Alkaline Phosphatase Level Change in Patients with Osteosarcoma: its Role as a Predictive Factor of Tumor Necrosis and Clinical Outcome

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ABSTRACT: **Background:** In osteosarcoma the histological response, measured by the percentage of tumor necrosis, constitutes one of the most significant predictive factors, with better survival in patients whose tumor necrosis is ≥ 90%.

Objectives: To determine if the decrease rate of serum alkaline phosphatase (SAP) levels during the first month of neoadjuvant chemotherapy could serve as a predictive indicator of tumor necrosis and clinical outcome.

Methods: We analyzed the medical files of 53 osteosarcoma patients (19 females, 34 males) (median age 16 years, range 8–24); the disease was metastatic in 12 and localized in the other 41.

Results: The histological responses were good in 38 patients (71.7%) and poor in 15 (28.3%). At a median follow-up of 50 months, 34 patients (64.2%) had no evidence of disease and 19 (35.8%) had died from the disease. High levels of SAP at diagnosis correlated with worse survival ($P = 0.002$). There was no difference in overall survival between patients whose SAP decrease rate was › 25% and those with a rate ‹ 25% ($P = 0.14$). Among female patients, "rapid" SAP responders had better survival than "slow" responders ($P = 0.026$). In patients with metastases the SAP decrease rate was positively correlated with survival ($P = 0.042$).

Conclusions: There was no evidence that "rapid" SAP responders had a higher percentage of tumor necrosis than "slow" responders, although female "rapid" SAP responders had a better prognosis than "slow" responders. Patients with metastases at presentation and "rapid" SAP response had better prognoses.

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KEY WORDS: alkaline phosphatase, necrosis, osteosarcoma, survival

Osteogenic sarcoma is the most common malignant bone tumor in children and young adults [1,2]. The majority of newly diagnosed patients are treated according to national or international protocols using a common therapeutic strategy comprising neoadjuvant chemotherapy with subsequent resection of tumor and adjuvant chemotherapy. Such a strategy enables determination of histological response to a given chemotherapy regimen. Only four chemotherapeutic agents used in the treatment of OS patients have been widely recognized in the last few decades as sufficiently efficacious: doxorubicin, cisplatin, ifosfamide, and high dose methotrexate [3,4]. Current disease-free survival in patients with non-metastatic OS is approximately 65%–70% [1,5]. Prognosis is significantly worse in patients with metastatic disease, with lung metastases being the most common [6]. Metastatic disease is considered the most significant prognostic factor in OS patients at presentation [6-9]. The well-recognized predictive factors are the percentage of necrosis as determined after neoadjuvant chemotherapy and completeness of tumor resection [10,11]. It is well known that patients with histological response to neoadjuvant chemotherapy (> 90% necrosis) have better chances of survival. Unfortunately, this predictive factor becomes relevant only after tumor resection.

Various patient and tumor characteristics have been evaluated as potential markers for predicting good or poor histological response to neoadjuvant chemotherapy, both clinically and in the laboratory. Among these, age [12], tumor volume [13], location [14], and levels of serum alkaline phosphatase [15,16] and lactate dehydrogenase [17,18] were found in several studies to be significant.

We are aware that a significant portion of newly diagnosed patients with osteogenic sarcoma have SAP levels within the normal range, but the SAP dynamics following neoadjuvant chemotherapy have not been properly investigated. The aim of our study was to determine if the rate of decrease of SAP during the first month of neoadjuvant chemotherapy could serve as a predictive indicator of histological response (measured by the percentage of tumor necrosis) and clinical outcome.

OS = osteogenic sarcoma
SAP = serum alkaline phosphatase

PATIENTS AND METHODS

We conducted a retrospective analysis of the medical files of 53 patients diagnosed with OS of the bones and treated at Meyer Children's Hospital (Rambam Health Care Campus) in Haifa or at Hadassah Medical Center in Jerusalem. Relevant demographic, clinical and laboratory data are summarized in Table I. The following criteria were used for inclusion in the analysis: biopsy-proven high grade OS (both localized and metastatic at presentation), age up to 25 years, known SAP and LDH levels before commencement of neoadjuvant chemotherapy and during therapy, known percentage of necrosis measured in all patients after resection of the primary tumor, and hepatic function tests within normal range. The study was approved by the local ethics committees of both hospitals.

LDH = lactate dehydrogenase

There were 34 males and 19 females. Median age was 16 years (range 8–24 years). At presentation 41 patients had localized OS and 12 had metastatic disease (11 with pulmonary metastases and 1 with bone and brain metastases). In all patients, a complete history was documented, and thorough physical examination, magnetic resonance imaging of the primary tumor, chest computed tomography, and bone scan with Tc99m for metastatic checkup were performed. SAP and LDH levels were determined before neoadjuvant chemotherapy as part of the routine laboratory evaluation in all patients. Neoadjuvant chemotherapy was administered according to three different protocols: EUROAMOS, AOST 0331 protocol (MAP regimen), POG/CCG Pilot Intergroup study (P9754), Pilot 1 with doxorubicin intensification without ifosfamide and Pilot 2 with doxorubicin intensification and ifosfamide. All patients were treated with chemotherapy consisting of high dose methotrexate (12 g/m^2), doxorubicin (75 mg/m^2) and cisplatin (120 mg/m^2)

Table 1. Patient data

No/ Gender	Age at diagnosis (yr)	Location of primary	Metastatic status at diagnosis	% necrosis	SAP at diagnosis	SAP after 1 month	SAP after 2 months	SAP after 3 months	LDH at diagnosis	Follow- up (months)	Clinical outcome
1/M	19	Tibia	Bone, bone marrow, brain	NA	1261	953	2020	NA	1437	2	DOD
2/F	14	Skull	No	100	143	151	124	115	481	130	NED
3/F	18	Fibula	Lung	100	117	57	50	52	168	126	NED
4/F	10	Femur	Lung	95	204	165	104	129	249	38	DOD
5/F	18	Femur	No	85	155	88	72	106	256	116	NED
6/M	12	Femur	No	95	265	142	120	136	201	115	NED
7/M	11	Femur	No	100	182	103	68	99	232	99	NED
8/F	22	Femur	No	100	202	111	83	109	431	98	NED
9/F	17	Head	No	20	1525	195	123	94	260	41	DOD
10/M	9	Head	No	15	178	183	152	158	164	88	NED
11/M	16	Humerus	No	95	288	164	97	137	200	91	NED
12/M	16	Femur	No	90	348	NA	NA	NA	456	81	NED
13/M	22	Femur	No	100	136	133	NA	NA	293	81	NED
14/F	8	Tibia	No	93	303	179	146	NA	269	94	NED
15/F	22	Radius	No	90	93	NA	NA	NA	89	71	NED
16/M	19	Femur	No	98	91	101	NA	NA	103	31	DOD
17/F	23	Femur	No	99	145	92	64	69	163	56	NED
18/M	14	Femur	No	100	245	167	183	184	180	52	NED
19/M	16	Femur	No	95	127	101	139	160	258	35	DOD
20/F	12	Tibia	No	100	219	148	120	102	221	50	NED
21/M	12	Humerus	Lung	100	975	165	142	123	462	48	NED
22/M	18	Pelvis	No	87	2507	1349	903	1598	261	23	DOD
23/M	12	Femur	No	99	163	78	72	75	225	50	NED
24/M	19	Mandible	No	5	134	112	114	110	214	51	NED
25/M	16	Femur	No	100	282	426	162	121	178	40	NED
26/F	13	Tibia	No	20	172	113	109	84	129	36	NED

No/ Gender	Age at diagnosis (yr)	Location of primary	Metastatic status at diagnosis	% necrosis	SAP at diagnosis	SAP after 1 month	SAP after 2 months	SAP after 3 months	LDH at diagnosis	Follow- up (months)	Clinical outcome
27/M	21	Pelvis	No	90	1355	347	172	NA	319	16	DOD
28/F	18	Femur	No	99	517	102	121	116	371	23	AWD
29/M	16	Femur	Lung	95	297	163	124	169	229	30	DOD
30/M	17	Femur	No	97	349	95	86	NA	402	28	NED
31/M	12	Femur	No	97	1244	240	141	146	248	17	NED
32/M	15	Femur	Lung	60	548	491	969	128	210	20	DOD
33/M	9	Femur	Lung	5	5658	983	214	1881	2376	35	DOD
34/M	24	Femur	No	90	143	81	81	134	418	77	NED
35/M	15	Tibia	No	100	129	124	78	95	496	72	NED
-36/F	13	Femur	No	90	1054	165	74	40	1548	71	NED
37/M	8	Femur	No	100	224	140	94	98	1879	63	NED
38/M	15	Femur	No	90	239	106	116	79	531	110	NED
39/M	22	Tibia	No	100	253	86	87	NA	587	7	DOD
40/F	14	Femur	Lungs	90	514	477	74	NA	2176	26	DOD
41/F	11	Femur	Lungs	100	308	115	81	102	635	72	NED
42/M	9	Femur	No	20	252	138	98	193	634	65	NED
43/M	17.5	Femur	No	95	129	99	100	104	876	54	NED
44/F	17.5	Tibia	No	95	122	87	73	89	346	42	NED
45/F	21.5	Humerus	No	95	153	95	86	75	463	89	NED
46/M	18	Femur	No	40	73	66	60	82	395	81	NED
47/F	14.5	Pelvis	Lungs	NA	5670	872	67	79	2231	3	DOD
48/M	13	Tibia	No	95	371	188	168	212	1244	36	DOD
49/M	9	Femur	No	70	233	159	111	297	844	9	DOD
50/F	23	Femur	No	70	151	110	76	101	584	50	DOD
51/M	17	Humerus	No	100	201	97	76	70	544	115	NED
52/M	16.5	Humerus	Lungs	NA	1139	913	213	93	665	14	DOD
53/F	17	Humerus	Lungs	NA	1951	3154	935	1149	1678	6	DOD

NED = no evidence of disease, DOD = dead of disease, SAP = serum alkaline phosphatase, NA = not available

with or without the addition of ifosfamide (14 g/m^2) depending on the protocol used for the given child.

SAP changes during neoadjuvant chemotherapy are shown in Figure 1A. Depending on the rate of SAP decrease after the start of neoadjuvant chemotherapy, patients were divided into two groups: those whose SAP level had decreased > 25% and those with < 25% decrease after the first month of therapy [Figure 1B]. The first group was designated "rapid" responders and the second as "slow" responders.

STATISTICAL ANALYSIS

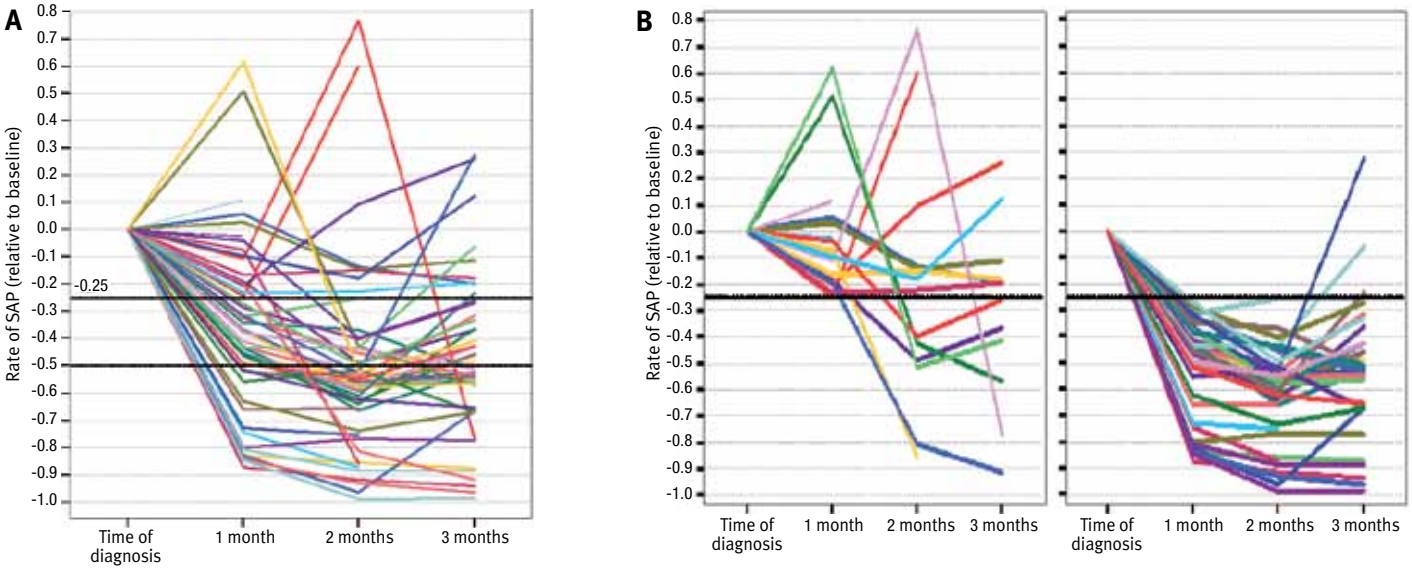
The decrease in SAP rate was calculated by dividing SAP level after every month (1st, 2nd, 3rd) by SAP level before treatment less 1. Hence, a rate decrease of -0.25 corresponds with a 25% decrease in SAP level. A comparison of the proportions was done using the Pearson chi-square test or Fisher's exact test, distributions of continuous variables by the Mann-Whitney test, and survival probabilities by the Kaplan-Meier method (log-rank test). A significance level of $P < 0.05$ was assumed to denote difference. Data analyses were performed using the SPSS 15.0 statistical software package (SPSS Inc, Chicago, IL, USA).

RESULTS

At a median follow-up of 50 months, 34 patients (64.1%) had no evidence of disease (median follow-up for NED was 72 months), 18 (34%) had died of disease, and 1 (1.9%) was alive with disease. For the purpose of analysis, DOD patients were combined with the alive-with-disease patient and designated the DOD group. There was no difference in survival between NED males and NED females: 22/34 (64.7%) and 12/19 (63.16%) respectively (not significant). Among patients with an

NED = no evidence of disease
DOD = died of disease

Figure 1. [A] Dynamics of SAP rate decrease after start of neoadjuvant chemotherapy, **[B]** Dynamics of SAP changes in two groups of patients: the "slow" responders (with no determined pattern, left figure), and the "rapid" responders (with a determined pattern, right figure)



elevated SAP level at diagnosis, survival was worse than among patients with a normal SAP level at diagnosis ($P = 0.002$).

There were 39 patients (73.6%) with good histological response (≥ 90% necrosis after neoadjuvant chemotherapy) and 14 (26.4%) with poor histological response (< 90% necrosis). Patients with initial metastatic disease were evenly distributed between the good and bad histological response groups. The mean age of patients with good histological response was 15.9 ± 4.3 years, those with poor response 14.9 ± 4.7 years (not significant).

There was an approximately equal gender distribution in both histological response groups, with two-thirds males in each group, and this correlated with gender distribution in this study (24/34 males and 13/19 females, 70.6% and 68.4% respectively, had good histological response). In the group with poor histological response were four children who did not undergo resection of their primary tumor due to progression of disease on neoadjuvant chemotherapy. This progression during therapy before definitive surgery indicates poor or even lack of response to chemotherapy. These four patients were diagnosed with metastatic disease at presentation (one with bone and brain metastases and the other three with lung metastases). There was also no difference in histological response between males and females ($P = 0.835$).

When we looked at the influence of SAP on survival, we found no significant difference in the decrease rate of SAP during the first month of neoadjuvant chemotherapy between the NED and DOD groups ($P = 0.14$) [Figure 2A]. Age did

Figure 2. [A] Overall survival according to pattern of SAP changes after start of neoadjuvant chemotherapy, **[B]** Overall survival according to pattern of SAP dynamics after start of neoadjuvant chemotherapy in patients with and without metastatic disease at presentation

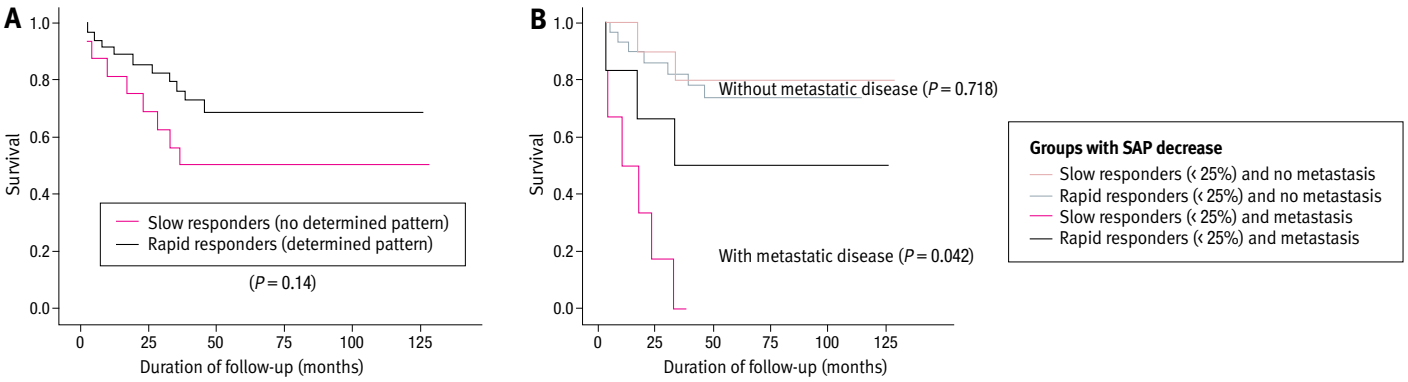
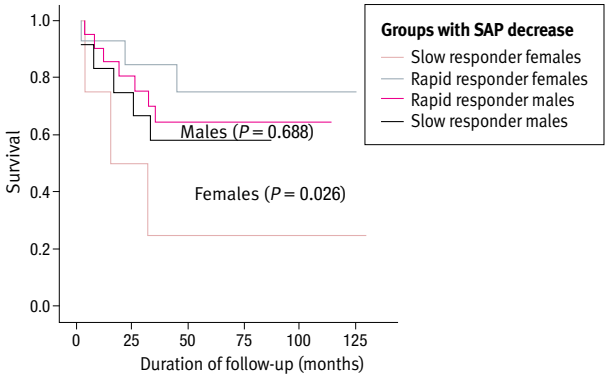


Figure 3. Overall survival according to pattern of SAP dynamics after start of neoadjuvant chemotherapy in male and female patients



not influence the SAP decrease rate. Regarding the influence of gender on correlation between SAP decrease rate and survival, we found that female "rapid" SAP responders had better survival than "slow" responders ($P = 0.026$). Such a correlation could not be seen in male patients [Figure 2B].

We were unable to show any statistical correlation between the rate of SAP decrease and percentage of necrosis on survival. Only in the subgroup of patients with metastatic disease at presentation was the rapid SAP decrease rate positively correlated with survival ($P = 0.042$) [Figure 3].

DISCUSSION

The most significant prognostic factor currently enabling physicians to predict the outcome of patients with osteogenic sarcoma is metastatic status at diagnosis, while histological response to neoadjuvant chemotherapy is the most important predictive factor. There is concern that patients whose tumors are relatively chemoresistant may develop distant metastases originating from the primary if these primaries are left in place for prolonged periods. It is plausibly explained that the commonly used therapeutic approach for such patients, i.e., neoadjuvant chemotherapy with subsequent definitive surgery, puts them at higher risk for developing metastatic spread, as their disease remains effectively untreated until surgery. In addition, we still do not have efficient second-line chemotherapy for the timely correction of presumed unresponsiveness to neoadjuvant chemotherapy. In such a situation, the only logical action is to proceed to tumor resection at an earlier stage of treatment, thereby diminishing the probability of metastatic dissemination during the ineffective neoadjuvant treatment phase.

Determination of reliable prognostic factors based on tumor characteristics at the time of initial diagnosis may facilitate the selection of those patients who should be offered resection of the primary tumor as a first step in their treatment. This will potentially decrease the chance of developing metastatic disease originating from a long-standing primary tumor that is unresponsive to chemotherapy.

Most current protocols used in the treatment of patients with OS usually defer definitive surgery for approximately 3 months after initiation of neoadjuvant chemotherapy. This strategy rests on the presumption that starting chemotherapy immediately allows the treating physician to address micrometastatic spread without delay, presumably improving the final outcome. Despite widespread approval of this approach, the real reason for the improved survival of these patients is still unclear. When the neoadjuvant approach was first introduced into clinical practice, it coincided with the introduction of new combinations of drugs, such as doxorubicin and ifosfamide [19,20]. Since then, the combination of three (doxorubicin, cisplatin and high dose methotrexate) or four drugs (with the addition of ifosfamide) has become the gold standard of chemotherapy in the treatment of OS patients. There has been no further improvement in survival for approximately 30 years. Critically appraising the literature of the past three decades, one should ask whether improvement in survival can be attributed to the neoadjuvant approach in the management of OS patients or whether it is the result of combined chemotherapy. In 2003, Goorin et al. [20] prospectively tested these two strategies on 100 OS patients and were unable to demonstrate superiority of one approach over the other [20]. Nothing more has been published comparing these two approaches since then.

Reliable tumor characteristics for predicting tumor response to chemotherapy will allow the treating physician to proceed to definitive surgery at earlier stages of therapy, thus minimizing the risk of future metastatic disease developing. It was recently shown that simple laboratory parameters, such as the number of lymphocytes routinely determined in OS patients, may serve as reliable predictors of outcome in this disease [21].

SAP is another such candidate, and its dynamics may potentially be used as a predictive tool with regard to histological response. Alkaline phospatase is a glycoprotein that has its source in bones, liver, kidney or placenta, although the glycolitic enzyme determined in the human serum of healthy persons derives mainly from bone or hepatic tissues. To exclude the possibility that hepatic disease was the reason for the high AP levels, we performed serial measurements of hepatic enzymes in all our OS patients and found them to be within normal range most of the time, despite very short-lived elevations immediately after high dose methotrexate administration. We believe that the source of SAP in our patients was mainly the skeletal system, since it continued to decrease in the majority of patients with no relation to the dynamics of other hepatic enzymes. Since not all OS patients with high AP levels at initial diagnosis will eventually have a poor histological response to neoadjuvant chemotherapy, we attempted a more "fine-tuned"

AP = alkaline phosphatase

evaluation of changes in AP levels during the course of chemotherapy given before definitive surgery. We found that the rate of decrease of SAP after initiation of chemotherapy did not correlate with histological response. We suggest that a decrease in SAP itself probably reflects a decrease in the size of the tumor, at least its soft tissue component, but this decrease does not translate into greater necrosis of tumor tissue. To verify this suggestion, it would be necessary to follow changes in volume of the soft tissue component during neoadjuvant chemotherapy. This may become the topic of a future study.

It has been postulated [22] that anticancer treatment exerts its tumoricidal effect not only by direct killing of malignant cells but also by changing the morphology of vessels supplying the tumor with nutrients and oxygen. It is possible that chemotherapy exerts its effect through the blood vessels that primarily supply pathological soft tissues surrounding bone tumorous matrix in the first stages of therapy. We were able to discern two groups of patients according to the dynamics of SAP after instituting neoadjuvant chemotherapy. The first group comprised patients whose SAP decreased more than 25% as compared to pretreatment levels, and the second group represented patients with less than a 25% decrease of SAP level or even increased levels despite chemotherapy. It would be intriguing to compare the dynamics of SAP level changes in correlation with the soft tissue component in pretreatment imaging studies. It is possible that patients who did not demonstrate a significant decrease in SAP levels in response to neoadjuvant chemotherapy had little or no soft tissue tumor mass along with the primary bone tumor.

Although we were unable to find a positive correlation between the rate of SAP decrease during the first month of neoadjuvant chemotherapy and survival, such a correlation was established in the subgroup of patients with metastatic disease, despite the small number of such patients. Metastatic disease usually presents as pulmonary nodules of various sizes and does not have a bone component characteristic for primary tumors. It can be speculated that this morphology predisposes the metastases to respond to chemotherapy at a higher degree than bony tumors. In clinical practice it is not uncommon to observe a significant decrease in number and size of lung metastases in response to neoadjuvant chemotherapy, despite the lack of such an effect on the primary bone tumor.

One of the drawbacks of our study was that by combining patients with localized and metastatic disease it was virtually impossible to determine the effect of SAP decrease rate on clinical outcome in all the patients. Combining both groups excluded the possibility of determining a possible correlation between SAP and clinical outcome. However, when we performed an additional analysis in the group of patients with metastatic disease only, we were able to show that the SAP decrease rate in the first month of chemotherapy could serve as a predictive indicator of clinical outcome [Figure 2B]. In the group of patients without metastatic disease this correlation was not found. The reason for this difference is unclear.

Further limitations of our study stem from its retrospective nature. Firstly, the number of analyzed cases is relatively small. Using various calculations, we could predict that the number of patients in the study should be increased approximately threefold to reach statistically significant results regarding the effect of SAP changes on survival. Secondly, not all pertinent data were available from the medical charts. We chose overall survival as a surrogate for disease progression instead of event-free survival or progression-free survival, but acknowledge that the latter two parameters may also be appropriate for such an analysis since they directly reflect the process of disease progression.

In addition, the patients in this study received chemotherapy according to similar but different therapeutic protocols. It is possible that slightly different protocols may lead to different responses to neoadjuvant chemotherapy, thereby influencing the results of the study. However, we note from the literature [23] that the percentage of patients who respond favorably to neoadjuvant chemotherapy is approximately the same, irrespective of the specific protocols used in various studies, implying that histological response is more a function of the intrinsic properties of the tumor itself than the effect of the same three or four drugs given in a slightly different combination and/or sequence.

In summary, we found no direct correlation between the SAP decrease rate and the survival of patients with osteosarcoma. Further studies directed at discovering new biological markers are needed to improve prediction of response to neoadjuvant chemotherapy and ultimate prognosis in this disease.

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References

1. Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer* 2009; 115: 1531-43.
2. Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. *Cancer Treat Res* 2009; 152: 3-13.
3. Ferrari S, Palmerini E, Staals EL, et al. The treatment of nonmetastatic high grade osteosarcoma of the extremity: review of the Italian Rizzoli experience. Impact on the future. *Cancer Treat Res* 2009; 152: 275-87.
4. Bruland S, Bauer H, Alvegaard T, Smeland S. Treatment of osteosarcoma. The Scandinavian Sarcoma Group experience. *Cancer Treat Res* 2009; 152: 309-18.
5. Federman N, Bernthal N, Eiber FC, Tap WD. The multidisciplinary management of osteosarcoma. *Curr Treat Options Oncol* 2009; 10: 82-93.
6. Pakos EE, Nearchou AD, Grimer RJ, et al. Prognostic factors and outcomes for osteosarcoma: an international collaboration. *Eur J Cancer* 2009; 45: 2367-75.
7. Clark JC, Dass CR, Choong PF. A review of clinical and molecular prognostic factors in osteosarcoma. *J Cancer Res Clin Oncol* 2008; 134: 281-97.

8. Glasser DB, Lane JM, Huvos AG, Marcove RC, Rosen G. Survival, prognosis, and therapeutic response in osteogenic sarcoma. The Memorial Hospital experience. *Cancer* 1992; 69: 698-708.
9. Bramer JA, van Linge JH, Grimer RJ, Scholten RJ. Prognostic factors in localized extremity osteosarcoma: a systematic review. *Eur J Surg Oncol* 2009; 35: 1030-6.
10. Bacci G, Mercuri M, Longhi A, et al. Grade of chemotherapy-induced necrosis as a predictor of local and systemic control in 881 patients with non-metastatic osteosarcoma of the extremities treated with neoadjuvant chemotherapy in a single institution. *Eur J Cancer* 2005; 41: 2079-85.
11. Raymond AK, Chawla SP, Carrasco CH, et al. Osteosarcoma chemotherapy effect: a prognostic factor. *Semin Diagn Pathol* 1987; 4: 212-36.
12. Harting MT, Lally KP, Andrassy RJ, et al. Age as a prognostic factor for patients with osteosarcoma: an analysis of 438 patients. *J Cancer Res Clin Oncol* 2010; 136: 561-70.
13. Bieling P, Rehan N, Winkler P, et al. Tumor size and prognosis in aggressively treated osteosarcoma. *J Clin Oncol* 1996; 14: 848-58.
14. Cho WH, Song WS, Jeon DG, et al. Differential presentations, clinical courses, and survivals of osteosarcomas of the proximal humerus over other extremity locations. *Ann Surg Oncol* 2010; 17: 702-8.
15. Bacci G, Picci P, Ferrari S, et al. Prognostic significance of serum alkaline phosphatase measurements in patients with osteosarcoma treated with adjuvant or neoadjuvant chemotherapy. *Cancer* 1993; 71: 1224-30.
16. Bacci G, Longhi A, Ferrari S, et al. Prognostic significance of serum alkaline phosphatase in osteosarcoma of the extremity treated with neoadjuvant chemotherapy: recent experience at Rizzoli Institute. *Oncol Rep* 2002; 9: 171-5.
17. Gonzalez-Billalabeitia E, Hitt R, Fernandez J, et al. Pre-treatment serum lactate dehydrogenase level is an important prognostic factor in high-grade extremity osteosarcoma. *Clin Transl Oncol* 2009; 11: 479-83.
18. Winkler K, Beron G, Delling G, et al. Neoadjuvant chemotherapy of osteosarcoma: results of a randomized cooperative trial (COSS-82) with salvage chemotherapy based on histological tumor response. *J Clin Oncol* 1988; 6: 329-37.
19. Bacci G, Picci P, Ferrari S, et al. Primary chemotherapy and delayed surgery for nonmetastatic osteosarcoma of the extremities. Results in 164 patients preoperatively treated with high doses of methotrexate followed by cisplatin and doxorubicin. *Cancer* 1993; 72: 3227-38.
20. Goorin AM, Schwartzentruber DJ, Devidas M, et al. Presurgical chemotherapy compared with immediate surgery and adjuvant chemotherapy for nonmetastatic osteosarcoma: Pediatric Oncology Group Study POG-8651. *J Clin Oncol* 2003; 21: 1574-80.
21. Moore C, Eslin D, Levy A, Roberson J, Giusti V, Sutphin R. Prognostic significance of early lymphocyte recovery in pediatric osteosarcoma. *Pediatr Blood Cancer* 2010; 55: 1096-102.
22. Jain RK. Normalization of tumor vasculature: an emerging concept in anti-angiogenic therapy. *Science* 2005; 307: 58-62.
23. Bacci G, Forni C, Ferrari S, et al. Neoadjuvant chemotherapy for osteosarcoma of the extremity: intensification of preoperative treatment does not increase the rate of good histologic response to the primary tumor or improve the final outcome. *J Pediatr Hematol Oncol* 2003; 25: 845-53.

“If you want to make peace with your enemy, you have to work with your enemy. Then he becomes your partner”

Nelson Mandela (1918-2013), activist, South African president, Nobel Peace Prize winner

“The art of progress is to preserve order amid change, and to preserve change amid order”

Alfred North Whitehead (1861-1947), English mathematician and philosopher. He is best known as the defining figure of the philosophical school known as process philosophy, which has found application to a wide variety of discipline, including ecology, theology, education, physics, biology, economics, and psychology, among others