Osteosarcoma and Ewing’s Sarcoma in Children and Adolescents: Retrospective Clinicopathological Study

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Aim. Almost 20 different prognostic factors have so far been investigated in childhood bone tumors, but apart from clinical stage the results have been inconclusive. We evaluated possible prognostic factors of malignant bone tumors in children and adolescents.

Methods. Seventy children and adolescents, who have been treated for bone sarcomas (36 with osteosarcoma and 34 with Ewing’s sarcoma) at the Children’s Hospital in Zagreb and the Orthopedic Clinic of the Zagreb University School of Medicine were included in the study. We analyzed 9 variables: clinical stage, patient age and sex, tumor size and location, location of metastases, chemotherapy response, concentration of lactate dehydrogenase (LDH) and erythrocyte sedimentation rate (ESR), and chondroid differentiation in osteosarcoma.

Results. The clinical stage was the most important prognostic factor for both type of tumors. Lactate dehydrogenase in patients with Ewing’s sarcoma (p=0.033) and in patients with osteosarcoma (p=0.015) as well as erythrocyte sedimentation rate (p<0.001 and p=0.014, respectively) may also be of predictive value.

Conclusion. Elevated erythrocyte sedimentation rate and high LDH concentration seem to be of importance in both Ewing’s sarcoma and osteosarcoma, regardless of their different pathogenesis or pathohistology.

Key words: adolescent; bone neoplasms; child; osteosarcoma; sarcoma, Ewing’s

Osteosarcoma and Ewing’s sarcoma are the two most common bone sarcomas in children. The frequency of childhood bone sarcomas in the world is 2-5% of all childhood tumors (1). Bone sarcomas show a somewhat irregular geographical distribution, with low rates in some Asian (Indian, Japanese, Chinese) and Latin American populations, and high rates in Europe and North America (2). In Europe and North America osteosarcoma is more frequent than Ewing’s sarcoma, but in Australia and New Zealand the ratio is opposite (3,4). Etiology is unknown for both sarcomas. The treatment of both tumors includes neoadjuvant chemotherapy, surgery, and sometimes, additional irradiation (1). This treatment regimen achieves a significant prognostic improvement (5). Contrasting the grim outlook from the past, long-term survival nowadays is around 70% for osteosarcoma (6) and up to 84% for Ewing’s sarcoma (7). Following this evolution, the search for prognostic factors to better define risk groups seems to be of great importance. Most studies pointed out that only the clinical stage was of prognostic significance (8).

About 20 different prognostic factors have been analyzed in many studies of Ewing’s sarcoma and osteosarcoma over the last twenty years. They included histological and clinical parameters (age, gender, location, grade, size of tumor, metastatic spread, chemotherapy response for both tumors, and chondral differentiation for osteosarcoma). In many of them, age, tumor size, and location showed to be statistically significant prognostic factors for both Ewing’s sarcoma and osteosarcoma (7-16). In some of the studies results were insignificant because of small patient groups (7,8,12,13).

So far, the only prognostic factors for Ewing’s sarcoma and osteosarcoma, as proven by most studies, are clinical stage (metastasis at the time of diagnosis), and grade of tumor necrosis following preoperative chemotherapy (7,8,10,11,14-27). Some patients with a favorable clinical stage develop metastases shortly after therapy. Therefore it seems necessary to establish prognostic factors in patients with nonmetastatic osteosarcoma and Ewing’s sarcoma, which can be determined before neoadjuvant chemotherapy, e.g., at the time of the first biopsy. Few studies that included biochemical parameters (sedimentation rate, alkaline phosphatase, lactate dehydrogenase for both tumors, and anemia and fever for Ewing’s sarcoma) showed that high concentrations of alkaline phosphatase in patients with osteosarcoma (15-17) and Ewing’s sar-
coma (18) was an adverse prognostic factor, as well as a high level of lactate dehydrogenase (15,17,18) and erythrocyte sedimentation rate (17-19). Some additional immunohistochemical and molecular studies were undertaken, yielding promising results: p53 for Ewing’s sarcoma (20) and DNA content (21), expression of Her-2 (22), CD44 (23), expression of MDR1 (24), and loss of heterozigosity (LOH) for Rb gene for osteosarcoma (25). Expression of MDR1 gene product seems to be a determining factor for tumor chemosensitivity (24).

In Croatia, the new therapeutic protocols including neoadjuvant chemotherapy were introduced in 1987. The aim of this study was to analyze which risk factors in children with osteosarcoma and Ewing’s sarcoma influenced their survival and to define a subpopulation of patients who would benefit from a more aggressive therapeutic approach.

### Patients and Methods

**Patients**

We included all children and adolescents who have been diagnosed for Ewing’s sarcoma or osteosarcoma at the Department of Pathology at the Zagreb University School of Medicine, and treated in the Children’s Hospital and the Orthopedic Clinic of Zagreb University School of Medicine from January 1988 until December 1999. There were 34 children and adolescents with Ewing’s sarcoma and 36 with osteosarcoma (Table 1).

For both sarcomas we divided the patients into two age groups: 1 (less than 12 years) and 2 (of or more than 12 years). This division was made to allow comparison with the results published in other studies (6,8,9).

Ewing’s sarcoma. The median age of patients was 11 years (range 1-17 years). There were 20 boys and 14 girls. There were 13 patients younger than 12 years of age. Three patients presented in the clinical stage 3 (metastatic disease) (29). Ten patients developed metastases one year after diagnosis. Nine patients had metastases in the lungs and 3 had metastases in the bones (navicular bone, vertebral bone, and rib). There was a single patient with lung and bone metastases. At the time of diagnosis, 26 patients had elevated sedimentation rate and 2 patients had elevated serum lactate dehydrogenase concentration.

Osteosarcoma. The median age of patients was 14 years (range 5-24 years). There were 21 boys and 15 girls. There were 18 patients younger than 12 years of age. A single patient was in the clinical stage 3 (metastatic disease). All other patients were in the clinical stage 2. Thirteen patients developed metastases one year after the diagnosis was made. The majority of metastases were in the lungs (n = 12) and 2 patients had metastases located in the vertebrae. At the time of diagnosis, 27 patients had elevated sedimentation rate and 2 patients had elevated serum lactate dehydrogenase concentration. Histologically, 11 tumors showed chondral differentiation.

**Tumor Volume**

The patients were classified into two groups according to the tumor size: 1 (0-10 cm³) and 2 (>11 cm³). We used 10 cm³ cut-off to allow comparison of our results with the results of other studies (6,7,11).

**Tumor Site**

For staging bone tumors, we avoided using the TNM staging system because these tumors never metastasize into lymph nodes. The stage is determined by Musculoskeletal Tumor Society Staging System of Malignant Bone Lesion (MTS) (29). MTS stage 1 includes the patients with low-grade sarcoma and localized disease, MTS stage 2 included the patients with high-grade sarcoma. Osteosarcoma and Ewing’s sarcoma are considered to be high-grade sarcomas. Clinical stage 3 indicated a metastatic spread.

**Treatment**

All but 3 patients with Ewing’s sarcoma and 2 patients with osteosarcoma underwent surgical procedure. The operative procedure performed was segmental resection or amputation. There were 27 partial limb resections for Ewing’s sarcoma and 19 partial limb resections for osteosarcoma. Four amputations of extremities were performed in patients with Ewing’s sarcoma and 14 with osteosarcoma. The tumor was measured and surface labeled sections created. The sections (formalin fixed and paraffin embedded, 3 μm thick) were stained with hematoxylin eosine and revised by a pathologist for diagnosis. Some of the tumors required additional immunohistochemical staining. During revision for this study, all Ewing’s sarcomas not previously stained were assessed for CD99 positivity.

According to the chemotherapy protocol, 31 patients with Ewing’s sarcoma and 32 patients with osteosarcoma received chemotherapy. Chemotherapy for patients with Ewing’s sarcoma was administered according to four chemotherapy protocols. The first protocol was adjuvant and comprised 45 weeks of treatment with a three drug regimen: ifosfamide (cumulative dose: 45 g/m²), uracitexan (12 mg/m²) and doxorubicin (450-630 mg/m²). A single patient received vincristine (10.5-12 mg/m²) instead of uracitexan. Second protocol was a neoadjuvant protocol where patients received ifosfamide, uracitexan, and doxorubicin during a two week period and then, after local treatment, they received five cycles of the same drugs. The third and fourth protocol differ from the second in the use of dactinomycin (0.7-2.2 mg/m²) instead of doxorubicin in the preoperative cycles in the third protocol and in the postoperative cycles of the fourth protocol. Three patients did not undergo chemotherapy treatment.

There were 3 chemotherapy protocols for the osteosarcoma patients. The first included two times of preoperative cisplatin (48-720 mg/m²) and doxorubicin (450-630 mg/m²) followed by the same drugs in the postoperative treatment. Second protocol consisted of the same induction drugs and in the postoperative treatment, methotrexate (42-144 mg/m²), BCD (bleomycin 0-120 mg/m²), cyclophosphamide 4.8-21.6 g/m², and dactinomycin

### Table 1. Univariate analysis for 70 patients with Ewing’s sarcoma and osteosarcoma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ewing’s sarcoma (n=34)</th>
<th>Osteosarcoma (n=36)</th>
</tr>
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<tr>
<td>Age (years):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>14</td>
<td>8</td>
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<tr>
<td>&gt;12</td>
<td>20</td>
<td>28</td>
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<tr>
<td>Gender:</td>
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<td></td>
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<tr>
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<td>20</td>
<td>23</td>
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<tr>
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<td>17</td>
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<tr>
<td>Tumor volume:</td>
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<td></td>
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<tr>
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<td>27</td>
<td>27</td>
</tr>
<tr>
<td>&gt;10 cm³</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Tumor location:</td>
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<td></td>
</tr>
<tr>
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<td>23</td>
<td>32</td>
</tr>
<tr>
<td>axial</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
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</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
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<td>13</td>
<td>14</td>
</tr>
<tr>
<td>bones</td>
<td>12</td>
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<tr>
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<td>high</td>
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<td>normal</td>
<td>8</td>
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</table>

*Abbreviations: LDH = lactate dehydrogenase; ESR = erythrocyte sedimentation rate.
1Log-rank test.
2The stage was determined by Musculoskeletal Tumor Society Staging System of Malignant Bone Lesion (MTS) (29).
0.7-2 mg/m²) were added. The third protocol consisted of methotrexate, doxorubicin, and BCD in pre- and postoperative treatment. Selected patients that entered the third protocol received cisplatin as well.

Eleven patients of those who received chemotherapy (8 with Ewing’s sarcoma and 3 with osteosarcoma) also received an additional irradiation therapy.

Histologic Response to Chemotherapy

The effect of neoadjuvant chemotherapy can be assessed quantitatively (measuring the necrotic area), semiquantitatively (scoring the necrotic area) or using descriptive elements. We used the Huvos criteria (30,31). Grade 1 indicates necrosis in less than 50% of the whole tumor area, Grade 2 indicates 50-90% of necrotic tumor area, Grade 3 indicates 91-99%, and Grade 4 more than 99% of necrotic area. We also measured necrosis area and scored it as high (more than 90% of necrotic area) or low (less than 90% of necrotic area) chemotherapy response. Twenty-six patients with Ewing’s sarcoma and 24 with osteosarcoma had chemotherapy. The tumor vanished completely in 7 cases of Ewing’s sarcoma and in 1 of osteosarcoma, 12 tumors did not show any postchemotherapy changes of which 8 were osteosarcomas. According to Huvos, there were 23 Ewing’s sarcoma and 20 osteosarcoma Grade 1, 6 Ewing’s sarcoma and 4 osteosarcoma Grade 2, 5 Ewing’s sarcoma and 3 osteosarcoma Grade 3 and 3 Ewing’s sarcoma and 8 osteosarcoma Grade 4 of chemotherapy response.

Biochemical Markers

Our patients were children aged from 6 months to 18 years and according to their age, elevated erythrocyte sedimentation rate (ESR) were considered to be values over 12 mm/h. For elevated lactate dehydrogenase (LDH) serum concentration we considered values higher than 573 U/L in girls and 544 U/L in boys under 12 years of age; 497 U/L in boys and girls between 13 and 14 years of age; and 441 U/L in boys over 14 years of age and 427 U/L in girls over 14 years of age.

Follow up

The follow-up period ranged between 6 months and 12 years (median 47.5 months). Within this period, 20 patients with osteosarcoma and 17 with Ewing’s sarcoma were free of disease, 14 patients with osteosarcoma and 13 with Ewing’s sarcoma had metastases, 3 patients with osteosarcoma and 1 with Ewing’s sarcoma had local recurrence. During this time, 14 patients with Ewing’s sarcoma and 11 with osteosarcoma died of the disease. Of those who died, 4 patients had lung metastases at the time of diagnosis.

Statistical Analysis

We examined the following factors for prognostic significance: age of patient at diagnosis, gender of patient, volume of tumor, site of primary tumor, clinical stage, site of metastasis, mode of treatment, histologic response after preoperative chemotherapy, serum LDH concentration, ESR, and chondral differentiation in osteosarcomas. Survival was measured from the time of diagnosis to the date of death or last follow up. The probability of survival was calculated with Kaplan Meyer estimate and differences between the groups were tested with the log-rank test. Statistical significance level was set at p<0.05. For statistical analysis we used Statistica 6 for Windows (StatSoft, Inc., Tulsa, OK, USA).

Results

Clinical Data

For Ewing’s sarcoma the 1-, 3-, and 5-year overall survival rates were 88.2%, 70.5%, and 64.7%, respectively. For osteosarcoma 1-, 3-, and 5-year survival rates were 100%, 86.1% and 77.7%, respectively (Fig. 1). All results are reported in terms of survival. Survival did not correlate with age neither in Ewing’s sarcoma nor in osteosarcoma group. There was no survival difference between genders neither in osteosarcoma nor Ewing’s sarcoma (Table 1).

There was a statistically significant difference in the survival of patients who had metastases at the time of diagnosis (clinical stage 3) and those who had no metastases at time of diagnosis (clinical stage 2) in patients with osteosarcoma and Ewing’s sarcoma, whether or not they developed metastases later (p=0.004 and p=0.034, respectively; Fig. 2).

The survival was the same for those with bone and those with lung metastases in both sarcomas. We have not found a significant difference in the survival between patients without metastases and those who...
had developed metastases at least one year after the diagnosis (Table 1).

**Tumor Volume**

The volume of Ewing's sarcomas ranged from 1.5 cm³ to 20 cm³ (median 5.5 cm³). The volume of osteosarcomas ranged from 1.5 cm³ to 32 cm³ (median 8 cm³). There was no correlation between tumor volume and survival (Table 1).

**Tumor Site**

The most frequent location was the femur, in both Ewing's sarcoma (n=7) and osteosarcoma (n=20), followed by the tibia (n=2; n=11, respectively), ribs (n=4; n=1), humerus (n=3; n=1), and fibula (n=1; n=1). Ewing's sarcoma was more often located in the axial skeleton (n=11); the tumors were found in the scapula, iliac bone, zygomatic bone, mandible, and vertebra were all Ewing's sarcoma, whereas osteosarcoma was more often located in the extremities (just one was found in the maxilla and one in the rib). The location of the tumor did not correlate with survival in either of the sarcomas (Table 1).

**Treatment**

There was no difference in the survival between patients with different surgical treatment (amputation vs resection) (Table 1).

There was no difference in the survival in Ewing's sarcoma group or osteosarcoma group between patients who received preoperative chemotherapy and those who did not receive it. Additionally, we investigated the response of the tumor to chemotherapy regimens (4 in Ewing's sarcoma and 3 in osteosarcoma) and we did not find significant difference among different regiments of chemotherapy (Table 1).

Out of all patients, 8 patients with Ewing's sarcoma and 6 with osteosarcoma received postoperative radiotherapy as an additional treatment. All 12 patients previously received chemotherapy. There was no survival difference between patients who have been treated by additional irradiation and patients treated only by chemotherapy (Table 1).

**Histologic Response of Primary Tumor after Chemotherapy**

Overall survival analysis showed that Huvos grade was not significantly associated with prognosis neither in Ewing's sarcoma nor in osteosarcoma. When grouping according to high versus low-grade response, patients with high-grade response did not have significantly better prognosis for both sarcomas from the rest of the patients (Table 1).

**Biochemical Markers**

In analyzed sarcomas, the survival correlated with pretreatment ESR and LDH concentration. All patients who had elevated LDH concentration died within two years. The overall survival was significantly better for those patients who had normal LDH concentration (p=0.033 for patients with Ewing's sarcoma; p=0.015 for patients with osteosarcoma) (Fig. 3) and ESR (p<0.001 for patients with Ewing's sarcoma; p=0.014 for patients with osteosarcoma) (Fig. 4).

**Chondral Metaplasia**

Osteosarcomas showing some degree of chondral metaplasia did not have any better prognosis than the ones without chondral metaplasia (Table 1).

**Discussion**

Our study showed that the clinical stage, serum LDH concentration, and ESR significantly correlated with survival in both Ewing's sarcoma and osteosarcoma. Concerning the response to preoperative chemotherapy, patients having more than 99% of necrotic tumor showed a tendency toward significantly better prognosis but the results did not reach statistical significance. The age and gender of the patient, and location and volume of the tumor showed no correlation with prognosis of Ewing's sarcoma or osteosarcoma. Chondral metaplasia was evident in some osteosarcomas but had no prognostic value.

Different parameters have been evaluated as prognostic factors in malignant bone tumors. Age, gender, tumor location, and tumor necrosis following preoperative chemotherapy were most commonly evaluated in Ewing's sarcoma and osteosarcoma. The age of the patients presented with osteosarcoma was investigated in five studies (8,10,11,13,22) showing significance in four of them. One of the oldest studies (13) showed that the patients with osteosarcoma of
Analysis of blood variables such as lactate dehydrogenase found that those relationships were maintained. In two of Ewing's sarcoma (9,15) and in three studies of osteosarcoma (8,13,22) the tumor volume was found to be an adverse prognostic factor in three studies in osteosarcoma (8,11,23). Five of seven studies (8,22) and in one study of Ewing's sarcoma (11) yielded differing and partially inconsistent results. To make a prospective single institution study very complicated. Most of the published studies are based on retrospective data (10,11,14-18).

Our study confirmed the prognostic significance of factors such as clinical stage, LDH, and ESR as others have also found. These studies also suggested that the older age and male gender, axial location of tumor, and larger tumor volume are associated with poorer outcome. However, our study found no correlation between those factors and prognosis. This discrepancy with the results of previous studies could be due to the population heterogeneity but more likely to a small number of patients.

The chemotherapy protocols have evolved through years and the need for a new and reliable parameter to which therapy can be induced started to appear. Chemotherapy protocols are numerous and differ according to the institution experience and the recent achievement in this area (26-28,30,31). The evolution of diagnostic tools and therapy protocols paired with the relative small number of patients make a prospective single institution study very complicated. Most of the published studies are based on retrospective data (10,11,14-18).

Despite of the fact that advances in therapy of osteosarcoma and Ewing's sarcoma increase the overall survival, still a substantial number of patients will soon develop a metastatic disease and/or respond weakly to chemotherapy, with the result of poor outcome. This has stimulated interest in the identification of early prognostic factors possibly guiding the therapeutic protocol from the onset. Elevated erythrocyte sedimentation rate and high LDH concentration seem to be of importance in both sarcomas that we analyzed no matter how different in pathogenesis or pathohistology they are. As it is obvious from the literature a plethora of different parameters has been analyzed by different groups on different patient groups yielding differing and partially inconsistent results. To our knowledge there is no study including all these potential prognostic factors for both osteosarcoma and Ewing's sarcoma patients. Therefore, this retrospective study represents the basis for a large-scale prospective study including all potential classical

![Figure 4](image-url)
blood parameters as well as the promising new ones such as Rb LOH (Loss of heterozygosity of the retino-blastoma gene) (25).

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