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Markers of bone formation and resorption in patients with mixed subtype osteosarcoma

Joanna Gajewska[1], Jadwiga Ambroszkiewicz[1], Teresa Klepacka[2], Magdalena Rychłowska-Pruszyńska[3], Teresa Laskowska-Klita[1]

Introduction. Several studies have indicated the clinical utility of specific bone turnover markers in patients with bone tumors, among them children with osteosarcoma both in the course of diagnosis and for monitoring therapy. According to the histological classification there are different subtypes of osteosarcoma. Among them the most frequent is the conventional mixed subtype in which different patterns are represented.
Aim. The aim of this study was to investigate selected bone turnover markers in patients with mixed subtype osteosarcoma during clinical treatment.
Subjects and methods. We studied 23 patients (7 girls, 16 boys) at the age 5-20 years with mixed subtype osteosarcoma. The tested group consisted of 12 patients with favourable (regression) and 11 patients with unfavourable (progression) prognosis. The levels of osteocalcin (OC), bone alkaline phosphatase (BALP) and the C-terminal cross-linking telopeptide of type I (CTX) collagen were measured in serum using ELISA kits at diagnosis, during preoperative and postoperative chemotherapy and after treatment.
Results. Values of bone turnover markers in patients with favourable and unfavourable prognosis at the moment of biopsy were similar. During chemotherapy and before treatment the values of OC, BALP and CTX were lower. However, in patients with unfavourable prognosis, as compared to those with favourable prognosis, the levels of tested parameters were higher. After clinical treatment the bone formation markers were 3-fold higher and the bone resorption marker was 2-fold higher in patients with progression, as compared to those with remission. The obtained differences between both groups were statistically significant for osteocalcin, bone alkaline phosphatase and the C-terminal cross-linking telopeptide of type I collagen - $p<0.05$, $p<0.02$, $p<0.002$, respectively.
Conclusions. Our results suggest, that bone turnover markers may be useful in the monitoring and in the assessment of the efficacy of therapy in children with mixed subtype osteosarcoma. However, further studies on bone metabolism are necessary to confirm the prognostic values of bone formation and resorption markers in different subtypes of osteosarcoma.

Key words: bone turnover markers, osteosarcoma, mixed subtype osteosarcoma

Introduction

Bone structure, adapted to meet the needs of human mechanics, depends mainly upon the bone turnover, which occurs throughout the entire life cycle. This is a modulating process, which allows to develop correct bone architecture capable of supporting weight and to repair microlesions. Studies aimed at searching for markers of bone turnover have drawn attention to protein molecules and their degradation products are released during bone turnover. They are said to reflect the activity of all turnover processes, which are active over a period of time throughout the entire skeleton, and provide a lot of valuable data useful both in monitoring the treatment of bone diseases and in predicting treatment outcome [1, 2]

Malignant tumors and bone metastases are often accompanied by imbalance in bone turnover in the form of increased bone formation and/or resorption. Until now the prognostic value of these markers had been evaluated in adult patients with malignancies that metastasize to the bones [3-5]. Bone metastases are associated with bone marrow infiltration and expansion of cancer cells, which in turn activates osteoclasts and promotes osteolysis and/or osteosclerosis [6]. These processes are accompanied by altered concentrations of markers of bone formation and resorption, the most important of which are bone alkaline phosphatase (BALP), osteocalcin (OC) and C-terminal cross-linking telopeptide of type I collagen (CTX) [7]. Increased BALP and OC activity has been observed in breast and prostate cancer patients with bone metastases

[1] Department of Clinical Biochemistry
[2] Department of Pathomorphology
[3] Clinical Department of Paediatric Oncological Surgery
Institute of Mother and Child, Warsaw, Poland

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[8, 9]. Marchei et al. have reported the high sensitivity and specificity of BALP in breast cancer patients, as its increased concentration would precede the development of scintigraphy-discernible bone metastases by a couple of months [8]. In the case of prostate cancer patients CTX serum concentrations were increased by 25% in metastases-free individuals and were 4 times higher in individuals with bone metastases, as compared to the control group [9]. The analysis of cross-linking telopeptides seems to be especially useful, as the levels of both CTX and NTX (N – terminal cross-linking telopepticle of type cologen) correlate positively with the number of metastatic lesions and with cancer progression [10, 11].

Our studies concerning the usefulness of the markers of bone formation and resoprtion confirm that they are good markers of disease progression in the monitoring of patients with mixed subtypes of osteosarcoma [12, 13]. Increased serum BALP activity and CTX and OC concentrations may be associated with poor prognosis in these patients. However these studies had been conducted on patients with bone sarcomas belonging to different histologic subtypes. It is well known that osteosarcomas are a histologically varied group, multipotential as to the differentiation of mesenchymal stroma. A characteristic feature of importance in tumor diagnostics is osteoid and/or bone production directly from the sarcomatic mesenchymal stroma without cartilage involvement. Thus, there has developed a whole classification system of osteosarcoma subtypes depending upon tumor histology. Among these the most common is the conventional mixed subtype, in which different structural elements appear alongside [14].

The aim of this study was to evaluate selected markers of bone turnover in the course of treatment in patients with the most common, histologically conventional, mixed subtype of osteosarcoma.

Material and methods

The studied group consisted of 23 patients (7 girls, 16 boys) aged between 5 and 20 years with a conventional, mixed form of osteosarcoma treated at the Clinical Department of Paediatric Oncological Surgery at the Institute of Mother and Child in Warsaw between the years 1999-2004. The patients were divided into 2 subgroups:

- a subgroup with good prognosis with disease remission (12 children),
- a subgroup with poor prognosis due to disease progression during treatment (11 children).

The patients were administered preoperative chemotherapy, underwent surgery and then were administered postoperative chemotherapy. Chemotherapy had been administered according to an EORTC (adriamycin, cis-platin) or SFOP (adriamycin, methotrexate, etopozide and ifosfamide) protocols. All the patients were evaluated for the concentration of bone formation and resorption markers before treatment onset, during pre- and postoperative chemotherapy and after the termination of treatment. The study protocol had been approved by the ethics committee of Institute of Mother and Child.

Venous blood had been collected from fasting patients in the morning hours. The serum obtained by centrifugation (300xg, 4°C, 10 min) was frozen in the temperature of -20°C until analysis (always under 2 months). OC and CTX measurements were performed immunoenzymatically using the N-mid Osteocalcin One Step Elisa kit and the Serum CrossLaps One Step Elisa kit (Osteometer Bio Tech, Denmark). BALP activity was evaluated using the immunoenzymatic ELISA method with the Alkphase-B kit (Metra Biosystem, USA). Statistica 6.0 software had been used for statistical analysis. Results are presented as median and range (25%-75%). The significance of the differences was tested with the Mann-Whitney test. The significance level was set at $p<0.05$.

Results

Changes of the parameters of bone turnover throughout the treatment course in relation to prognosis have been presented in Figures 1, 2 and 3.

At the time of biopsy the concentration of osteocalcin was similar in patients with good and poor prognosis (Figure 1). In the course of preoperative chemotherapy its serum concentrations fell by 30-35% in both the groups. During postoperative chemotherapy these values were increased by 15% in patients with good prognosis and by as much as 30% in patients with poor prognosis. After the termination of treatment patients with good prognosis reported with a similar concentration of osteocalcin (median 44.2 μg/L), while in the poor prognosis group this value was approx. three-fold higher (median 122.8 μg/L; $p < 0.05$)

In the course of preoperative treatment BALP activity decreased by 40%, as compared to the values at diagnosis, and did not undergo any significant changes in the course of adjuvant chemotherapy. However, after treatment completion BALP activity was 3 times higher in the poor prognosis group (median 104,8 U/L; $p<0.02$) than in the good prognosis group (median 37.5 U/L) (Figure 2).

CTX levels decreased by approx. 85% in the course of preoperative chemotherapy in patients with good prognosis, then, during postoperative treatment they increased by some 15% and remained at that level after treatment completion. In the case of patients with poor prognosis the decrease during preoperative chemotherapy was much less significant, approx. 30%, while the increase during postoperative chemotherapy was also approx. 30%. After the completion of treatment patients with progression reported with twofold values of the marker of bone resorption (median: 15528 pmol/L; $p<0.002$) as compared to patients with remission (median: 8195 pmol/L) (Figure 3).

Discussion

We have presented two groups of children with osteosarcoma – with good and poor prognosis (the latter have suffered from disease progression during treatment). We have analyzed the dynamic changes of bone turnover markers before, during and after treatment. The concentrations of OC, BALP and CTX before treatment did not differ significantly between the two groups. In

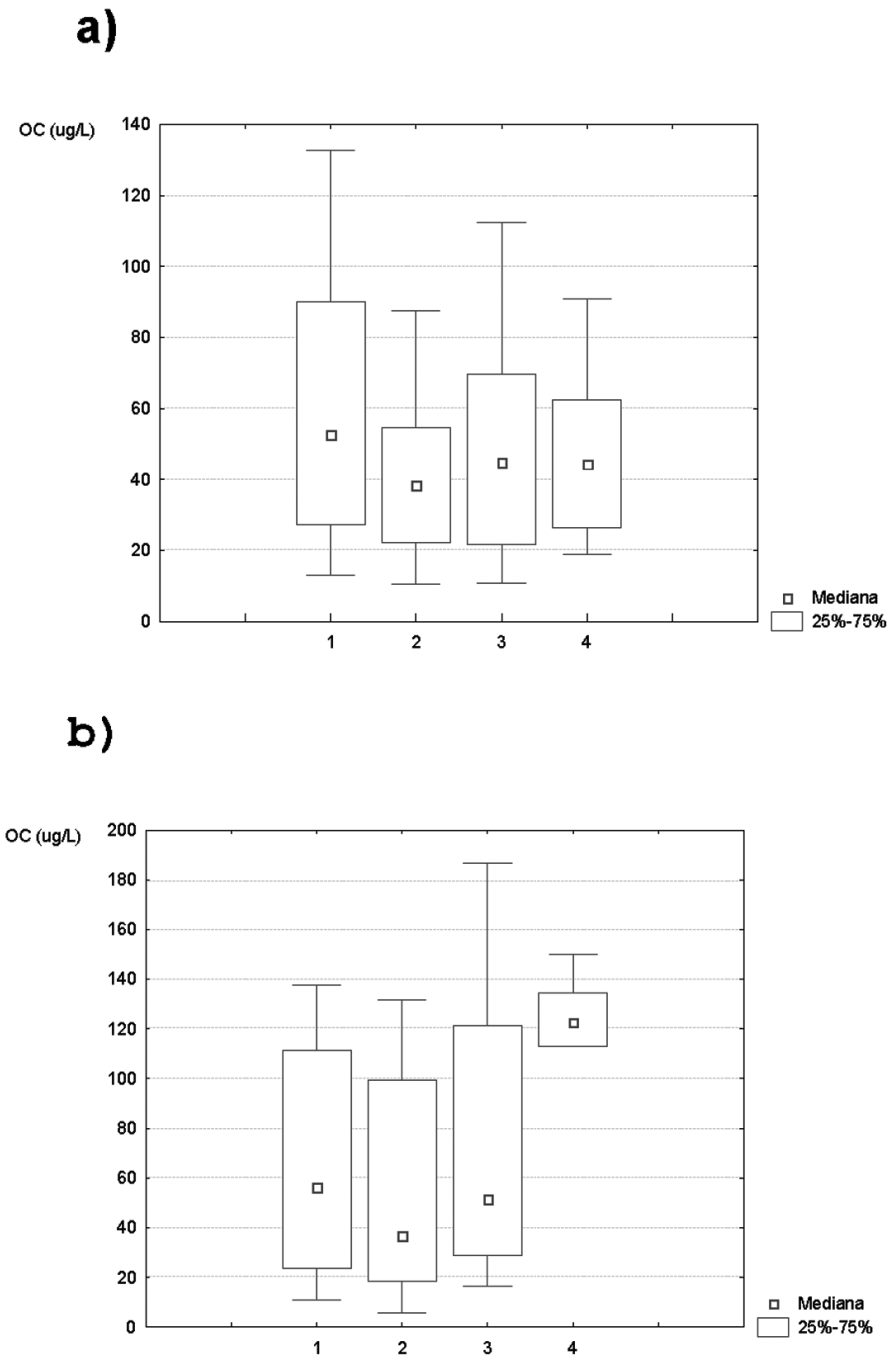

Figure 1. The concentration of OC in patients with favourable (a) and unfavourable (b) prognosis during diagnosis (1), preoperative chemotherapy (2), postoperative chemotherapy (3) and after treatment (4)

the course of preoperative chemotherapy and during its postoperative phase the levels of all the 3 markers of bone metabolism decreased significantly in a majority of patients, although, commonly, they were higher in patients with poor prognosis, as compared to the patients with good prognosis. The greatest differences between the groups were observed after treatment completion. In patients with progression the levels of the 2 markers of bone formation were three times as high, while the level of CTX was twice as high, as compared to patients with remission. Literature data shows that patients with prostate, lung, breast cancer and with Ewing's sarcoma present an increase in BALP activity [8, 10, 15, 16]. Fontana et al. [9] and Jung et al. [17] have shown that in patients with prostate cancer high BALP activity correlate with bone metastases. It has also been reported that the activity of this enzyme correlates positively not only with the number of radiologically confirmed metastatic sites, but also with the degree of disease advancement [8, 16, 18]. These observations and own results obtained from osteosarcoma patients suggest that in these cases the process of bone formation may be increased, all the more so because the second marker of bone formation – osteocalcin – appears to play a diagnostic role in

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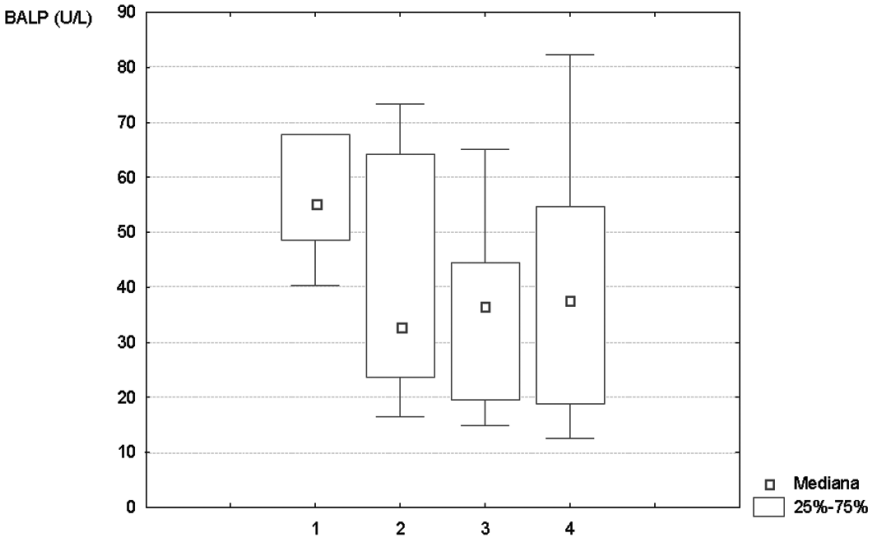

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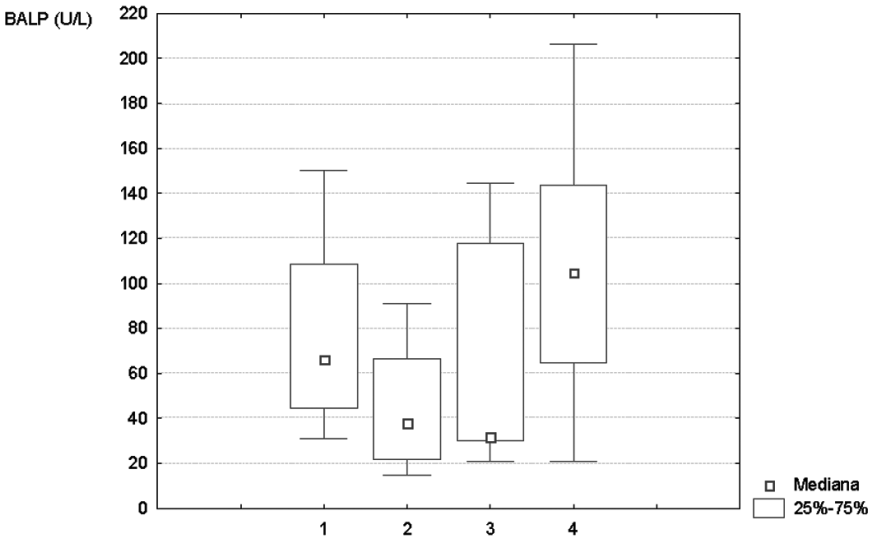
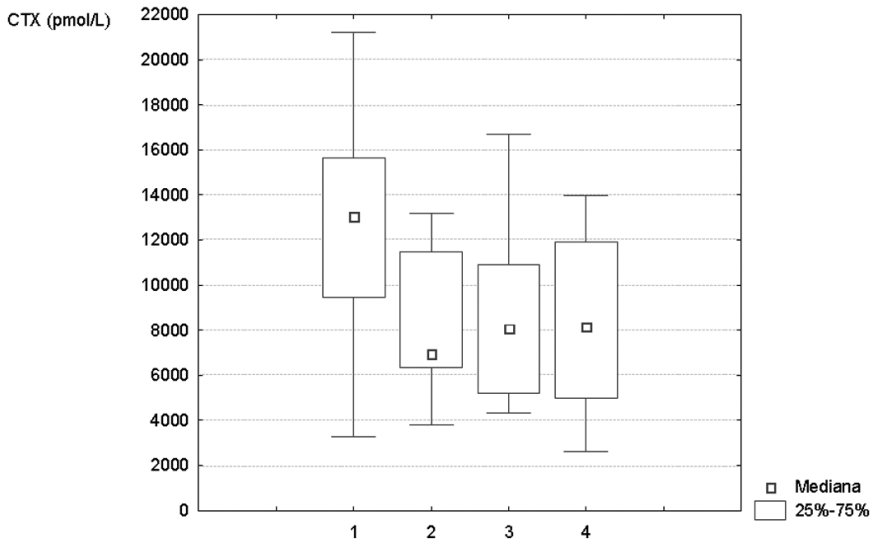


Figure 2. The activity of BALP in patients with favourable (a) and unfavourable (b) prognosis during diagnosis (1), preoperative chemotherapy (2), postoperative chemotherapy (3) and after treatment (4)

osteosarcoma progression. In the course of previous immunohistochemical studies Fanburg et al. [19] and Nagoya et al. [20] have postulated the usefulness of BALP and OC in the diagnosis of osteosarcoma.

There are also discussions in literature concerning the prognostic value of bone resorption markers. It seems that their increased secretion is the effect of intensified resorption induced by the neoplastic process. In adult patients with primary tumors of the prostate, lung, breast, kidney, throat and the alimentary tract the markers of bone resorption are elevated [8, 15, 21-23]. This is especially true in patients with metastases, in whom CTX concentration may be almost 4 times as high as in the control group [9]. Berruti et al. [10] and Demers et al. [11] have shown that the bone resorption markers correlate positively with the number of metastatic sites and with tumor progression. Body et al. [24] suggest that the evaluation of the efficacy of anticancer treatment using the markers of bone resorption may be faster and more specific than using conventional radiological and scintigraphic techniques. A decrease in the concentration of these markers is a symptom of a positive reaction to treatment, while their increase suggests disease progression [25]. This is consistent with our findings, both

a)



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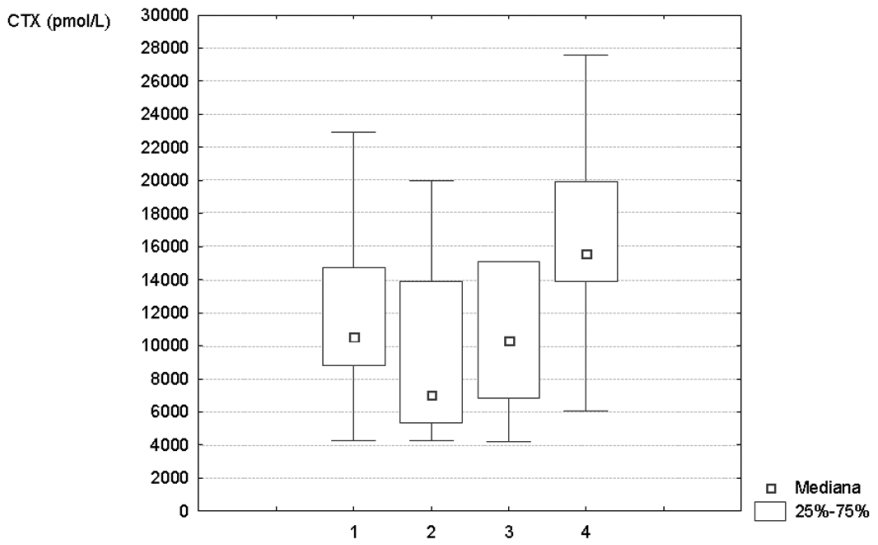

Figure 3. The concentration of CTX in patients with favourable (a) and unfavourable (b) prognosis during diagnosis (1), preoperative chemotherapy (2), postoperative chemotherapy (3) and after treatment (4)

previous and those presented in this paper, i.e. regarding patients with conventional mixed type osteosarcoma [12]. Therefore it is possible that bone resorption markers may have a prognostic value in children with osteosarcoma, as is the case with adult osteosarcoma patients. These observations correspond with those of Ferrari et al. [26], who have found a higher secretion of bone resorption markers in urine in osteosarcoma patients. They have suggested that in children with osteosarcoma intense bone resorption may be associated with the degree of tumor aggression. The prognostic value of bone turnover markers in children and adolescents with different subtypes of osteosarcoma has never been the subject of systematic studies. The data presented above shows that the values of both formation and resorption markers in patients with a mixed subtype of osteosarcoma often accompanies disease progression, while their decrease may suggest a positive reaction to clinical treatment. These markers could therefore be used not only in the course of anticancer therapy in order to assess the response to treatment but also after the completion of treatment as a part of routine follow-up in order to discern early stage metastatic tumors. However only further studies on bone metabolism performed both on

cases of mixed subtype and in other subtypes of osteosarcoma may allow to assess the prognostic value of bone formation and resorption markers.

Conclusions

1. Anticancer treatment administered to children and adolescents with conventional mixed subtype osteosarcoma may decrease the rate of bone turnover due to the inhibition bone formation as well as bone resorption processes.
2. In patients with conventional, mixed subtype osteosarcoma high values of bone formation and resorption markers during and after treatment are associated with poor prognosis and may be a sign of disease progression.

Joanna Gajewska PhD
Department of Clinical Biochemistry
Institute of Mother and Child
Kasprzaka 17a, 01-211 Warsaw, Poland
e-mail:biochem@imid.med.pl

References

1. Szulc P, Seeman E, Delmas PD. Biochemical measurements of bone turnover in children and adolescents. *Osteoporos Int* 2000; 11: 281-94.
2. Seibel MJ. Molecular markers of bone turnover: biochemical, technical and analytical aspects. *Osteoporos Int* 2000; Suppl 6: 18-29.
3. Vinholes J, Coleman R, Eastell R. Effects of bone metastases on bone metabolism: implication for diagnosis imaging and assessment of response to cancer treatment. *Cancer Treat Rev* 1996; 22: 289-331.
4. Souberbielle JC, Cormier C, Kindermans C. Bone markers in clinical practice. *Curr Opin Rheumatol* 1999; 11: 312-9.
5. Seregeni E, Martinetti A, Ferrari L et al. Clinical utility of biochemical marker of bone remodelling in patients with metastases of solid tumors. *Q J Nucl Med 2001*; 5: 7-17.
6. Goltzman D. Osteolysis and cancer. *J Clin Invest* 2001; 107: 1219-20.
7. Demers L.M. Biochemical markers in the menagement of patients with metastatic bone disease. *Clin Chem* 1999; 45: 1131-2.
8. Marchei P, Santini D, Bianco V et al. Serum ostase in the follow-up of breast cancer patients. *Anticancer Res* 1995;15: 2217-2222.
9. Fontana A., Delmas P.D.: Markers of bone turnover in bone metastases. *Cancer* 2000; 88: 2952-60.
10. Berruti A, Dogliotti L, Gorzegno G et al. Differential patterns of bone turnover in relation to bone pain and disease extent in bone in cancer patients with skeletal metastases. *Clin Chem* 1999; 45: 240-7.
11. Demers L.M., Costa L., Lipton A. Biochemical markers and skeletal metastases. *Cancer* 2000; 88: 2919-26.
12. Ambroszkiewicz J, Gajewska J, Laskowska-Klita T et al. Biochemiczne wskaźniki procesu resorpcji kości u dzieci z osteosarcoma. *Med Wieku Rozwoj* 2004; 8: 225-33.
13. Gajewska J, Ambroszkiewicz J, Rychłowska-Pruszynska M et al. Markery kościotworzenia u dzieci z osteosarcoma. *Med Wieku Rozwoj* 2004; 8: 235-43.
14. Bertoni F, Bacchini P. Classification of bone tumors. *Eur J Radiol* 1988; 27: 74-6.
15. Plebani M, Bernardi D, Zaninotto M et al. New and traditional serum markers of bone metabolism in the detection of skeletal metastases. *Clin Biochem* 1996; 29: 67-72.
16. Alatas F, Alatas O, Metintas M et al. Usefulness of bone markers for detection of bone metastases in lung cancer patients. *Clin Biochem* 2002; 35: 293-6.
17. Jung K, Lein M, Stephan C et al. Comparison of 10 serum bone turnover markers in prostate carcinoma patients with bone metastatic spread: diagnostic and prognostic implications. *Int Cancer* 2004; 111: 783-91.
18. Berruti A, Piovesan A, Torta M et al. Biochemical evaluation of bone turnover in cancer patients with bone metastases: relationship with radiograph appearances and disease extension. *Br J Cancer* 1996; 73: 1581-7.
19. Fanburg JC, Rosenberg AE, Weaver DL et al. Osteocalcin and osteonectin immunoreactivity in the diagnosis of osteosarcoma. *Am J Clin Pathol* 1997; 108: 464-73.
20. Nagoya S, Uede T, Wada T et al. Detection of bone-type alkaline phosphatase by monoclonal antibodies reacting with human osteosarcoma-associated antigen. *Jpn J Cancer Res* 1991; 82: 862-70.
21. Kir Z.O, Oner P, Iyidogan YO et al. Serum prolidase I activity and some bone metabolic markers in patients with breast cancer: in relation to menopausal status. *Clin Biochem* 2003; 36: 289-94.
22. Noguchi M, Yahara J, Noda S. Serum levels of bone turnover markers parallel the results of bone scintigraphy in monitoring bone activity of prostate cancer. *Urology* 2003; 61: 993-8.
23. Vinholes J, Coleman R, Eastell R. Effects of bone metastases on bone metabolism: implication for diagnosis imaging and assessment of response to cancer treatment. *Cancer Treat Rev* 1996; 22: 289-331.
24. Body JJ, Dumon JC, Gineyts M et al. Comparative evaluation of markers of bone resorption in patients with breast cancer-induced osteolysis before and after bisphosphonate therapy. *Br J Cancer* 1997; 75: 408-12.
25. Coleman RE. Monitoring of bone metastases. *Eur J Cancer* 1998; 34: 252-9.
26. Ferrari S, Zolezzi C, Pratelli L et al. Urinary excretion of pyridinium cross-links and serum osteocalcin levels in patients with primary high-grade osteosarcoma. *Calcif Tissue Int* 2003; 73: 1-4.

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