65, 0.13 and 0.34; α=0.0167). From the frequency distribution
it can be calculated that the predictive value of a Very High pre-ct AP for a poor chemo response was 80% (table 5.2).

5.3.3 Post-ct AP values and the relation to Local Recurrence, Survival, and Chemotherapy Response

Post-chemotherapy AP values were available in 86 patients. This was Normal in 69, High in 13 and Very High in 4 patients. Again, no relationship was found with local recurrence, this being 8%, 15% and 0% respectively (p=0.18). Patients with a Normal post-ct AP had a survival of 68%. This was significantly better compared to patients with High or Very High post-ct AP who survived in 39% and 25% respectively (p=0.0007) (figure 5.3).

Post-ct AP values did correlate with chemotherapy response (good versus poor) p=0.049 in chi square test). Necrosis after chemotherapy was significantly lower when post-ct AP was elevated, with a mean necrosis of 70% in the Normal group, 38 and 47% in the High, and Very High group (p=0.0023 comparing the Normal and High group, and p=0.38 comparing the Normal and the Very High group). The predictive value of a elevated post-ct AP for a poor chemotherapy response was 100% (table 5.3).
5.3.4 Normalisation of AP values after chemotherapy, and the relation to Local Recurrence, Survival, and Chemotherapy Response

In 35 patients AP was not elevated before chemotherapy (not applicable), in 24 AP did normalise, and in 17 AP did not normalise after chemotherapy. Local recurrence did not differ between these groups (12%, 4% and 13%, p=0.43). Survival was 65% in the “not applicable” group, and 74% in the group where AP did normalise. The group where AP remained raised after chemotherapy had a significantly worse survival of 35% (p=0.0015) (figure 5.4).

The group of patients who’s AP normalised after chemotherapy, had a larger proportion of good responders to chemotherapy (p=0.018 in chi-square test). Necrosis after chemotherapy response was also significantly higher in patients where AP normalised over chemotherapy or where normalisation was not applicable (mean necrosis of 71% and 76% respectively), compared to patients where AP was still elevated (mean necrosis of 40%) (p=0.0004 comparing normalised to not normalised, and p<0.0001 comparing not applicable to not normalised). The predictive value of AP not normalising over chemotherapy for a poor chemotherapy response was 100% (table 5.4).

Table 5.3 Post-chemotherapy Alkaline Phosphatase (post-ct AP) and Chemotherapy Response

<table>
<thead>
<tr>
<th></th>
<th>Normal post-ct AP (n=69)</th>
<th>High post-ct AP (n=13)</th>
<th>Very High post-ct AP (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good responders</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poor responders</td>
<td>46</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>No info on response</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>% Necrosis (sd)</td>
<td>70 (29)</td>
<td>38 (24)</td>
<td>47 (6)</td>
</tr>
</tbody>
</table>

FIGURE 5.3 Kaplan-Meier survival curve showing the likelihood of survival from time of diagnosis for patients with Normal, High or Very High post-chemotherapy Alkaline Phosphatase (post-ct AP) (p=0.0007).
5.3.5 Osteosarcoma Subtype related to Alkaline Phosphatase levels and to Local Recurrence, Survival, and Chemotherapy response

No statistical difference in local recurrence or survival was found between patients with different subtype of osteosarcoma (p=0.12 and p=0.95 respectively in log rank test).

No correlation was found between osteosarcoma subtype and chemotherapy response (good or poor, p=0.55 in chi-square test) or percentage of necrosis after chemotherapy (not significant in all comparisons in anova post hoc test).

Pre-chemotherapy AP did correlate with subtype. Patients with an osteoblastic type of osteosarcoma were more frequently in the Very High pre-ct AP group.

Table 5.4 Normalisation of Alkaline Phosphatase (AP) and Chemotherapy Response

<table>
<thead>
<tr>
<th>Normalising: Not applicable (n=35)</th>
<th>Normalising: Yes (n=24)</th>
<th>Normalising: No (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good responders</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Poor responders</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>No info on response</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>% Necrosis (sd)</td>
<td>76 (26)</td>
<td>71 (29)</td>
</tr>
</tbody>
</table>

5.3.5 Osteosarcoma Subtype related to Alkaline Phosphatase levels and to Local Recurrence, Survival, and Chemotherapy response

No statistical difference in local recurrence or survival was found between patients with different subtype of osteosarcoma (p=0.12 and p=0.95 respectively in log rank test).

No correlation was found between osteosarcoma subtype and chemotherapy response (good or poor, p=0.55 in chi-square test) or percentage of necrosis after chemotherapy (not significant in all comparisons in anova post hoc test).

Pre-chemotherapy AP did correlate with subtype. Patients with an osteoblastic type of osteosarcoma were more frequently in the Very High pre-ct AP group.

Table 5.5 Osteosarcoma Subtype and Pre-chemotherapy Alkaline Phosphatase (pre-ct AP)

<table>
<thead>
<tr>
<th>Type</th>
<th>Normal pre-ct AP</th>
<th>High pre-ct AP</th>
<th>Very High pre-ct AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoblastic (n=31)</td>
<td>45%</td>
<td>19%</td>
<td>36%</td>
</tr>
<tr>
<td>Chondroblastic (n=21)</td>
<td>57%</td>
<td>33%</td>
<td>10%</td>
</tr>
<tr>
<td>Fibroblastic (n=14)</td>
<td>86%</td>
<td>0%</td>
<td>14%</td>
</tr>
<tr>
<td>Conventional ns (n=15)</td>
<td>47%</td>
<td>33%</td>
<td>20%</td>
</tr>
<tr>
<td>telangiectatic (n=6)</td>
<td>50%</td>
<td>50%</td>
<td>0%</td>
</tr>
<tr>
<td>Small cell (n=1)</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>High grade periosteal (n=1)</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>
(p=0.03 in chi square test) (table 5.5). This difference disappeared in the analysis of post-chemotherapy AP and normalisation of AP (p=0.43 and p=0.12).

5.4 Discussion

Assessment of the prognosis of individual patients with a high grade osteosarcoma is important for decision making and counselling of patients and/or their parents. Information that can be obtained early in treatment, preferably before operation, will be most helpful. Type and timing of surgery and chemotherapy possibly could be individualised to the patient’s needs if more accurate prognostication were available. Individual estimation of the prognosis however remains difficult. The strongest parameters still are stage at diagnosis and tumour necrosis after chemotherapy(3;6;10;13;17). The latter can only really be established after surgery, although imaging methods are being evaluated to predict necrosis before resection(18;19). Chemotherapy response remains a significant and independent predictor in several published multivariate analysis. Other factors that may have prognostic value are age, sex, tumour size and site, histological subtype and surgical margin after resection. They are however not consistently reported to be significant, and mostly do not hold in multivariate analyses(6).

Alkaline phosphatase has specifically been addressed as a prognostic factor by several authors. The enzyme has been shown to be produced directly by human osteosarcoma cells(20), and its level can be raised in patients with osteosarcoma(21). Thorpe et al showed a correlation of AP levels and prognosis in a small patient group(11). This study was done before the era of neoadjuvant chemotherapy. More recently studies with larger patient populations confirmed the prognostic value of alkaline phosphatase. Bacci et al reported pre-treatment AP levels to have a predictive value for survival(7), but not for chemotherapy response(22). Post neoadjuvant chemotherapy levels were reported to “normalize in most patients” in these studies, but it remains unclear what these post-chemotherapy levels meant regarding survival or chemotherapy response. In 2001 Ferrari(10), and in 2002 both Bacci(8) and Stokkel(9) also showed prognostic value for pre-treatment AP levels. In the last 2 mentioned papers this appeared to be an independent prognostic factor in multivariate analysis. Contradictory to these results was the study of Pochanugool who did not find any correlation between pre-treatment AP levels and survival(13). None of these studies however looked at the AP levels after chemotherapy and before surgery. Juergens did study post-chemotherapy AP levels and found them to be predictive for chemotherapy response, but did not study its value for predicting survival(12).
In accordance with most of the abovementioned papers our study shows that elevated pre-treatment AP over twice the upper normal level is predictive of a worse survival. Moreover, the predictive value of a Very High pre-treatment AP for a poor chemotherapy response was 80%. The AP level after chemotherapy but before surgery, seems even more useful. Survival decreased stepwise with post-ct AP values being normal, moderately raised or severely raised. The predictive value of any elevated post-ct AP for a poor chemotherapy response was 100%. A decrease of AP levels after chemotherapy appeared not to correlate with improved survival unless AP returned to normal, in which case survival was the same as in patients with a normal AP at diagnosis. We did not find a significant relationship between AP levels at any stage and local recurrence.

Osteosarcoma subtype appeared not to be predictive of Local Recurrence and Survival in this patient group, and did not correlate with chemotherapy response. This is contradictory to the results of Hauben et al (23) who found a significantly higher proportion of good chemotherapy responders in patients with a fibroblastic, and a lower proportion in patients with a chondroblastic subtype, and a trend for better survival in the chondroblastic group. This contradiction is remarkable because part of the patients in the current study were included in the above mentioned study. An explanation could be that in the current study only adults were included whereas Hauben et al only included patients under the age of 40. Moreover they reported on a large group of patients from different institutions, whereas our study only included a limited group from one institution. We did find a correlation of subtype with pre-chemotherapy AP levels, osteoblastic tumours showing more often a Very High pre-ct AP. This is in accordance with what one would expect, because osteoblastic tumours probably result in a higher turnover of bone.

One limitation in the present study was the fact that the patients in whom we were unable to find recorded AP levels had more local recurrences and a worse survival. No satisfactory reason for this difference could be found. A possible reason could have been that these patients were treated to a lesser extent in chemotherapy trials. This could explain the worse treatment results as well as less laboratory values being present. No such difference however could be found in these patient groups. In other respects this patient group did not differ from the other groups. Because the groups with pre-ct, post-ct, or both AP values were comparable we believe that our conclusions about the predictive value of AP are valid. Furthermore it should be emphasized that only patients over the age of 18 were studied, making our results only valid for adults, whereas the majority of osteosarcoma patients is under the age of 18 (58% in our population).

We conclude that alkaline phosphatase, measured before chemotherapy, after chemotherapy, and the change of alkaline phosphatase after chemotherapy are possibly valuable factors in predicting chemotherapy response and survival in high
grade osteosarcoma in adults. This factor is cheap and easy to determine and could, together with other factors, play a role in improving individual prognostication. It should therefore be determined systematically in a prospective manner in order to further evaluate its usefulness.

Conflict of interest: No financial or personal relationships with any of the authors exists that could inappropriately influence this work.
Reference List


Do Pathological Fractures influence Survival and Local Recurrence Rate in Bony Sarcomas?

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R.J. Grimer\textsuperscript{2}
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6.1 Introduction

Pathological fracture through a bony sarcoma can theoretically worsen prognosis by spreading tumour via the fracture haematoma, or by spreading micro-metastases. It can also lead to joint involvement. If the fracture is not recognized as being pathological, there is a risk of inappropriate procedures, delaying diagnosis and potentially spreading the disease more than necessary. The literature is unclear about the implications of a pathologic fracture on the outcome for patients with bony sarcomas (1-6). The aim of the current study was to establish whether pathological fracture had any influence on surgical management, local recurrence or survival in patients treated for a localised high grade extremity sarcoma of bone (osteo-, Ewing’s, or chondrosarcoma). For osteosarcoma and chondrosarcoma the influence of subtype was established as well.

6.2 Patients and methods

A retrospective survey was performed, using a prospectively kept database in which patient, tumour, treatment and outcome details were recorded. We included all patients, treated between 1983 and 2003, for a localised, primary, high grade, bony osteosarcoma, chondrosarcoma or Ewing’s sarcoma of an extremity. All patients were treated in the Royal Orthopaedic Hospital in Birmingham (UK), which is a national referral centre for bone tumours. We excluded those patients who did not receive “standard treatment”. In osteosarcoma and Ewing’s sarcoma this consisted of pre-operative chemotherapy, followed by resection of the tumour, and post-operative chemotherapy. For osteosarcoma chemotherapy was administered according to the protocol of the European Organisation for Research and Treatment of Cancer (EORTC) current at the time (7;8), for Ewing’s sarcoma according to the protocol of the UKCCSG or the EICESS groups (9;10). Tumours, located in, or extending into the proximal half of the humerus and femur were considered to be proximal”, the others “distal”. Radical and wide margins, according to Enneking (11), were considered to be “adequate”, marginal or intralesional margins “inadequate”. Chemotherapy response was defined according to the protocol of the European Osteosarcoma Intergroup as good if less than 10% of viable tumour was found in the resection specimen (12;13). Outcome parameters were local recurrence and estimated 10-years overall survival. We compared the outcome in patients with and without a pathological fracture through the tumour, occurring before or during treatment. To evaluate safety of limb saving surgery in patients with a fracture, type of surgery was evaluated in respect to local recurrence and survival.
Other evaluated prognostic variables included proximity of the tumour, surgical margins, and chemotherapy response (for osteo- and Ewing's sarcomas). The influence of subtype on the occurrence of fracture and on survival was established for osteosarcoma (telangiectatic as opposed to “other” subtypes), and for chondrosarcoma (dedifferentiated as opposed to grade 2 or grade 3).

In a total of 620 eligible patients who were treated for osteosarcoma in the mentioned period, 83 had metastatic disease at diagnosis and were excluded. The fraction of patients with metastasis at diagnosis was equal in the fracture and non-fracture group (15% and 13% respectively, p=0.58, chi square). Fifty three further patients were excluded, 45 because they did not receive standard treatment (41 had no or incomplete chemotherapy, 4 no resection), and 8 because they died of an unrelated cause. Thus 484 patients were analysed. The mean follow-up in survivors was 117 months (7-252 months). Completeness of follow-up was 97% after 2 years and 94% after 3 years.

For chondrosarcoma, 152 patients were treated, 13 of them had metastatic disease at diagnosis. Again no difference in percentage of patients with and without fracture was found between these 2 groups (13% and 7%, p=0.30, chi square). A further 9 patients were excluded, 2 because resection was impossible, 7 because of unrelated death. This left 130 patients with localised, primary, high grade chondrosarcoma of an extremity to analyse. All included patients were diagnosed with a chondrosarcoma grade 2 or 3, or a dedifferentiated chondrosarcoma. The mean follow-up in survivors was 81 months (3-263 months). Completeness of follow-up was 88% after 2 years and 76% after 3 years.

Of the 223 patients with Ewing's sarcoma, 52 had metastatic disease at the time of diagnosis. The percentage of metastatic disease in the fracture and no fracture groups did not differ significantly (14% and 11%, p=0.56, chi square). Fifteen patients were excluded because they did not receive standard treatment (5 had palliative chemotherapy, 7 had chemo- and radiotherapy but no surgery, 3 did not have chemotherapy). This left 156 patients with Ewing's sarcoma to analyse. The mean follow-up in survivors was 120 months (19-253 months). Completeness of follow-up was 99% after 2 years and 92% after 3 years.

**Statistical analysis**

Comparability of the groups with and without fracture was assessed with the Chi-Square test for nominal variables and with a Mann/Whitney test for age. Local recurrence was compared between the groups with a Chi-square test as well. Overall survival was determined by Kaplan Meier survival analysis and compared between groups with a log rank test. For assessment of (independent) predictive value of factors a Cox proportional hazards model was used (level of significance p≤0.05).
6.3 Results

6.3.1 Osteosarcoma: Patient and tumour characteristics and treatment. Comparability of fracture- and control groups

Of the 484 patients in the osteosarcoma group, 56 had a fracture (12%). The groups with or without a fracture were comparable regarding sex and age at diagnosis. The site of the osteosarcoma in both groups was predominantly in the distal femur. The second most common place was the proximal tibia, followed by the humerus (figure 6.1-A). Location of the tumours in the bone was different in both groups: in the fracture group 41% of the tumours were proximal, compared to only 13% of tumours in the control group (p<0.01). The fraction of telangiectatic subtype was higher in the fracture group (23% versus 6% in the control group, p<0.01). In the group of patients with a telangiectatic osteosarcoma the incidence of fracture was higher (34%) than in the group with other subtypes (10%).

Treatment in both groups was comparable. Adjuvant radiotherapy was given in 9% of patients in the fracture group and in 8% in the non-fracture group (p=0.90). All fractures were treated conservatively apart from 1 which was treated with osteosynthesis elsewhere, which did not influence further treatment. Limb saving surgery was done in the majority of cases and percentages of ablative and limb saving surgery were comparable between the 2 groups, as were surgical margins and chemotherapy response (see table 6.1).

FIGURE 6.1 Number of fractures and total number of tumours on specified sites (% of fractures between brackets) in the eligible patients: 484 with osteosarcoma (A), 130 with chondrosarcoma (B), and 156 with Ewing’s sarcoma (C).
6.3.2 Chondrosarcoma: Patient and tumour characteristics and treatment. Comparability of fracture- and control groups

Of the 130 analysed chondrosarcoma patients 33 (25%) had a fracture. The groups of patients with or without a fracture were comparable concerning age, sex, treatment and surgical margin. The fracture group showed a tendency towards more proximal tumours and towards a higher fraction of dedifferentiated subtypes, but these differences did not reach the level of significance. Tumour site was different from that in osteosarcoma, the majority being localised at the proximal, rather than the distal femur or proximal tibia (figure 6.1-B; Table 6.1).

6.3.3 Ewing’s sarcoma: Patient and tumour characteristics and treatment. Comparability of fracture- and control groups

In the Ewing’s sarcoma group, 16 of 156 (10%) had a fracture. Again, the groups with or without a fracture were comparable for age, sex, treatment and surgical margin. Also in Ewing’s sarcoma the fracture group showed significantly more proximal tumours (Figure 6.1-C; Table 6.1).

Table 6.1 - Comparability of fracture- and no-fracture group in Osteosarcoma, Chondrosarcoma, and Ewing’s sarcoma

<table>
<thead>
<tr>
<th></th>
<th>Osteosarcoma Fracture (n=56)</th>
<th>Osteosarcoma No-fracture (n=428)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>36/20</td>
<td>254/174</td>
<td>0.48</td>
</tr>
<tr>
<td>Median age (yrs+range)</td>
<td>16 (4-57)</td>
<td>16 (5-57)</td>
<td>0.52</td>
</tr>
<tr>
<td>Proximal tumour (%)</td>
<td>23 (41)</td>
<td>54 (13)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Teleangiectatic subtype1 (%)</td>
<td>13 (23)</td>
<td>25 (6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Grade 2:</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Dedifferentiated (%)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Grade 3 (%)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Grade 2 (%)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Limb salvage (%)</td>
<td>44 (79)</td>
<td>357 (83)</td>
<td>0.37</td>
</tr>
<tr>
<td>Adequate margin (%)</td>
<td>35 (63)</td>
<td>288 (67)</td>
<td>0.58</td>
</tr>
<tr>
<td>Poor Chemotherapy response (%)</td>
<td>43 (78)</td>
<td>320 (75)</td>
<td>0.91</td>
</tr>
<tr>
<td>Adjuvant radiotherapy (%)</td>
<td>5 (9)</td>
<td>36 (8)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

1 For osteosarcomas, 2 For chondrosarcomas
6.3.4 Local Recurrence and overall survival in osteosarcoma

The local recurrence rate was similar (eg 14 %) in the fracture group and the control group in osteosarcoma patients (p=0.96). Comparing local recurrence between patients in the fracture group only, treated with ablative or limb saving surgery, revealed no statistically significant differences with local recurrence in 17% of the ablative group and 14% of the group treated with limb saving surgery (p=0.79 in Chi-Square test). The estimated 10-year overall survival in the entire group of osteosarcoma patients was 55 %. The overall survival in the group with a fracture was lower (34%) than in the control group (58%; p<0.01). Table 6.2 shows the results of univariate analysis. It appears that, apart from fracture, proximal tumour location, poor chemotherapy response, inadequate margin, and ablative surgery are correlated with worse survival. Comparing survival in fracture and control group, shows that fracture is a predictor of worse survival in most subgroups, but not in patients with proximal tumours, telangiectatic subtype, good chemotherapy response, or ablative surgery. In multivariate analysis (table 6.3), fracture, proximal tumour location, ablative surgery, inadequate margin, and poor chemotherapy response appear to be independent predictors of worse survival.
6.3.5 Local Recurrence and Overall survival in Chondrosarcoma

The local recurrence rate was not statistically different between the fracture and the control group in chondrosarcoma, although a tendency towards more local recurrence in the fracture group seemed to exist (local recurrence in fracture group 33%, in control group 20%, p=0.11). No statistical difference in local recurrence was found in the fracture group comparing ablative and limb saving surgery (39% in the ablative group, and 20% in the group treated with limb saving surgery; p=0.28 in Chi-Square test).

Table 6.2 Estimated 10 year survival in osteosarcoma (%); Univariate analysis

<table>
<thead>
<tr>
<th>All patients (n=484)</th>
<th>Fracture (n=56)</th>
<th>No-fracture (n=428)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>55</td>
<td>34</td>
<td>58</td>
</tr>
<tr>
<td>Proximal tumours</td>
<td>39</td>
<td>33</td>
<td>42</td>
</tr>
<tr>
<td>Distal tumours</td>
<td>58</td>
<td>36</td>
<td>60</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.0002</td>
<td>0.3757</td>
<td>0.0043</td>
</tr>
<tr>
<td>Telangiectatic</td>
<td>47</td>
<td>43</td>
<td>52</td>
</tr>
<tr>
<td>Other subtype</td>
<td>56</td>
<td>33</td>
<td>58</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.6284</td>
<td>0.7886</td>
<td>0.9975</td>
</tr>
<tr>
<td>Poor chemo response</td>
<td>48</td>
<td>24</td>
<td>51</td>
</tr>
<tr>
<td>Good chemo response</td>
<td>79</td>
<td>66</td>
<td>81</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>&lt;0.0001</td>
<td>0.0233</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inadequate margin</td>
<td>43</td>
<td>21</td>
<td>46</td>
</tr>
<tr>
<td>Adequate margin</td>
<td>61</td>
<td>42</td>
<td>63</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.0024</td>
<td>0.3752</td>
<td>0.0045</td>
</tr>
<tr>
<td>Limb saving surgery</td>
<td>59</td>
<td>37</td>
<td>62</td>
</tr>
<tr>
<td>Ablative surgery</td>
<td>35</td>
<td>21</td>
<td>37</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>&lt;0.0001</td>
<td>0.3181</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 6.3 - Overall survival in osteosarcoma; Multivariate analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds ratio</th>
<th>Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Fracture</td>
<td>0.59</td>
<td>0.401 - 0.869</td>
<td>0.0076</td>
</tr>
<tr>
<td>Distal Tumour</td>
<td>0.649</td>
<td>0.456 - 0.924</td>
<td>0.0166</td>
</tr>
<tr>
<td>“Other” Subtype¹</td>
<td>1.25</td>
<td>0.749 - 2.087</td>
<td>0.3937</td>
</tr>
<tr>
<td>Ablative Surgery</td>
<td>2.48</td>
<td>1.743 - 3.528</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adequate Margin</td>
<td>0.56</td>
<td>0.412 - 0.761</td>
<td>0.0002</td>
</tr>
<tr>
<td>Good Chemo Response</td>
<td>0.336</td>
<td>0.208 - 0.543</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

¹as opposed to osteoblastic subtype

6.3.5 Local Recurrence and Overall survival in Chondrosarcoma

The local recurrence rate was not statistically different between the fracture and the control group in chondrosarcoma, although a tendency towards more local recurrence in the fracture group seemed to exist (local recurrence in fracture group 33%, in control group 20%, p=0.11). No statistical difference in local recurrence was found in the fracture group comparing ablative and limb saving surgery (39% in the ablative group, and 20% in the group treated with limb saving surgery; p=0.28 in Chi-Square test).
The estimated 10-year overall survival in the entire group with chondrosarcoma was 57%. The overall survival in the fracture group was lower (35%) than in the control group (63%) (p=0.04). Apart from fracture, only dedifferentiated and grade 3 subtypes were correlated with worse survival in univariate analysis (table 6.4). As in osteosarcoma, patients treated with ablative surgery, showed a worse survival, 

<table>
<thead>
<tr>
<th>Table 6.4 - Estimated 10 year survival in chondrosarcoma (%)</th>
<th>Univariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>All patients (n=130)</td>
</tr>
<tr>
<td>All patients</td>
<td>57</td>
</tr>
<tr>
<td>Proximal tumours</td>
<td>50</td>
</tr>
<tr>
<td>Distal tumours</td>
<td>65</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.2378</td>
</tr>
<tr>
<td>Dedifferentiated</td>
<td>36</td>
</tr>
<tr>
<td>Grade 3</td>
<td>48</td>
</tr>
<tr>
<td>Grade 2</td>
<td>67</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inadequate margin</td>
<td>60</td>
</tr>
<tr>
<td>Adequate margin</td>
<td>53</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.3093</td>
</tr>
<tr>
<td>Limb saving surgery</td>
<td>60</td>
</tr>
<tr>
<td>Ablative surgery</td>
<td>43</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.1208</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 6.5 - Overall survival in chondrosarcoma; Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
</tr>
<tr>
<td>No Fracture</td>
</tr>
<tr>
<td>Distal Tumour</td>
</tr>
<tr>
<td>Subtype¹</td>
</tr>
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<td>Dedifferentiated</td>
</tr>
<tr>
<td>Grade 2</td>
</tr>
<tr>
<td>Global Wald test</td>
</tr>
<tr>
<td>Ablative Surgery</td>
</tr>
<tr>
<td>Adequate Margin</td>
</tr>
</tbody>
</table>

¹Wald test related to grade 3
but this difference was not statistically significant. Studying subgroups, fracture correlated with worse survival only in distally located tumours, although a tendency existed in all subgroups. In multivariate analysis, subtype appeared to be the only independent predictor of survival (table 6.5).

### 6.3.6 Local Recurrence and Overall survival in Ewing’s sarcoma

In patients with a Ewing’s sarcoma, no difference between the groups was found for local recurrence, which was 0% and 9% for fracture and no-fracture group

<table>
<thead>
<tr>
<th>Table 6.6 - Estimated 10 year survival in Ewing’s sarcoma (%)</th>
<th>Univariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>All patients (n=156)</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------</td>
</tr>
<tr>
<td>All patients</td>
<td>65</td>
</tr>
<tr>
<td>Proximal tumours</td>
<td>70</td>
</tr>
<tr>
<td>Distal tumours</td>
<td>63</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.2887</td>
</tr>
<tr>
<td>Poor chemo response</td>
<td>64</td>
</tr>
<tr>
<td>Good chemo response</td>
<td>62</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.2878</td>
</tr>
<tr>
<td>Inadequate margin</td>
<td>68</td>
</tr>
<tr>
<td>Adequate margin</td>
<td>67</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.4396</td>
</tr>
<tr>
<td>Limb saving surgery</td>
<td>66</td>
</tr>
<tr>
<td>Ablative surgery</td>
<td>66</td>
</tr>
<tr>
<td>p-value ↑</td>
<td>0.428</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Table 6.7 - Overall survival in Ewing’s sarcoma; Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>No Fracture</td>
</tr>
<tr>
<td>Distal Tumour</td>
</tr>
<tr>
<td>Ablative Surgery</td>
</tr>
<tr>
<td>Adequate Margin</td>
</tr>
<tr>
<td>Good Chemo Response</td>
</tr>
</tbody>
</table>
respectively (p=0.22). All patients in the fracture group were treated with limb saving surgery.
Fracture in Ewing’s sarcoma was not correlated with estimated 10-year overall survival, which was 75% and 64% for fracture and no-fracture group (p=0.50). None of the studied factors showed any statistically significant correlation with survival in univariate (table 6.6) or multivariate analysis (table 6.7).

6.4 Discussion

Although a fracture through a bony sarcoma may theoretically have an adverse effect on the outcome of the disease after treatment(14;15), this is not consistently reflected by the literature. Zeifang and colleagues studied 336 patients with bony sarcomas of different type and stage, 30 of whom had a fracture. They found a similar local recurrence rate but a worse survival in patients with a fracture. Limb saving surgery was considered safe if an adequate resection margin can be obtained(5). This is confirmed by Ebeid and colleagues, who presented a series of 31 patients with a fracture through different types of stage IIIB tumours of bone, who were all treated with limb saving surgery. Local recurrence in this series occurred in 6%. Survival was 81% and seemed worse for patients who sustained their fracture during chemotherapy as opposed to those who presented with a fracture. The follow-up period however in this study was short and the type of tumour differed, making this comparison rather weak(16).

For osteosarcoma the literature is contradictory on the implications of pathological fracture. In 1996 Abudu presented a series of patients with a fracture who showed similar survival to a comparable group in the literature without a fracture(1). Glasser and colleagues reported worse survival for patients with a fracture(17). Scully showed an unfavourable influence of fracture on both local recurrence and survival in a multicentre evaluation(4). This study could be biased by the fact that a considerable part of the patients did not receive pre-operative chemotherapy, as was correctly noted in a comment by Bacci(18). Bacci himself did not find a difference for either local recurrence or survival(3). Both Abudu(1) and Scully(4) compared oncological outcome between patients who presented with a fracture, and those who sustained it during treatment, and both did not find any difference. Abudu reported a higher chance of local recurrence if patients with a fracture were treated with limb saving surgery. This difference disappeared however after correction for surgical margin. Scully(4) as well as Bacci(3) compared limb saving and ablative surgery in their studies and did not find any difference in oncological outcome between them.
In the current study, fracture- and control group consisted of osteosarcoma patients, treated in the same period in one institution. All had similar treatment. The groups were comparable, apart from the fact that tumours in the fracture group were located more proximally, and that telangiectatic subtype was more frequent in the fracture group. This is consistent with the concept that proximal tumours, as well as telangiectatic subtypes, are more aggressive (19). Fracture was found to be an independent predictor for worse survival, but no difference in local recurrence was found. This seems to indicate that it is probably not spreading of tumour cells in the fracture haematoma that leads to a worse prognosis. More likely, the fracture is a symptom of a more aggressive tumour, and therefore heralds a lower survival chance.

For chondrosarcoma several authors highlighted the importance of local control (20-25), but few mentioned the influence of pathological fracture. Lee et al (2) reported on 227 patients with chondrosarcoma, 141 of which were high grade. Pathological fractures occurred predominantly in the group with high grade tumours (38 compared to 46 in the entire group). In their series, fracture did not have an influence on oncological outcome, although it is not clear how exactly this analysis was done. The authors did not comment on the type of surgery in comparison to local recurrence or oncological outcome. They did find a correlation between achieved surgical margin and survival in high grade lesions, as is reported in other publications. In most publications, histological grade is found to be an independent, prognostic factor in chondrosarcoma. In our patients with high grade non metastatic chondrosarcoma, surgical margins were comparable between fracture and control group. No difference in local recurrence was found. Patients with a fracture did show a lower survival rate. In multivariate analysis however, only subtype appeared to be an independent predictor of survival, which decreased with increasing grade of malignancy. This fits with the idea that a fracture is more likely to occur in a more aggressive tumour, which would also be supported by the abovementioned literature. The fact that more patients with dedifferentiated chondrosarcomas are in the fracture group in our study supports this theory.

For Ewing’s sarcoma, Wagner reported that tumours in the proximal femur are at higher risk for fracture and that late fracture, occurring after completion of therapy, should raise the suspicion of local recurrence (26). They did not report on the difference in survival or local recurrence between patients with or without a fracture. Fuchs found similar results concerning location, and reported no significant difference in survival or local recurrence comparing 14 patients with a fracture through a Ewing’s sarcoma, sustained before or during treatment, with the entire group of patients (27). It is not clear in this paper whether the fracture patients are included
in the control group and whether fracture and no-fracture group were matched for
treatment, stage, and other characteristics. Hoffmann and colleagues compared 42
fracture patients with a control group of 350 patients with Ewing’s sarcoma or PNET
stage 2, and found no difference in relapse free or overall survival(6).

Our study seems consistent with the abovementioned. None of the studies compares
ablative and limb saving surgery in fractured Ewing’s sarcoma patients, which in
our study does not reveal a difference. The explanation why Ewing’s sarcoma does
not show a difference in overall survival between fracture and no-fracture group,
whereas osteo-and chondrosarcoma do show worse survival in fractured patients,
could be that Ewing’s sarcoma generally is more chemotherapy -sensitive(28). This
idea is strengthened by our finding that in osteosarcoma, good chemotherapy
responders do not show a difference in survival between fracture and control group.

Another factor that might influence both survival and the chance of pathological
fracture in all of the three studied tumours is tumour volume. Bacci and colleagues
found tumour volume to be an independent predictor for survival in osteosarcoma,
but in their patients pathological fracture did not correlate with survival, not even in a
univariate analysis(29). Scully found exactly the opposite, worse survival for fractured
patients, but no influence of tumour size(4). For chondrosarcoma Lee and colleagues
report a correlation of larger tumour volume with a worse prognosis, but they did
not find fracture to be of influence(2). Hoffmann and colleagues found no influence
of fracture in Ewing’s sarcoma, and worse survival for patients with larger tumours.
Volume, however, lost its predictive value in the fracture group(6). Unfortunately we
did not have sufficient information about tumour volume in the three studied patient
groups to establish the influence of tumour volume.

We conclude that a pathological fracture in a bony sarcoma does not increase the
chance of local recurrence, provided oncological principles are adhered to and the
tumour and fracture site can be excised with clear margins. Overall survival is worse
in patients with a fracture in osteo- or chondrosarcoma, but not in Ewing’s sarcoma.
Fracture is an independent predictor of survival in osteosarcoma only. The fracture
is probably not in itself the cause of the lower survival, but rather a symptom of a
more aggressive tumour. The influence of tumour volume should be further studied.
Limb saving surgery in fractured patients does not seem to have an influence on
local recurrence or survival and therefore is thought to be safe, as long as adequate
margins can be obtained. We recommend that patients with a pathological fracture
through a bony sarcoma be treated by non-operative stabilisation of the fracture
(e.g. by means of a splint) and appropriate analgesia, followed by chemotherapy
according to the standard protocol. After this, resection of the tumour should, as
usual, be done with wide margins. We have not been able to clarify the benefit, or otherwise, of adjuvant radiotherapy following limb salvage after a pathological fracture.

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Reference List


Summary

chapter 7
7.1 Summary of introduction and general aim of the thesis

Before the 1970’s, surgery was the sole available therapy for high grade extremity osteosarcoma, in 90% of cases involving amputation of the inflicted limb. Survival was only 10 to 15%(1-3). This has changed with the introduction of chemotherapy in the 1970’s, and since the 1980’s, the combination of pre-operative chemotherapy, surgery, and postoperative chemotherapy, results in a survival rate of around 70%(4-9). Down-staging of the tumor, decreasing in volume if reacting on chemotherapy, facilitates the resection. The development of imaging techniques, especially magnetic resonance imaging, has facilitated pre-operative planning (10;11). Nowadays, surgery can be limb saving in about 90% of cases (4;6;7). Reconstruction methods after resection have improved, and range from biological to complete endoprosthetic replacements. Each of these has advantages and disadvantages(12;13). A complete biological reconstruction, knows on the long run good results with vital bone, allowing a normal life with little restrictions concerning the limb(14;15). A long period of non weight bearing, however, up to 18 months postoperatively, is often required, as are sometimes re-operations(16;17). On the other hand, reconstruction with a cemented endoprosthesis allows the patient full weight bearing quickly, usually after 1 or 2 weeks. On the long run, however, there is always a risk of peri-prosthetic fracture, infection, and eventually loosening (12;18;19).

The increased survival has resulted in a growing number of long term survivors(20). These may experience long term health effects of treatment, especially if they were children at the time of treatment. Late effects sometimes are mild, but may have a severe impact on quality of life or even be fatal (21;22).

The combination of factors mentioned above is leading in decision making for osteosarcoma patients. In each patient the optimal treatment has to be chosen, with the highest chance of survival, and the least possible late adverse effects of treatment. The surgical treatment should be planned balancing expected rehabilitation time and chance of survival.

Adequate estimation of the survival chance of the individual patient is essential for making these considerations. This should ideally be possible early in treatment, allowing early assignment to the best chemotherapeutic and surgical treatment. Unfortunately, prognostication for individual patients remains difficult. Many prognostic factors have been reported, but the value of different factors for the
individual patient is not clear (23). Extend, importance, and etiology of late effects of treatment need to be further clarified.

**General aim of this thesis** was to establish the available prognostic factors predicting survival chance after treatment of high grade osteosarcoma, and to assess the value of these for the individual patient. Special focus was on factors which can be assessed before, or in an early stage of treatment and which are easy to assess. Furthermore, it was investigated which adverse late effects of treatment occurred in patients who were treated for high grade osteosarcoma in childhood, and who survived more than 5 years after the end of treatment. The impact of these effects was established as well as the relation of the effects with the sort of treatment patients underwent.

### 7.2 Summary of chapters 2 to 6

**Chapter 2: A systematic review of the literature** In chapter 2 a systematic review of the literature concerning prognostic factors in high grade osteosarcoma is presented, trying to identify evidence-based prognostic factors in the literature since 1992 and to establish pooled relative risks of factors. Of 1777 “hits”, 93 papers were studied in depth. Only 7 papers were of sufficient quality to use in a meta-analysis. Poor chemotherapy response (pooled RR = 2.37), large tumor volume (pooled RR = 1.36) and ablative surgery (pooled RR= 2.18) were independent predictors of a bad outcome. Further factors that are presumably predicting a worse outcome, but could not be pooled, are inadequate excision margin, age under 14 years, male gender, high alkaline phosphatase, local recurrence, p-Glycoprotein expression, and absence of Erb2 expression.

Conclusion: Poor chemotherapy response, large tumor volume and ablative surgery were independent predictors of a bad outcome. The literature is abundant but only few papers are of sufficient quality to allow hard conclusions. Because of heterogeneity of the studies pooling results is hardly possible. Because of the relatively small sample sizes in most studies, even very powerful prognostic factors may not have become significant and may have been left unreported.

**Chapter 3: Survival, prognostic factors, and late effects of treatment in children** Chapter 3 evaluates survival, prognostic factors, and late effects of treatment in children, treated for non-metastatic high grade extremity osteosarcoma, in our institute, the Emma Children’s Hospital (EKZ)/Academic Medical Centre in Amsterdam.
For the analysis of survival and prognostic factors a retrospective survey was performed on all consecutive patients, treated between 1985 and 2006, who were younger than 18 years of age at the time of diagnosis. For the assessment of late effects of treatment, patients who survived more than 5 years after the end of treatment, were seen at an especially established outpatient clinic. Adverse effects were graded according to the Common terminology Criteria for Adverse Events version 3.0 (CTCAE) from grade 1 (mild) to grade 5 (fatal).

Seventy patients were included. The 5 year survival was 75%. Tumor size and chemotherapy response were the only factors found to independently predict overall survival. Thirty-nine of the 40 patients, surviving more than 5 years after the end of treatment had one or more adverse effect. In 2 patients this was fatal (1 cardiac and 1 bone marrow related effect). Musculoskeletal adverse effects were found in 95% of survivors, and in 70% this concerned a grade 4 (disabling) effect. This was the result of the treatment strategy, with obligatory adequate tumour resection. In the studied period a large proportion of patients underwent rotationplasty, a grade 4 event. The second most frequently found type of adverse long term effect was auditory (in 35% of patients). This was mostly mild or moderate; some patients however suffered from severe hearing loss in speech frequencies. Cardiac effects occurred in 20% of patients. These also mostly were mild or moderate, but 1 patient died of cardiomyopathy, 18 years after the end of treatment. Patients with ifosfamide in the treatment regimen, had significantly more cardiac adverse effects. One patient died of a myelodysplastic syndrome, 11 years after the end of treatment. Other types of adverse effects were rare and mostly not very important.

Conclusion: Survival was good (75%) in this patient group. Chemotherapy response and tumor size were of prognostic value. Late adverse effects were common, quit often disabling, and mainly musculoskeletal.

Chapter 4: The value of Colour Doppler Ultrasound for predicting chemotherapy response and survival

In chapter 4 it was investigated whether chemotherapy response and survival could be predicted with Colour Doppler Ultrasound (CDUS) after chemotherapy, but before resection of the tumor. Color Doppler ultrasound (CDUS) is a non-invasive, short procedure. The ultrasound system used in our study is available in most modern hospitals. CDUS was performed in 21 consecutive patients, treated in our institution for a high grade extremity osteosarcoma, before and after pre-operative chemotherapy. The Peak Systolic Velocity (PSV) in the soft tissue component of the tumor and the Quotient of Resistive Index of the feeding artery and contra lateral control (QRI) were assessed. After surgery, a pathologist,
unaware of CDUS results, assessed histological response to chemotherapy in the resection specimen.
QRI-change after chemotherapy was significantly higher in histological responders compared to non-responders. There was no significant difference in PSV-change comparing any of the subgroups, and neither QRI nor PSV were directly predictive for survival.

Conclusion: CDUS appeared useful in predicting chemotherapy response (sensitivity 83%, specificity 86%), especially for negative response (predictive value for poor response 92%), but not for survival.

**Chapter 5: The predictive value of serum alkaline phosphatase for predicting chemotherapy response and survival**

Alkaline phosphatase (AP) is easily assessable and cheap. We aimed to determine the value of alkaline phosphatase for predicting chemotherapy response, local recurrence, and survival in patients with high grade osteosarcoma.
Alkaline phosphatase is directly produced by osteosarcoma cells but the serum level of it is also influenced by the occurrence of fractures and by bone growth. For this reason patients aged under 18 years and patients with pathological fractures were excluded. A retrospective study was performed in 132 consecutive adult patients, treated for high grade, non metastatic osteosarcoma in the Royal Orthopaedic Hospital (Birmingham, UK) between 1983 and 1999.
Alkaline phosphatase levels were recorded before chemotherapy (pre-ct AP), after chemotherapy but before surgery (post-ct AP), and the change in the level of alkaline phosphatase before and after chemotherapy was recorded. The alkaline phosphatase values were divided into three categories: Normal (below the upper normal limit), High (raised, but less than twice the upper limit), and Very High (raised more than twice the upper limit).
We found that elevated pre-treatment AP over twice the upper normal level (“Very High”) was predictive of a worse survival. Moreover, the predictive value of a Very High pre-treatment AP for a poor chemotherapy response was 80%. The AP level after chemotherapy, but before surgery, seems to be even more useful. Survival decreased stepwise with post-ct AP values being normal, moderately raised or severely raised. The predictive value of any elevated post-ct AP for a poor chemotherapy response was 100%. A decrease of AP levels after chemotherapy appeared not to correlate with improved survival unless AP returned to normal, in which case survival was the same as in patients with a normal AP at diagnosis. We did not find a significant relationship between AP levels at any stage and local recurrence.
Conclusion: Alkaline phosphatase, measured before chemotherapy, after chemotherapy, and the change of alkaline phosphatase after chemotherapy are valuable factors in predicting chemotherapy response and survival in high grade osteosarcoma in adults.

Chapter 6: The influence of pathological fracture on surgical management, local recurrence and survival  This was established in a retrospective review of 770 patients with a high grade, non metastasized bony sarcoma of an extremity. It concerned 484 patients with an osteosarcoma, 130 patients with a chondrosarcoma, and 156 with a Ewing’s sarcoma.

Alongside pathological fracture, other prognostic factors that were analyzed were proximity of the tumor, subtype (for osteosarcoma and chondrosarcoma), chemotherapy response (for osteosarcoma and Ewing’s sarcoma), surgery type and achieved surgical margin.

Fracture occurred in 12 % of osteosarcoma patients, in 25% of chondrosarcoma patients, and in 10 % of patients with a Ewing’s sarcoma. The groups of patients with or without a fracture were in all 3 tumors comparable for sex, age at diagnose, and treatment, including achieved surgical margin. For all 3 tumors however, the fracture groups had more proximally located tumors (significantly so in osteo- and Ewing’s sarcoma). In osteosarcomas, the fracture group had a higher proportion of telangiectatic subtypes, and in chondrosarcomas the fracture showed a tendency towards more dedifferentiated subtypes. Limb salvage was done in 79% of patients with a fracture compared to 84% of patients without (p=0.17). No difference in local recurrence was found between fracture and control group in any of the 3 tumors. Comparing the group of patients where limb saving surgery was done with those who were treated with ablative surgery no difference in local recurrence was found either.

In a univariate analysis, survival in the fracture group was lower than in the control group for osteosarcoma (34% versus 58%, p<0.01) and chondrosarcoma (35% versus 63%, p=0.04), but not for Ewing’s (75% versus 64%, p=0.80). In a multivariate analysis, fracture remained a significant predictor of survival for osteosarcoma, but not for chondrosarcoma, where dedifferentiated subtype appeared to be decisive.

Of the other tested prognostic factors in the osteosarcoma patients, proximity of the tumor, surgery type, surgical margin and chemotherapy response, showed an independent predictive value for survival in the multivariate analysis. In chondrosarcoma only histological grade was independently predictive for survival, and in Ewing’s sarcoma none of the tested prognostic factors showed correlation with survival.
Conclusion: A pathological fracture in the studied bony sarcomas does not increase the chance of local recurrence. Overall survival is worse in patients with a fracture in osteo- or chondrosarcoma, but not in Ewing’s sarcoma. Fracture is an independent predictor of survival in osteosarcoma only. Limb saving surgery in fractured patients does not seem to have an influence on local recurrence or survival and therefore is thought to be safe, as long as adequate margins can be obtained.
Reference List


General Discussion
and
Future Perspectives
8.1 Increased survival, possibilities of reconstruction in limb saving surgery, and investment by the patient

High grade Osteosarcoma is the most common primary malignant bone tumor. It is rare with an incidence of about 3 per million, with a peak in the second decade of life(1;2).

Before the 1970’s, surgery was the only therapeutic option for patients, mainly, in 90% of cases, involving amputation of the inflicted limb. Survival was poor, about 10 to 15%(3-5). After the introduction of adjuvant chemotherapy in the early 1970’s, survival increased to around 50%. Since the 1980’s, the combination of pre-operative, neo-adjuvant chemotherapy, surgery, and postoperative chemotherapy, results in a survival rate of around 70%(6-11).

This improvement of survival is generally attributed to the effect of chemotherapy. For the choice of the pre-operative chemotherapy regimen, patients are stratified in different risk groups, depending on the stage of the disease at diagnose. After assessment of chemotherapy response, determined in the resected tumor, stratification is repeated, and chemotherapy regimen post-operatively is, if necessary, changed(6;12).

Apart from improving survival, pre-operative chemotherapy also proved to be of use in down-staging of the tumor; If the tumor reacts on chemotherapy, it decreases in volume in a number of cases. This facilitates the resection, or even makes this first possible.

Another aspect that has chanced in the last decennia is the development of imaging techniques. First computed tomography, and especially later magnetic resonance imaging, has facilitated pre-operative planning of the resection(13;14).

With all this, the surgical therapy has changed from predominantly ablative to predominantly limb saving. In about 90% of cases the limb can nowadays be preserved(6;8;9). After resection, a deficit of bone, and usually also muscles and tendons, results. Reconstruction methods have greatly improved. There now is a spectrum of possibilities for reconstruction, from biological, with bone lengthening or vascularized autografts, to complete endoprosthetic replacements. Occasionally, amputation or rotationplasty still is necessary. Each of these reconstruction methods has advantages and disadvantages(15;16). A complete biological reconstruction with, for instance, replacement of the resected bone with a vascularized fibular graft, knows on the long run excellent results with vital bone. This allows the patient eventually to lead a normal life with little or no restrictions concerning the limb(17;18). The investment for the patient, however, is high. A long period of non
weight bearing, up to 18 months postoperatively, is often required, as are sometimes re-operations\(^\text{(19;20)}\). The same goes for segmental bone transport after resection. On the other end of the spectrum, reconstruction with a cemented endoprosthesis allows the patient to mobilize fully weight bearing quickly, usually already after 1 or 2 weeks. On the long run however the patient has to always take into account that he is walking with an endoprosthesis. Risk of peri-prosthetic fracture, infection, and eventually loosening is always there and will hamper the patient more or less for the rest of his life\(^\text{(15;21;22)}\).

8.2 Increased number of survivors and late effects of treatment

The increase in survival of high grade osteosarcoma has resulted in a growing number of long term survivors\(^\text{(23)}\). More and more is reported about long term health effects these survivors may encounter as a result of the intensive treatment they had, especially if they were children at the time of treatment. Late effects may concern secondary tumors, cardiac, neurologic or bone marrow problems, which can be invalidating or even life threatening. They can also consist of auditory, metabolic, fertility, psychological or musculoskeletal impairments, which can have a moderate, but sometimes severe impact on quality of life\(^\text{(24;25)}\).

8.3 The importance of individual prognostication

The combination of factors mentioned above is leading in decision making for osteosarcoma patients. In each patient the optimal treatment has to be chosen, with the highest possible realistic chance of survival, combined with the best possible functional result after surgery, giving the least possible late adverse effects of treatment. In order to make these considerations optimally, adequate information about the survival chance of the individual patient is essential. Accurate estimation of the survival chance could improve stratification in risk groups, and ideally should be possible early in treatment, allowing early and more adequate assignment of patients to the best chemotherapy schedule. Surgery needs to be tailored to the patient, and should be planned taking into account the expected survival chance of the patient. It is well worth motivating a patient with a high survival chance to have a biological reconstruction done, and bear with a long and intensive rehabilitation period. On the long run the result will be rewarding.
On the other hand, someone with a low chance of survival should be enabled to have a good quality of life on the short term, and should not be hindered by an unnecessary long and intensive rehabilitation. More knowledge about the relation of type and cumulative dose of chemotherapy, and of the followed surgical strategy, with late adverse effects of treatment, could lead to a more integrated decision about the total treatment, which is optimizing survival chance and minimizing late adverse effects.

### 8.4 General aim and scope of the thesis

Prognostication for individual patients remains difficult. Many prognostic factors have been reported in literature, but most of these reports do not meet the standard of modern evidence based medicine, and the value of different factors for the individual patient is not clear\(^{(26;27)}\). Extend, importance, and etiology of late effects of treatment need to be further clarified.

General aim of this thesis was to establish the available prognostic factors predicting survival chance after treatment of high grade osteosarcoma, and to assess the value of these for the individual patient. Special focus was on factors which can be assessed before, or in an early stage of treatment and which are easy to assess. Furthermore, it was investigated which adverse late effects of treatment occurred in patients who were treated for high grade osteosarcoma in childhood and who survived more than 5 years after the end of treatment. The impact of these effects was established as well as the relation of the effects with the type of treatment patients underwent.

It was decided to limit the scope of the thesis to patients with high grade, non metastatic osteosarcoma of an extremity. The reason for this was to enable studying homogeneous patient populations and rule out bias by treatment variations, allowing a proper comparison of groups regarding prognostic factors and late adverse effects. Patients, who present with metastasis, were excluded because they often are treated in a non standardized way, from a curative to sometimes only palliative perspective. For the same reason, we focused on patients with an extremity osteosarcoma. Axial osteosarcoma behaves differently, and also is treated variably. Because both chemotherapy and surgery are considered essential in the treatment of osteosarcoma, only patients were included who completed the full treatment, consisting of chemotherapy and surgery.
8.5 Discussion of the chapters of the thesis

Systematic review of the literature

Chapter 2 presents a systematic review of the literature concerning prognostic factors in high grade osteosarcoma. We tried to identify evidence-based prognostic factors in the literature since 1992 and to establish pooled relative risks of factors. Factors that were already known, and regarded a poor sign for survival, such as more proximal location and large tumors, appear to be inconsistently reported (7-9;11;28-41). Chemotherapy response, the only proven independent factor in an earlier review (26), is reported to predict outcome by most, but not all authors (28;30;42-45). Newly reported factors are mostly “indirect” factors. Most promising among these seem to be the high expression of p-glycoprotein (46-52), expression of the human epidermal growth factor receptor 2 (HER2) (28;53-56), expression of vascular endothelial growth factor (VEGF) (42;57-59), and loss of heterogeneity of the Rb-gene (60).

These factors may be of true predictive value, but it is important to keep in mind that the majority of the studies had methodological flaws (61). Because of this, simply counting the papers with a supposed predictive value is dangerous. Valid conclusions can not yet be drawn from this part of the literature.

Only 7 papers were of sufficient quality to use in a meta-analysis. Poor chemotherapy response, large tumor volume and ablative surgery could be used in a meta analysis and appear to be predictors of a bad outcome. For absence of an adequate surgical margin, although proven to be an independent factor in 2 studies (8;9), there was vast heterogeneity, so no pooling could be performed. This heterogeneity of studies and of reported data is a major problem in comprehending the literature. Different cut off points for various factors may make pooled results less reliable. Authors report on different sets of factors in their multivariate analyses, making pooling of results less valuable. Because of the relatively small sample sizes in most studies, even very powerful prognostic factors may not have become significant (62), and may have been left unreported. If the non-significant results would have been available, and could have been pooled, more precise estimates of the effect might have been possible. Moreover, usually the actual figures about prognostic factors only were published if they were significant, thus, the pooled relative risks that are calculated from these publications might be overestimated (outcome bias).

From the available information in the literature one may assume that chemotherapy response is an independent prognostic factor, a poor response increasing the risk for dying of the disease probably approximately 2.4 times. Further factors that are presumably independently predicting a worse outcome are large tumors, inadequate
excision margin, ablative surgery, age under 14 years, male gender, high alkaline phosphatase, local recurrence, p-Glycoprotein expression, and absent Erb2 expression.

We concluded that there is a need for methodologically high quality studies with more uniform study design and reporting. All results should be reported, whether significant or not. It would be most useful if raw data could be made available on line or in collaborative databases.

**Survival, prognostic factors, and late effects of treatment in the paediatric cohort, treated in the Emma Children's Hospital/AMC Amsterdam** Chapter 3 evaluates survival, prognostic factors for survival, and the occurrence of late effects of treatment in children, treated for non-metastatic high grade extremity osteosarcoma, in our institute, the Emma Children's Hospital (EKZ)/Academic Medical Centre in Amsterdam. The 5 year survival rate was 75%. This seems slightly higher than what is reported in other series(8;9;63). The explanation for this might be that we only included patients with localised extremity osteosarcoma, who were treated completely with pre-operative chemotherapy, resection, and post-operative chemotherapy. This was decided to allow proper comparison of prognostic factors, unhindered by treatment variations. Patients with metastatic disease or axial osteosarcoma are very often treated in a non-standardized way(64;65). Tumor size and chemotherapy response were the only factors found to independently predict overall survival, which is in accordance with earlier literature on prognostic factors(27).

All patients but one, surviving more then 5 years after the end of treatment, had one or more adverse effect. This is a high proportion compared to earlier literature, which mentions adverse effects in approximately two-thirds of patients in survivors of childhood cancer in general(23;24). Other publications, however, show that survivors of bone tumors are at higher risk for late health effects than survivors of other cancers(25;66;67). In 2 patients the adverse effect was fatal. Apart from the 2 fatal effects, which were cardiac and bone marrow related, the musculoskeletal ones were the most frequent and the most severe. No less than 95% of survivors suffered from a musculoskeletal adverse effect, and in 70% this concerned a grade 4 (disabling) effect. This is the inevitable result of the treatment strategy, in which adequate tumor resection is obligatory. In the studied period a relatively large proportion of patients underwent rotationplasty. In the CTCAE classification, absence of a limb is defined as a grade 4 event. Although it seems reasonable to score rotationplasty as such, there are studies which show comparable functional results of rotationplasty in comparison with endoprosthetic replacement, and better than after amputation(68;69). More recently, reconstruction methods have improved considerably. Over 90% of patients can be treated with limb saving surgery(15). This
will doubtlessly have a favourable effect on long term outcome and will decrease burden of long term effects.

The second most frequently found type of adverse long term effect was auditory. This is not surprising, because all of the used chemotherapy regimen contained cisplatin, which is known for its ototoxicity, especially in young children(70-73). Although mostly mild or moderate, some patients suffer severe hearing loss in speech frequencies, which can be socially invalidating.

Cardiac effects occurred in 20 % of patients. These mostly were mild or moderate, but 1 patient died of cardiomyopathy, even as long as 18 years after the end of treatment. Late cardiac toxicity has been reported before, and, although uncommon, is known to have a high mortality(74). Usually, this is attributed to the use of anthracyclines. Alkylating agents, such as ifosfamide, seem to add to this effect(25;74). This is confirmed in our study; patients with ifosfamide in the treatment regimen had significantly more cardiac adverse effects. Prevention of toxicity should be aimed for if possible by lowering the cumulative dose, or by the use of cardio protective agents. Long term follow-up and alertness, should allow early detection and treatment(75-77). The myelodysplastic syndrome, which was fatal in 1 patient, long (11 years) after the end of treatment, is another effect which, although rare, justifies long term alertness.

Large scale prospective evaluation is necessary to evaluate possible prognostic factors, and for the development of more accurate ones. Attention should also be directed at adverse effects in survivors, especially at the long term results of surgical procedures. Better prognostication, for survival and for adverse effects, should lead to a more individually planned treatment strategy in order to improve both oncologic outcome and long term health status of survivors.

The value of Colour Doppler Ultrasound for predicting chemotherapy response and survival  

In chapter 4 it was investigated whether chemotherapy response and survival could be predicted with Colour Doppler Ultrasound (CDUS) after chemotherapy, but before resection of the tumor. The percentage of tumor necrosis after chemotherapy still is the most consistently reported independent predictor of survival(27), but can only be assessed in the resection specimen after resection of the tumor when this can be pathologically evaluated. Chemotherapy response is an important factor in planning further therapeutic strategy after preoperative chemotherapy, both in planning of type and timing of surgery, and in further chemotherapy treatment. Earlier, pre-surgical, prediction of histological response would have obvious advantages. Clinical and conventional radiological methods, including conventional MRI, have proven not to be reliable in predicting chemotherapy response(78;79). The value of skeletscintigraphy remains controversial(80-82).
Dynamic MRI does seem to enable accurate prediction response after completion of chemotherapy (83-86). Reports about dynamic Thalium-scintigraphy and Positron Emission Tomography are promising (87-92).

A disadvantage of the above-mentioned methods is that they are time-consuming, more or less invasive, and requiring patient compliance. Therefore they are not particularly suitable for children, for whom a simple and short procedure, non-invasive, is desirable.

Color Doppler ultrasound (CDUS) is such a non-invasive, short procedure (93). The ultrasound system used in our study is available in most modern hospitals. The procedure is simple and lasts at the most 20 minutes; there is no need for the patient to lie still. Because of the short duration of the procedure and the availability of the equipment, logistic planning is easy. These are clear advantages of the CDUS method compared to dynamic MRI. A conventional MRI will still have to be carried out pre-operatively as well, to establish extent of the tumor and relation to surrounding tissues after chemotherapy. For prediction of chemotherapy response however, CDUS would be much shorter and less invasive than a dynamic MRI. Apart from this, it is less costly. In circumstances where one has no, or little, access to dynamic MRI, CDUS could possibly be of use even more.

CDUS appeared useful in predicting chemotherapy response (sensitivity 83%, specificity 86%), especially for negative response (predictive value for poor response 92%), but not for survival.

A possible explanation for this could be the fact that CDUS, similar to other methods, does not detect small amounts of remaining viable tumor cells, because these have no effect on the vascularity of the tumor or of the afflicted limb. These small amounts of viable cells could also be the reason that chemotherapy response, although until now the most powerful prognostic factor, is not in all cases predictive.

CDUS is a relatively simple procedure, predicting chemotherapy response before surgery is carried out. The method is suitable for patients, especially children, with an extremity osteosarcoma. CDUS is widely available and not costly. It could be a useful tool for therapeutic considerations pre-operatively, and should be used more routinely in assessment of chemotherapy response.

The value of serum alkaline phosphatase for predicting chemotherapy response and survival Even more than CDUS, alkaline phosphatase (AP) is easily assessable and cheap. Earlier reports on prognostic factors in osteosarcoma mention the predictive value of this enzyme (40; 94; 95), but only of the serum alkaline phosphatase measured before chemotherapy. Strange enough, no authors have established its predictive value for survival after chemotherapy, although the
biological status of the tumor after chemotherapy seems the most important factor for survival. In our study we aimed to determine the value of alkaline phosphatase for predicting chemotherapy response, local recurrence, and survival in patients with high grade osteosarcoma.

Alkaline phosphatase is directly produced by osteosarcoma cells\(^{(96;97)}\). The serum level of it, however, is also influenced when fractures occur, and by bone growth. Both of these cause serum alkaline phosphatase to rise considerably, regardless of the tumor activity. For this reason, in our study only patients were included, aged 18 years or older, and patients with pathological fractures were excluded.

We found that elevated pre-treatment AP over twice the upper normal level (“Very High”) was predictive of a worse survival. Moreover, the predictive value of a Very High pre-treatment AP for a poor chemotherapy response was 80%. This is in accordance with other studies, addressing pre-treatment AP levels\(^{(40;94;98;99)}\). The AP level after chemotherapy, but before surgery, seems to be even more useful. This has not been reported before\(^{(100)}\). Survival decreased stepwise with post-ct AP values being normal, moderately raised or severely raised. The predictive value of any elevated post-ct AP for a poor chemotherapy response was 100%. A decrease of AP levels after chemotherapy appeared not to correlate with improved survival unless AP returned to normal, in which case survival was the same as in patients with a normal AP at diagnosis. We did not find a significant relationship between AP levels at any stage and local recurrence.

We concluded that alkaline phosphatase, measured before chemotherapy, after chemotherapy, and the change of alkaline phosphatase after chemotherapy are valuable factors in predicting chemotherapy response and survival in high grade osteosarcoma in adults. This factor is cheap and easy to determine and could, together with other factors, play a role in individual prognostication. Alkaline phosphatase before and after chemotherapy should be determined routinely in adults, and further evaluated for its prognostic value in children.

**The influence of pathological fracture on surgical management, local recurrence and survival**

A pathological fracture through a bony sarcoma is thought to decrease the survival chance by spreading tumor via the fracture haematoma, or by spreading micro-metastases. It can also lead to joint involvement. The literature is unclear about the implications of a pathologic fracture on the outcome for patients with bony sarcomas\(^{(101-106)}\). The aim of the current study was to establish whether a pathological fracture had any influence on surgical management, local recurrence or survival in patients treated for a localised high grade extremity sarcoma of bone. Apart from osteosarcoma, this was investigated for Ewing’s and high grade
Chondrosarcoma. Alongside pathological fracture, other prognostic factors were analyzed.

The groups of patients with or without a fracture were in all 3 tumors comparable for sex, age at diagnose, and treatment, including achieved surgical margin. For all 3 tumors however, the fracture groups had more proximally located tumors (significantly so in osteo- and Ewing’s sarcoma). Moreover, in osteosarcomas, the fracture group had a higher proportion of telangiectatic subtypes, and in chondrosarcomas the fracture group showed a tendency towards more dedifferentiated subtypes. This is not surprising, because if bone defects are located proximally in a limb, especially in the lower limb, the risk of fracture seems higher(107). Also, telangiectatic osteosarcomas are usually more destructive to the cortical bone than other types(108).

No difference in local recurrence was found between fracture and control group in any of the 3 tumors. Comparing the group of patients where limb saving surgery was done with those who were treated with ablative surgery revealed no difference in local recurrence either.

In a univariate analysis, survival in the fracture group was lower than in the control group for osteosarcoma and chondrosarcoma, but not for Ewing’s. In a multivariate analysis, fracture remained a significant predictor of survival for osteosarcoma, but not for chondrosarcoma, where dedifferentiated subtype appeared to be decisive.

For osteosarcoma, the literature on implications of pathological fracture is inconsistent. Glasser and Scully report worse survival for patients with a fracture, but in the study of Scully, a considerable part of the patients did not receive pre-operative chemotherapy so these results are biased(11;109). Bacci(103) found no difference in survival, neither did Abudu(101). The latter found a higher chance of local recurrence in fracture patients that were treated with limb saving surgery, but this difference disappeared after correction for surgical margin. Both Scully and Abudu compared oncological outcome for limb saving and ablative therapy and found no difference. Our findings are in accordance with this. It seems that it is not spreading of tumor cells in the fracture hematoma that leads to a worse prognosis, but rather that fracture is a symptom of a more aggressive osteosarcoma.

In chondrosarcoma, the importance of adequate resection is stressed by several authors(110-115). In a large study concerning pathological fracture in chondrosarcoma no influence of fracture on the oncologic outcome was found(102). Surgical margin and tumor grade did correlate with survival. In our patients the importance of tumor grade was confirmed. Survival was less in patients with a fracture, but in the multivariate analysis only grade appeared to be an independent prognostic factor.
For Ewing’s sarcoma our study seems consistent with earlier literature, in which authors find no difference in oncologic outcome between patients with or without a fracture(106;116). None of the studies compares ablative and limb saving surgery in fractured Ewing’s sarcoma patients, which in our study does not reveal a difference. The explanation why Ewing’s sarcoma does not show a difference in overall survival between fracture and no-fracture group, could be that Ewing’s sarcoma generally is more chemotherapy -sensitive(117). This idea is strengthened by our finding that in osteosarcoma, good chemotherapy responders do not show a difference in survival between fracture and control group.

Unfortunately we did not have sufficient information about tumor volume in the three studied patient groups to establish the influence of tumor volume. This factor might influence both survival and the chance of pathological fracture. Earlier reports about volume and pathological fracture in regard to survival, regrettably are very much contradictory (8;102;106;109).

We conclude that a pathological fracture in a bony sarcoma does not increase the chance of local recurrence. Overall survival is worse in patients with a fracture in osteo- or chondrosarcoma, but not in Ewing’s sarcoma. Fracture is an independent predictor of survival in osteosarcoma only. Probably it is not the fracture in itself that causes the lower survival. It is rather a symptom of a more aggressive tumor. The influence of tumor volume should be further studied. Limb saving surgery in fractured patients does not seem to have an influence on local recurrence or survival and therefore is thought to be safe, as long as adequate margins can be obtained.

We recommend that patients with a pathological fracture through a bony sarcoma be treated by non-operative stabilisation of the fracture (e.g. by means of a splint) and appropriate analgesia, followed by chemotherapy according to the standard protocol. After this, resection of the tumor should, as usual, be done with wide margins.

8.6 General remarks and future perspectives

Both in our literature review as in our own research it appears that the identification of prognostic factors is hampered by the rareness of the disease, resulting in relatively small patient groups in single institutions, and even in multicentered trials. Pooling of results of different research groups is hardly possible because of the lack of raw data. Even the actual numbers of non-significant factors are often not made available. Another problem is the inconsistent methodology and reporting of studies.
Possible prognostic factors may thus remain undiscovered. Pakos et al succeeded in gathering raw retrospective data from 10 large centres, treating osteosarcoma (63). This has clarified the value of several prognostic factors. Missing information however made it impossible in this large retrospective review to identify other factors. Large cooperative prospective studies as those of the European Osteosarcoma Intergroup (EOI), the Cooperative Osteosarcoma Study group (COSS), and the European and American Osteosarcoma Study Group (EURAMOS), will hopefully improve this situation. Joining forces, also in experimental research, or at least sharing the raw data of results, could greatly improve our knowledge and bring about new factors, which are more reliable for individual prognostication. Eventually it should be possible to design a nomogram, in which a number of reliable factors are combined, which can give a reliable prediction of survival chance for the individual patient. This nomogram should have the possibility to include treatment factors. With such a nomogram, treatment, chemotherapeutic as well as surgical, could be tailored to the individual patient, taking survival chance, rehabilitation time, and the chance of late effects of treatment into account. Thus an optimal combination of therapies could be offered to each single patient.
Reference List


Chapter 9

Recommendations
9.1 Recommendations for clinical practice

1. More effort should be taken to estimate survival chance for each individual patient. Treatment strategy should balance survival chance, investment by the patient (treatment related morbidity and rehabilitation), and late effects of treatment.

2. Special attention should be given to the surgical procedures of resection and reconstruction; adverse late effects are predominantly caused by the results of surgery.

3. Prognostication should be done more extensively after chemotherapy, but before surgery. Estimation of survival chance and chemotherapy response should be given a more important role in the planning of surgery.

4. Estimation of chemotherapy response after chemotherapy, but before surgery, should be done routinely by Colour Doppler Ultrasound or by dynamic MRI scanning. CDUS is more easily available and more patient friendly, and is therefore preferable, depending on the clinical setting.

5. In adults, serum alkaline phosphatase should be routinely assessed before, and after chemotherapy, and used in survival chance assessment.

6. Patients with a pathological fracture through a bony sarcoma should be treated by non-operative stabilisation of the fracture, after which standard treatment should be carried out.
9.2 Recommendations for future research

1. In reporting research on prognostic factors of osteosarcoma, authors should be encouraged to mention significant results as well as non-significant results in order to make pooling of literature more feasible.

2. More effort should be put in making studies methodologically compatible, in the sense of more uniform inclusion, end points, and reporting.

3. Raw data should be made available. A web based cooperative prospectively kept database could reveal much information and should be aimed for.

3. The value of colour Doppler ultrasound, dynamic MRI, and other imaging techniques for predicting chemotherapy response before surgery should be further investigated.

4. Serum alkaline phosphatase and its sub fractions should be further evaluated for their value of predicting survival in adult, and paediatric osteosarcoma patients.

5. Large scale prospectively conducted cooperative studies should be directed at prognostic factors. With the identified independant prognostic factors a nomogram should be developed in which the survival chance for the individual patient can be estimated.
Nederlandse Samenvatting en Aanbevelingen
Samenvatting van introductie en doel van het proefschrift

Vóór de zeventiger jaren was operatief ingrijpen de enige beschikbare therapie voor patiënten met een hooggradig osteosarcoom van een extremiteit. In 90% van de gevallen betekende dit amputatie van het aangedane ledemaat. De overleving was slechts 10 tot 15%(1-3). Deze situatie is veranderd met de introductie van chemotherapie in de zeventiger jaren. Sinds de tachtiger jaren resulteert de, nog steeds vigerende, standaard behandeling met chemotherapie preoperatief, resectie van de tumor en tenslotte chemotherapie postoperatief in een overleving van rond de 70% van de patiënten(4-9). Als er een goede reactie is op preoperatieve chemotherapie wordt de tumor soms kleiner (down-staging) waardoor de resectie van de tumor technisch makkelijker wordt. De verdere ontwikkeling van beeldvormende technieken, in het bijzonder van de Magnetic Resonance Imaging (MRI) heeft het mogelijk gemaakt preoperatief de resectie van een tumor goed te plannen(10;11). Tegenwoordig kan in 90% van de gevallen het ledemaat na resectie van de tumor behouden blijven(4;6;7). De mogelijkheden voor reconstructie zijn sterk verbeterd en variëren van biologische tot compleet endoprosthetische oplossingen. Elk van deze heeft voor- en nadelen(12;13). Een complete biologische reconstructie kent op de lange termijn een goed resultaat met levend bot. Dit staat een actief leven toe, vrijwel zonder beperkingen wat betreft het ledemaat(14;15). Vaak is dit hierbij echter een lange periode niet belastbaar, soms tot 18 maanden na de operatie. Soms zijn ook re-operaties nodig(16;17). Anderzijds kan een reconstructie met een gecementeerde endoprothese snel volledig belast worden, meestal na 1 tot 2 weken. Op de lange termijn echter blijft er bij deze optie altijd risico op peri-prothetische fractuur, infectie en uiteindelijk loslating(12;18;19).

De verbetering in overleving heeft geleid tot een groeiend aantal overlevenden(20). Dezen kunnen te maken krijgen met lange termijn gezondheidseffecten van de behandeling, vooral als ze behandeld zijn in hun kinderjaren. Late effecten kunnen mild zijn maar kunnen ook de kwaliteit van leven compromitteren of zelfs fataal zijn(21;22).

In de besluitvorming bij osteosarcoompatiënten is de combinatie van bovengenoemde factoren leidend. Voor elke patiënt moet de optimale behandeling worden gezocht, met de grootste kans op overleving, het best mogelijke functionele resultaat en de kleinste kans op ongunstige late effecten van de behandeling. Bij de gekozen chirurgische behandeling moet een balans worden gevonden tussen de verwachte revalidatie tijd en de kans op overleving.
Om deze overwegingen goed te kunnen maken is een adequate inschatting van de kans op overleving van de individuele patiënt essentieel. Idealiter zou dit mogelijk moeten zijn in een vroeg stadium zodat voor patiënten op tijd de best passende chemotherapie en chirurgische strategie kunnen worden gekozen en eventueel aangepast. Helaas blijkt voorspellen van het beloop voor de individuele patiënt nog steeds moeilijk te zijn. Er zijn veel prognostische factoren beschreven in de literatuur maar de waarde van veel factoren voor individuele voorspellingen is onbekend(23). Daarnaast is er nog veel onduidelijk over het voorkomen en de etiologie van late effecten van behandeling.

Het doel van dit proefschrift was vast te stellen welke factoren er beschikbaar zijn om de overlevingskans na behandeling van een hooggradig osteosarcoom in te schatten en om de waarde van deze factoren voor de individuele patiënt te bepalen. Speciale aandacht was gericht op factoren die vóór, of vroeg in de behandeling kunnen worden bepaald en op factoren die eenvoudig te bepalen zijn. Daarnaast werd onderzocht welke ongunstige late effecten van behandeling optraden bij patiënten die langer dan 5 jaar na het einde van de behandeling overleefden. Zowel de ernst van deze effecten werd bestudeerd als de relatie ervan met de behandeling die de patiënt onderging.

Samenvatting van Hoofdstuk 2 tot en met 6

**Hoofdstuk 2: Systematische review van de literatuur**

Hoofdstuk 2 presenteert een systematische review van de literatuur betreffende prognostische factoren bij het hooggradig osteosarcoom. Hierin wordt getracht evidence-based prognostische factoren te identificeren in de literatuur sinds 1992 en om “pooled” relatieve risico’s (RR) van deze factoren te bepalen. Uit 1777 “hits” bij de literatuur search werden 93 artikelen diepgaand bestudeerd. Slechts 7 hiervan waren van voldoende kwaliteit om in een meta-analyse te betrekken. Slechte respons op chemotherapie (“pooled” RR = 2.37), groot tumor volume (“pooled” RR = 1.36) en ablatieve chirurgie (“pooled” RR 2.18) bleken onafhankelijke voorspellers van een slechte prognose te zijn. Andere factoren die waarschijnlijk een slechte uitkomst voorspellen maar die niet in een “pooled analysis” konden worden betrokken zijn inadequate resectie marge, leeftijd (bij diagnose) lager dan 14 jaar, mannelijk geslacht, hoog serum alkalisch phosphatase, locaal recidief, p-Glycoproteine expressie en afwezigheid van Erb2 expressie.

Conclusie: Slechte respons op chemotherapie, groot tumor volume en ablatieve chirurgie zijn onafhankelijke voorspellers van een slechte prognose. Hoewel de
literatuur overvloedig is zijn slecht enkele artikelen van voldoende kwaliteit om harde conclusies te trekken. Vanwege de heterogeniciteit van de studies is “pooling’ van de resultaten nauwelijks mogelijk. Omdat de meeste studies een relatief kleine patiëntent popula tie betreffen kan het zijn dat zelfs vrij sterke prognostische parameters niet significant naar voren komen daarom niet worden gerapporteerd.

Hoofdstuk 3: Overleving, prognostische factoren en late effecten van behandeling bij kinderen

Hoofdstuk 3 evalueert overleving, prognostische factoren en ongewenste late effecten van behandeling in kinderen die in het Emma Kinderziekenhuis (EKZ/AMC) werden behandeld voor een niet gemetastaseerd hooggradig osteosarcoom van een extremiteit.

Voor de analyse van overleving en prognostische factoren werd een retrospectief onderzoek verricht onder alle opeenvolgende patiënten, jonger dan 18 jaar bij diagnose die behandeld werden tussen 1985 en 2006. Voor onderzoek naar de late effecten van behandeling werden patiënten met een overleving langer dan 5 jaar na het einde van de behandeling gezien op een speciaal ingestelde polikliniek.

Ongewenste late effecten van behandeling werden gegradeerd volgens de “Common Terminology Criteria for Adverse Events” versie 3.0 (CTCAE), variërend van graad 1 (mild) tot graad 5 (dodelijk).

Zeventig patiënten werden geïncludeerd. De 5 jaars overleving was 75%. Grootte van de tumor en chemotherapie respons waren de enige factoren waarvan een onafhankelijke voorspellende waarde voor overleving kon worden vastgesteld.

Negenendertig van de 40 patiënten, met een overleving van 5 jaar of meer, hadden een of meerdere ongewenste effecten van behandeling. In 2 patiënten was dit effect dodelijk (1 maal cardiaal en 1 maal beenmerg gerelateerd). Ongewenste late effecten van het steun- en bewegingsapparaat werden gevonden in 95% van de overlevenden.

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In 70% hiervan betrof dit een graad 4 effect (invaliderend). Dit heeft te maken met de behandелingsstrategie, waarbij resectie van de tumor met een ruime marge obligaat is. In de bestudeerde periode werd in een groot deel van de patiënten na resectie gereconstrueerd door middel van een omkeerplastiek. Dit telt in de CTCAE als een graad 4 effect. De tweede meest voorkomende soort van ongewenste late effecten betrof het auditive systeem (in 35% van de patiënten). Meestal was dit een mild of matig invaliderend effect maar sommige patiënten bleken een ernstig gehoorsverlies in frequenties van het spraakgebied te hebben.

Cardiale late effecten werden gevonden in 20% van de patiënten. Ook deze waren meestal mild of matig ernstig. Eén patiënt echter overleed aan de gevolgen ervan (cardiomyopathie), 18 jaar na het einde van de behandeling. Patiënten waarbij ifosfamide in het chemotherapie schema was gegeven hadden significant meer late cardiale effecten.
Eén patiënt overleed aan een myelodysplastisch syndroom, 11 jaar na de beëindiging van de behandeling. Andere soorten van ongewenste late effecten van behandeling waren zeldzaam en meestal niet ernstig.

Conclusie: De overleving in deze patiëntenpopulatie was goed (75%). Chemotherapie respons en tumor grootte waren van prognostische waarde. Late ongewenste effecten van behandeling kwamen veel voor, waren vaak invaliderend en betroffen vooral het steun- en bewegingsapparaat.

**Hoofdstuk 4: De waarde van Colour Doppler Ultrasound (Echo-Doppler) voor het voorspellen van chemotherapie respons en overleving**

Onderzocht werd of chemotherapie respons en overleving kunnen worden voorspeld met Colour Doppler Ultrasound (CDUS) na chemotherapie maar vóór resectie van de tumor. CDUS is een non-invasieve methode die relatief weinig tijd kost(24). De apparatuur die in ons onderzoek werd gebruikt is beschikbaar in de meeste moderne ziekenhuizen. CDUS werd vóór en na chemotherapie verricht in 21 opeenvolgende patiënten die in het AMC werden behandeld voor een hooggradig osteosarcoom van een extremiteit. De “Peak Systolic Velocity” (PSV) in de weke delen component van de tumor en het “Quotient of Resistive Index” (QRI) van de voedende arterie werden bepaald. Na resectie van de tumor werd de histologische respons op chemotherapie bepaald in het resectiepreparaat door een gespecialiseerde patholoog die niet op de hoogte was van de CDUS resultaten.

De verandering van QRI na chemotherapie was significant hoger in goede histologische responders vergeleken met non-responders. Er was geen significant verschil in PSV-verandering tussen de subgroepen. Nog QRI- nog PSV-verandering correleerde direct met overleving.

Conclusie: CDUS kan chemotherapie respons voorspellen (sensitiviteit 83%, specificiteit 86%), vooral slechte respons (voorspellende waarde voor slechte respons 92%) maar heeft geen voorspellende waarde voor overleving.

**Hoofdstuk 5: De voorspellende waarde van het serum alkalische phosphatase voor chemotherapie respons, locaal recidief en overleving**

Alkalische phosphatase is eenvoudig in het serum te bepalen met weinig kosten. Doel in dit hoofdstuk was om de voorspellende waarde van het serum alkalische phosphatase voor chemotherapie respons, locaal recidief en overleving te bepalen. Alkalische phosphatase wordt geproduceerd door de cellen van een osteosarcoom. Het serum gehalte ervan wordt echter ook beïnvloed indien er een fractuur optreedt en door groei. Om deze redenen werden patiënten, jonger dan 18 jaar, en patiënten
met een pathologische fractuur geëxcludeerd. Een retrospectief onderzoek werd uitgevoerd in een groep van 132 patiënten met een hooggradig, niet gemetastaseerd, osteosarcoom die opeenvolgend waren behandeld in het Royal Orthopaedic Hospital in Birmingham (United Kingdom) tussen 1983 en 1999.

Voor elke patiënt werden de serum waarden van alkalische phosphatase opgezocht van vóór aanvang van de chemotherapie (pre-ct AP), na chemotherapie maar vóór resectie (post-ct AP) en het verschil tussen deze 2 waarden werd bepaald. De alkalische phosphatase waarde werd verdeeld in 3 categorieën: Normaal (onder de bovenste normaalwaarde grens), Hoog (verhoogd maar minder dan 2x de bovengrens) en Zeer Hoog (meer dan 2x de bovengrens).

Een Zeer Hoog prechemotherapie alkalische phosphatase bleek voorspellend te zijn voor een lagere overlevingskans. De voorspellende waarde van een Zeer Hoog alkalische phosphatase voor een slechte chemotherapie respons was 80%. De waarde van alkalische phosphatase na chemotherapie maar vóór resectie bleek van nog meer prognostisch belang. De overleving nam stapsgewijs af met postchemotherapie AP waarden die Normaal, Hoog, of Zeer Hoog waren. De voorspellende waarde van een verhoogd postchemotherapie AP in welke mate dan ook voor een slechte chemotherapie respons was 100%. Het dalen van de alkalische phosphatase na chemotherapie bleek niet te correleren met een betere overleving tenzij de waarde tot normaal terug kwam. In de groep patiënten waarbij de alkalische waarde wel tot normaal daalde was de overleving gelijk aan die in de groep met normaal AP bij diagnoste. Er kon geen significante correlatie worden aangetoond tussen de AP waarde op enig moment en het ontstaan van locaal recidief.

Conclusie: De alkalische phosphatase serum waarde vóór chemotherapie, na chemotherapie en de verandering van de waarde na chemotherapie zijn waardevolle voorspellers van chemotherapie respons en overleving bij volwassen patiënten met een hooggradig osteosarcoom.

**Hoofdstuk 6: De invloed van een pathologische fractuur op chirurgische strategie, locaal recidief en overleving**

Dit werd onderzocht door middel van een retrospectief onderzoek onder 770 patiënten met een hooggradig ossaal sarcoom van een extremiteit zonder metastasen bij diagnose. Het betrof 484 patiënten met een osteosarcoom, 130 met een chondrosarcoom en 156 met een Ewing’s sarcoom.

Andere prognostische factoren die werden bestudeerd waren proximaliteit van de tumor, subtype (voor osteo- en chondrosarcoom), chemotherapie respons (voor osteo- en Ewing’s sarcoom), type resectie (ablatief vs. ledemaatsparend) en de chirurgische marge na resectie.
Een pathologische fractuur werd gezien in 12% van de osteosarcoom patiënten, 25% van de chondrosarcoom patiënten en 10% van de patiënten met een Ewing’s sarcoom. De groepen patiënten met en zonder fractuur waren bij alle 3 de tumoren vergelijkbaar voor geslacht, leeftijd en behandeling, inclusief de bereikte resectiemarge. Bij alle drie tumor soorten echter bleek dat in de groep patiënten met een fractuur de tumoren meer proximaal waren gelokaliseerd (significant verschil in osteo- en Ewing’s sarcoom). In de osteosarcoom patiënten was er een groter percentage teleangiectatische subtypes in de fractuur groep en in de chondrosarcoom patiënten was er een tendens naar meer gededifferentieerde subtypes in de fractuurgroep. Ledemaat sparende chirurgie werd verricht in 79% van de patiënten met een fractuur en in 84% van de patiënten zonder fractuur (p=0.17). In geen van de drie tumor soorten werd er een verschil gezien in het optreden van locaal recidief tussen fractuur en controle groep. Vergelijking van de groep die met ledemaatsparende chirurgie werd behandeld met de ablatief behandelde groep gaf evenmin een verschil in locaal recidief te zien.

In een univariate analyse bleek het overlevingspercentage significant lager te zijn in de fractuur groep vergelijk met de controle groep bij het osteosarcoom (34% versus 58%, p<0.01) en het chondrosarcoom (35% versus 63%, p=0.04) maar niet bij het Ewing’s sarcoom (75% versus 64%, p=0.80). In een multivariate analyse bleef pathologische fractuur een significante voorspeller voor een slechtere prognose voor het osteosarcoom maar niet voor het chondrosarcoom. Bij de laatste bleek de aanwezigheid van een gededifferentieerde subtype bepalend te zijn.

Van de andere onderzochte prognostische factoren bij het osteosarcoom lieten proximaliteit van de tumor, type chirurgie, resectiemarge en chemotherapie respons een significante onafhankelijke voorspellende waarde zien voor overleving. Bij het chondrosarcoom bleek uitsluitend de histologische graad cq het subtype van de tumor een onafhankelijke voorspeller van de prognose te zijn. Bij het Ewing’s sarcoom liet geen van de onderzochte factoren een correlatie met de overleving zien.

Conclusie: Een pathologische fractuur in de bestudeerde ossale sarcomen geeft geen verhoogde kans op locaal recidief. De kans op overleving is lager in patiënten met een fractuur in geval van een osteo- of chondrosarcoom maar niet in geval van een Ewing’s sarcoom. Alleen bij het osteosarcoom is een pathologische fractuur een onafhankelijke voorspeller van een slechtere prognose. Ledemaatsparende chirurgie bij patiënten met een fractuur lijkt geen invloed te hebben op locaal recidief of overleving en wordt daarom als veilig beschouwd mits een adequate resectiemarge kan worden verkregen.
Aanbevelingen voor de kliniek

1. Er zou meer moeten worden gedaan om de overlevingskans in te schatten voor de individuele patiënt met een hooggradig osteosarcoom. Bij de behandeling moet een optimale balans worden gezocht tussen overlevingskans, investering door de patiënt (behandelingsgerelateerde morbiditeit en revalidatie) en late effecten van behandeling.

2. Speciale aandacht zou uit moeten gaan naar de chirurgische procedures bij resectie en reconstructie; ongewenste late effecten van behandeling worden voornamelijk veroorzaakt door de resultaten van deze chirurgie.

3. Inschatting van de prognose zou meer uitgebreid moeten worden gedaan na chemotherapie maar vóór resectie van de tumor. De inschatting van de kans op overleving en van de chemotherapie respons zou een belangrijker rol moeten spelen in de planning van de operatie.

4. Inschatting van de chemotherapie respons ná chemotherapie maar vóór resectie zou routinematig moeten worden gedaan met Colour Doppler Ultrasound of met dynamische MRI. CDUS is over het algemeen beter beschikbaar en meer patiënt vriendelijk en zou daardoor de voorkeur kunnen hebben, afhankelijk van de klinische setting.

5. Bij volwassenen zou het serum alkalische phosphatase routinematig moeten worden bepaald vóór en na chemotherapie en moeten worden gebruikt bij het inschatten van de kans op overleving.

6. Patiënten met een pathologische fractuur door een ossaal sarcoom moeten worden behandeld door middel van non operatieve stabilisatie van de fractuur, gevolgd door standaard behandeling van de tumor.

Aanbevelingen voor toekomstig wetenschappelijk onderzoek

1. Bij de publicatie van onderzoek betreffende prognostische factoren bij het osteosarcoom moeten auteurs worden aangemoedigd om zowel significante als niet significante resultaten te vermelden om meta-analyse van de literatuur beter mogelijk te maken.

2. Er moet meer getracht worden studies methodologisch vergelijkbaar op te zetten in de zin van meer uniforme inclusie, end points en rapportage.

3. De waarde van Colour Doppler Ultrasound, dynamische MRI en andere imaging technieken om de chemotherapie response te voorspellen vóór de resectie van de tumor moet verder worden geëxploreerd.

4. De prognostische waarde van het serum alkalische phosphatase en de subfracties daarvan moet verder worden geëvalueerd voor volwassen en pediatrische osteosarcoma patiënten.

5. Er moet prospectief onderzoek worden gedaan naar prognostische factoren in grote samenwerkingsverbanden. Met de hierin vastgestelde onafhankelijke factoren kan een nomogram worden ontwikkeld waarin de overlevingskans voor de individuele patiënt kan worden geschat.
Reference List


Acknowledgements
Recipe for a thesis:

What you do:
* Write a couple of scientific papers
* Put them in order
* Put a staple through them

What you need:
* The Idea
* Facilitators
* Stimulators
* Inhibitors
* The New Idea
* Endurance
* Wise Advise
* Sturdy Co-Operators
* Enthousiastic Co-Authors
* An Eminent Reading Committee
* Trust And Support
* Concentration
* Distraction
* Criticasters
* Honesty
* Friendship
* Fun
* Booze
* Love

I am very thankful for the help of good friends, fine colleagues, a lovely family, and a super wife, who supplied me with the ingredients when I needed them. Without them life wouldn’t be much fun and preparing a thesis would have been impossible. All of you mean a lot to me!


Thanks !!!
Addendum:
Overview of Prognostic Factors, reported in the Literature from 1992 to 2006
Addendum

Prognostic factors, reported in the literature from 1992 to 2006

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Authors reporting worse survival</th>
<th>Authors reporting no difference</th>
<th>Authors reporting better survival</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient related factors</strong></td>
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<tr>
<td>Age (young)</td>
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<td>Bielack(28); Ferrari(29);</td>
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<td><strong>Indirect factors (biochemical, genetic, radiological)</strong></td>
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<td>Albumin ASAT/ALAT Calcium</td>
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<td>Collagen markers</td>
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<td>Wiklund(33)</td>
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<td>Gamma GT</td>
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<tr>
<td>Insulin Growth factor</td>
<td>Rodriguez-Galindo(34)</td>
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<tr>
<td>High APE expression</td>
<td>Trieb(35)</td>
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<tr>
<td>Bone Morphogenetic protein production</td>
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<td>Bone scan Thallium</td>
<td>Kaste(37); Stokkel(4);</td>
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<td>Calponin gene expression</td>
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<td>Yamamura(38)</td>
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<tr>
<td>CD44 v6 expression</td>
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<td>CDS6 and CD99;</td>
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<td>Okada(18);</td>
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<tr>
<td>Genetic imbalances</td>
<td>Entz(39); Tarkkanen(40);</td>
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## Addendum

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Authors reporting worse survival</th>
<th>Authors reporting no difference</th>
<th>Authors reporting better survival</th>
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<tbody>
<tr>
<td>Cyclin D1 expression (high)</td>
<td>Benassi(41)</td>
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<tr>
<td>c-myc / c-fos proto-oncogene</td>
<td>Gamberi(42)</td>
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<td>DNA content</td>
<td>Adler(43); Kusazaki(44); Neuburger(45)</td>
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<td>FDG Pet scan (High uptake)</td>
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<td>Glutathione-S-Transferase</td>
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<td>Uozaki(25);</td>
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<td>Heat shock protein 27</td>
<td>Uozaki(47)</td>
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<td>HER2/erbB-2 expression</td>
<td>Morris(48); Onda(49); Scotland(50)</td>
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<td>Ki 67 expression (high)</td>
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<td>Jong(53)</td>
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<td>Lactate Dehydrogenase (high)</td>
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<td>Lung resistance-related protein</td>
<td>Uozaki(25);</td>
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<td>Methylation of p14 gene</td>
<td>Oh(54)</td>
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<td>Metrotrexate serum level in 24 h</td>
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<td>Smeland(22);</td>
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<td>MMP expression (high)</td>
<td>Uchibori(55)</td>
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<td>Nm23 protein</td>
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<td>Liao(56)</td>
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<tr>
<td>Nuclear Survivin localisation</td>
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<td>Trieb(57)</td>
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<td>p-53 expression (high)</td>
<td>Shiraishi(58); Uozaki(47)</td>
<td>Gorlick(51); Park(19); Wunder(59);</td>
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<td>P-glycoprotein expression (high)</td>
<td>Baldini(10); Baldini(60); Chan(61); Hornicek(62); Scotland(50); Serra(63); Yamamoto(64)</td>
<td>Gorlick(51); Park(19);</td>
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<tr>
<td>p-53 / p-glycoprotein Co-expression</td>
<td>Park(19);</td>
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<tr>
<td>Proliferating Cell Nuclear Antigen (high)</td>
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<td>Shiraishi(58)</td>
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<tr>
<td>Loss of heterozygosity of Rb gene</td>
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<td>Feugeas(65)</td>
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### Addendum

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<tr>
<td>pRb expression (high)</td>
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<td>TGF-β3</td>
<td>Kloen(66)</td>
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<td>Presense of TMM</td>
<td>Ulaner(67)</td>
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<td>VEGF High/positive</td>
<td>Charity(12); Kaya(68); Kaya(69); Lee(70)</td>
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**Tumor related factors**

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<tr>
<th>Bone marrow infiltration / Mircometastasis</th>
<th>Bruland(71); Hermann(72)</th>
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<tbody>
<tr>
<td>Local Tumor Extent</td>
<td>Spanier(23); Wuisman(73)</td>
</tr>
<tr>
<td>Haemangio-pericytoma like pattern</td>
<td>Okada(18);</td>
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<tr>
<td>Grade</td>
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<td>Subtype</td>
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<td>Bacci(1); Bacci(8); Bauer(11); Baldini(10); Ger(13); Glasser(15); Hauben(74); Okada(18); Park(19); Smeland(22); Tomer(24);</td>
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<td>Micro Vessel Density (high)</td>
<td>Mantadakis(17); Kreuter(75)</td>
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<tr>
<td>Osteoclast-like giant cells present</td>
<td>Okada(18);</td>
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<tr>
<td>Pathological fracture</td>
<td>Bacci(6); Bacci(1); Bacci(31); Glasser(14); Glasser(15); Scully* 93;</td>
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<tr>
<td>Pleomorfism</td>
<td>Okada(18);</td>
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<tr>
<td>Rosette-like features</td>
<td>Okada(18);</td>
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<tr>
<td>Skip metastasis</td>
<td>Glasser(14);</td>
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<tr>
<td>Site</td>
<td>Bielack(28); Bauer(11); Bentzen(2); Glasser(14); Glasser(15); Mantadakis(17); Szendroi(30); Weeden(26); Akatsuka(5); Bacci(6); Bacci(7); Bacci(1); Bacci(31); Bacci(8); Bacci(9); Baldini(10); Ferrari(29); Ger(13); Kuryu(16); Okada(18); Rehan(21); Smeland(22); Spanier(23); Stokkel(4); Tomer(24); Uozaki(25); Wunder(27);</td>
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<tr>
<td>Prognostic Factor</td>
<td>Authors reporting worse survival</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Size / volume (large)</td>
<td>Bacci(1); Bacci(9); Bielack(28); Bieling(76); Ferrari(29); Okada(18); Rehan(21); Smeland(22); Stokkel(4); Szendroi(30); Wunder(59); Wunder(27)</td>
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<tr>
<td>Spontaneous tumor necrosis</td>
<td>Bjornsson(78)</td>
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<tr>
<td><strong>Treatment related factors</strong></td>
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<tr>
<td>Local recurrence</td>
<td>Scully(77); Weeden(26);</td>
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<tr>
<td>Response to chemotherapy (poor)</td>
<td>Bacci(6); Bacci(1); Bacci(31); Bacci(9); Baldini(60); Bielack(28); Bieling(76); Ferrari(29); Glasser(14); Glasser(15); Okada(18); Rehan(21); Scully(77); Szendroi(30); Tomer(24); Uozaki(47); Wunder(59);</td>
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<tr>
<td>Surgery type (limb saving)</td>
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<tr>
<td>Surgical margins (inadequate)</td>
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<tr>
<td>Treatment delay / time to diagnosis.</td>
<td>Akatsuka(5); Bielack(28);</td>
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<td></td>
<td>Ger(13);</td>
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Reference List


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Vd Sande MAJ, Bramer JAM, Jutte PC, Schreuder HWB, Dijkstra PDS
Accepted for publication in Nederlands Tijdschrift voor Geneeskunde