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Pre- and post-chemotherapy alkaline phosphatase levels as prognostic indicators in adults with localised osteosarcoma

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Abstract

The prognostic value of alkaline phosphatase (AP) measured before and after chemotherapy, but before surgery was established in a retrospective survey of patients. The patients were 18 years or older, with non-metastatic high-grade osteosarcoma. Pre-chemotherapy AP was available in 89 cases, post-chemotherapy AP in 86 patients, and both in 71 cases. AP was classified as Normal (<100% upper limit), High (100% $\leqslant$ AP < 200%) or Very High (AP $\geqslant$ 200%). Osteosarcoma subtype was predominantly conventional. No correlation was found between subtype and chemotherapy response, local recurrence or survival. Pre-chemotherapy AP was raised more in the osteoblastic subtype. Post-chemotherapy AP and normalisation were the same among different subtypes. AP was not correlated with local recurrence. Normal or High pre-chemotherapy AP correlated with better survival at 10 years (64% and 70%) than Very High pre-chemotherapy AP (37%, $P = 0.005$). Post-chemotherapy AP correlated with survival (68%, 39% and 25% in the Normal, High and Very High group, $P = 0.0007$) and response to chemotherapy ($P = 0.049$). A pre-chemotherapy AP above twice Normal correlated with worse survival. If AP decreased after chemotherapy, but was still raised, survival was better, but still worse than if AP normalised. A raised post-chemotherapy AP predicts poor chemotherapy response.

Keywords: Osteosarcoma; Adult; Prognosis; Alkaline phosphatase; Subtype; Response to chemotherapy; Survival; Local recurrence

1. Introduction

Although survival in high-grade osteosarcoma has improved considerably since the introduction of chemotherapy, 30–40% 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 changes in the level of alkaline phosphatase after chemotherapy were recorded. Attention was given to different osteosarcoma subtypes to study both influence on alkaline phosphatase levels and on outcome.

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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 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. 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. Two-hundred and sixty-one of them were under the age of 18, leaving 187 (42%) adult patients. Of these patients, 18 had a pathological fracture and were excluded. 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 pre-operative chemotherapy because of immediate necessity of resection, 2 refused post-operative 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 post-operative 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". As osteosarcoma subtype could influence AP 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 normalise, 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.

2.1. Statistical analysis

Comparability of the groups of different "AP-availability" was assessed with the χ^2-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 $P \leqslant 0.05$). Chemotherapy response (good or poor) was compared between groups with a χ^2-test Tumour necrosis after chemotherapy (%) was compared between groups using ANOVA with a Bonferroni post hoc analysis (level of significance $P = 0.0167$).

3. Results

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 (Table 1 and Fig. 1). 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 intralesional 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

Table 1
Comparability of groups with different availability of alkaline phosphatase (AP)

	Only pre-ct AP available ($n = 18$)[a]	Only post-ct AP available ($n = 15$)[b]	Both APs available ($n = 71$)	No AP available ($n = 28$)	*P*-value
Sex male/female	11/7	11/4	51/20	22/6	0.64
Median age years (range)	20 (18–57)	18 (18–55)	22 (18–56)	22 (18–44)	Ns
Proximal/distal tumour	8/10	7/8	36/35	10/18	0.61
Limb saving surgery (%)	10 (56)	12 (80)	58 (82)	20 (71)	0.12
Adequate surgical margin (%)	13 (72)	11 (73)	51 (72)	18 (67)	0.96
Local recurrence (% Kaplan-Meier)	8	7	10	38	0.02
10-Year survival (% Kaplan-Meier)	50	53	64	25	<0.0001

[a] Pre-ct AP = alkaline phosphatase before the start of chemotherapy.
[b] Post-ct AP = alkaline phosphatase after pre-operative chemotherapy but before surgery.

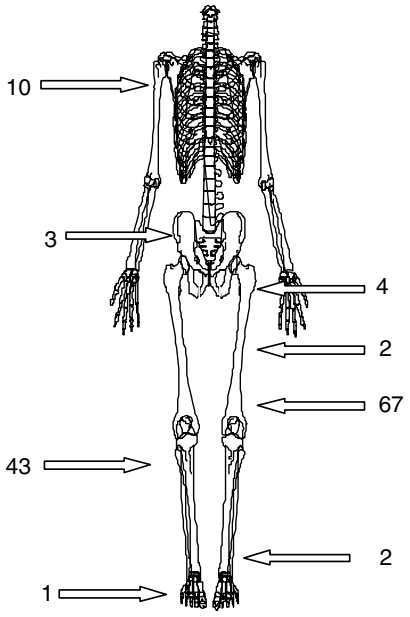

Fig. 1. Tumour site in the 132 eligible patients.

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 χ^2-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.

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%, $P = 0.005$) (Fig. 2). No statistically significant correlation was found between pre-ct AP and chemotherapy response (good *versus* poor) ($P = 0.26$ in χ^2-test) (Table 2). 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

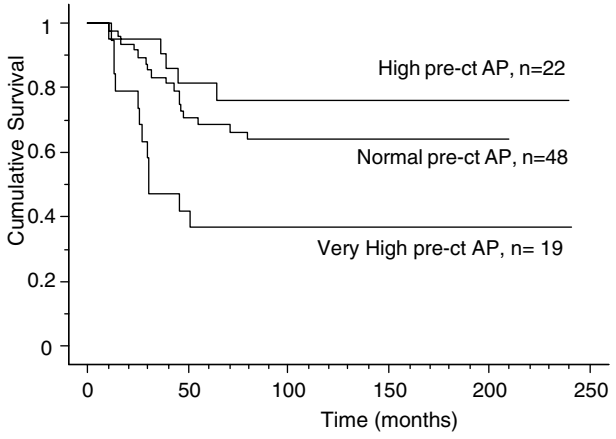

Fig. 2. Kaplan-Meier survival curve showing the likely hood of survival from time of diagnosis for patients with Normal, High or Very High pre-chemotherapy alkaline phosphatase (pre-ct AP) ($P = 0.005$).

Table 2
Pre-chemotherapy alkaline phosphatase (pre-ct AP) and chemotherapy response

	Normal pre-ct AP ($n = 48$)	High pre-ct AP ($n = 22$)	Very High pre-ct AP ($n = 19$)
Good responders	14	6	2
Poor responders	31	15	16
No info on response	3	1	1
% Necrosis (s.d.)	71 (26)	68 (32)	59 (24)

59%. This difference did not reach the level of significance in the ANOVA analysis ($P = 0.65$, 0.13 and 0.34; $\alpha = 0.0167$).

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 was 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 ($P = 0.0007$) (Fig. 3). Post-ct AP values did correlate with chemotherapy response (good *versus* poor) ($P = 0.049$ in χ^2-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 an elevated post-ct AP for a poor chemotherapy response was 100% (Table 3).

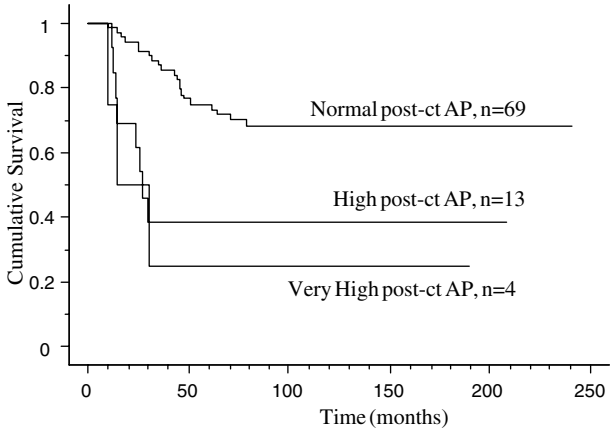

Fig. 3. Kaplan-Meier survival curve showing the likely hood of survival from time of diagnosis for patients with Normal, High or Very High post-chemotherapy alkaline phosphatase (post-ct AP) ($P = 0.0007$).

Table 3
Post-chemotherapy alkaline phosphatase (post-ct AP) and chemotherapy response

	Normal post-ct AP ($n = 69$)	High post-ct AP ($n = 13$)	Very High post-ct AP ($n = 4$)
Good responders	20	0	0
Poor responders	46	12	3
No info on response	3	1	1
% Necrosis (s.d.)	70 (29)	38 (24)	47 (6)

3.4. Normalisation of AP values after chemotherapy, and the relation to local recurrence, survival, and chemotherapy response

In 35 patients AP levels were 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% ($P = 0.0015$) (Fig. 4). The group of patients who's AP normalised after chemotherapy, had a larger proportion of good responders to chemotherapy ($P = 0.018$ in χ^2-test). Necrosis after chemotherapy response was also significantly higher in patients where AP normalised after 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 after chemotherapy for a poor chemotherapy response was 100% (Table 4).

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 χ^2-test) or percentage of necrosis after

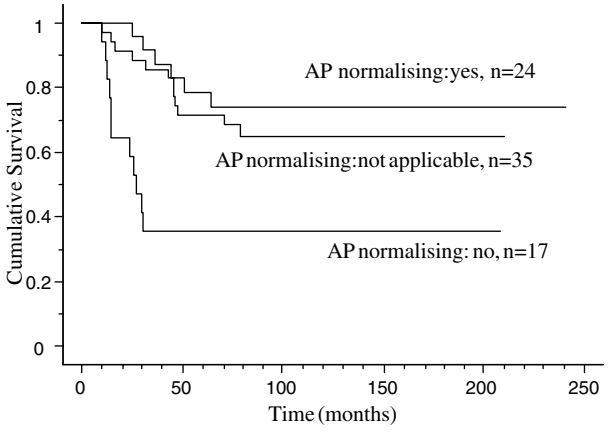

Fig. 4. Kaplan-Meier survival curve showing the likely hood of survival from time of diagnosis for patients in whom alkaline phosphatase (AP) normalised after chemotherapy, those where it did not, and those where it could not because it was not raised before chemotherapy (not applicable) ($P = 0.0015$).

Table 4
Normalisation of alkaline phosphatase (AP) and chemotherapy response

	AP normalising		
	Not applicable ($n = 35$)	Yes ($n = 24$)	No ($n = 17$)
Good responders	13	7	0
Poor responders	20	17	15
No info on response	2	0	2
% Necrosis (s.d.)	76 (26)	71 (29)	40 (22)

Table 5
Osteosarcoma subtype and pre-chemotherapy alkaline phosphatase (pre-ct AP)

	Normal pre-ct AP (%)	High pre-ct AP (%)	Very High pre-ct AP (%)
Osteoblastic ($n = 31$)	45	19	36
Chondroblastic ($n = 21$)	57	33	10
Fibroblastic ($n = 14$)	86	0	14
Conventional ns ($n = 15$)	47	33	20
Telangiectatic ($n = 6$)	50	50	0
Small cell ($n = 1$)	0	100	0
High grade periosteal ($n = 1$)	0	100	0

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 ($P = 0.03$ in χ^2-test) (Table 5). This difference disappeared in the analysis of post-chemotherapy AP and normalisation of AP ($P = 0.43$ and $P = 0.12$).

4. Discussion

Assessment of the prognosis of individual patients with 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 patients' needs if more accurate prognostication were available. Individual estimation of the prognosis however remains difficult. The strongest parameters are still stage at diagnosis and tumour necrosis after chemotherapy [17,3,6,10,13]. 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.* [11] showed a correlation of AP levels and prognosis in a small patient group. 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.* [7] reported pre-treatment AP levels to have a predictive value for survival, but not for chemotherapy response [22]. Post-neoadjuvant chemotherapy levels were reported to "normalise in most patients" in these studies, but it remains unclear what the significance of these post-chemotherapy levels was regarding survival or chemotherapy response. In 2001 Ferrari [10], and in 2002 both Bacci and Stokkel also showed prognostic value for pre-treatment AP levels [8,9]. In the last two 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 [23] who found a significantly higher proportion of good chemotherapy responders in patients with 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 abovementioned study. An explanation could be that in the current study only adults were included whereas Hauben 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 be 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 emphasised 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 possible 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 statement

None Declared. No financial or personal relationships with any of the authors exists mat could inappropriately influence this work.

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