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Prognostic Significance of BsALP in Healing of Long Bone Fractures

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A B S T R A C T

The aim of this work was to assess the relationship of both total alkaline phosphatase (ALP) and bone-specific alkaline phosphatase (BsALP) with the course and outcome of operatively treated long-bone fractures The activity of total ALP and BsALP was measured in 41 patients with a long bone fracture, comprising 26 men and 15 women. All patients were treated operatively. Total ALP and BsALP levels were measured in sera on day 1, 7, 14 and 21 after sustaining injury. Patient monitoring included X-rays. According to the outcome, patients were divided into two groups: the fast healing group and the slow healing group. The levels of total ALP and BsALP showed parallel trends in the course of this study. Depending on the healing outcome, on day 7 an increase in the case of slow healing, or a decrease in the case of fast healing, for both BsALP and total ALP was observed. No difference was found between various sites of bone fracture. This is an important result indicating the prognostic significance of total ALP and BsALP measurement in the monitoring of long bone fracture healing. In addition, an early change in the level of these enzymes was associated with the efficiency of the performed surgery.

Key words: *prognostic significance, BsALP, long bone fractures*

Introduction

The analysis of alkaline phosphatase (ALP) activity and determination of its bone isoenzyme (BsALP) are biochemical techniques, in which the interpretation of results and validity in patients with bone fractures has not been worked out in detail. Scientific researches conducted so far have shown that total activity of alkaline phosphatase increases parallel to the formation of the subperiosteal callus[1]. Silberman and coworkers have found that alkaline phosphatase participates in the process of bone formation only with one of its isoenzymes – namely with the so-called bone-specific alkaline phosphatase (BsALP)[2]. Sarmiento and coworkers have also come to the same conclusion[3].

The level of BsALP in osteoblast-line cells and in bones is proportional to bone formation[4]. Clinical studies have also proved that the level of BsALP in the serum may provide an index of the bone formation rate[5]. Several authors have reported that the maximum increase in the BsALP level occurs on day 21 after injury regardless of the treatment method (conservative or operative treatment with compressive osteosynthesis)[6,7]. Using statistical analysis and the method of correlation, a significant correlation was found between BsALP and total ALP concentrations at the level of 1.1% as early as seven days after injury[6]. Patients treated conservatively had the highest increase in the ALP activity after injury, whereas those treated by stable osteosynthesis had the lowest increase in the ALP activity. Based on these findings, it may be assumed that the activity of ALP depends upon the stability of bone fragments after a fracture has occurred. This has also been confirmed by other studies showing that unstable osteosynthesis allows greater mobility of fracture fragments as compared to stable osteosynthesis, whereas conservative treatment of fractures provides the greatest mobility of bone fragments[8]. Laurer and coworkers have examined the activity of BsALP after various types of trauma. They have demonstrated that the initial decrease in the BsALP activity is not only due to response to the fracture alone but is obviously part of a general stress response to trauma and opera-

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tion. The subsequent increase in the BsALP activity depends on the site of fracture and grows parallel to the applied osteosynthesis[9].

Bowles and coworkers have not found a continuous increase either of total ALP or BsALP from day 1 to week 10 post injury. Their measurements have shown that the total ALP activity increases and the BsALP activity decreases significantly by the fourth day post injury, and on the eight day post injury it returns to the level measured on the first day to rise continuously up to the tenth week[10]. In their later researches conducted in tibial fractures, they have also found a decline in the BsALP activity during the first week post injury, followed by a significant increase in week 5[11].

These results differ from those obtained by Leung and coworkers, who have measured the BsALP activity in 49 adult patients with fractures of long bones, but have not registered the initial decline of BsALP activity[12]. Studying total ALP concentrations in tibial fractures Oni and coworkers also failed to observe the initial decrease, while the initial increase in the ALP level was insignificant[13]. Although all authors agree that the presence of BsALP seems important for bone healing, its concentration and activity during the process of healing has been rarely and insufficiently researched. Therefore, the aim of our study was to examine the significance of alkaline phosphatase (ALP), particularly of bone-specific alkaline phosphatase (BsALP), for the prognosis of the course and rate of bone healing.

Materials and Methods

The activity of BsALP was measured in the sera of 41 patients with fractures of long bones, who were hospitalized and treated at our institution. There were 26 males (63.4%) and 15 females (36.6%), with the mean age of 37.3 yrs and 47.9 yrs respectively. Only completely healthy patients were included into the study (based on preceding medical history and actual laboratory findings). The BsALP activity was measured in the sera every seven days during four weeks.

Measurements of total ALP (necessary for the calculation of BsALP) were performed on the spectral flow photometer OPTON PM2DL, with 1-cm cuvettes, wave length of 405 nm and at 37 °C.

The laboratory method developed by Da Francesca-Wolheim was used for the determination of ALP activity[14]. The BsALP activity was determined in accordance with the method described by S.B. Rosalki and A.Y. Foo in 1984[15].

Parameters necessary for the calculation of fracture extent, i.e. the size of fractured bone area were measured on x-rays. The volume of periosteal callus was also calculated using parameters measured on x-rays following complete healing of the fracture. X-rays were obtained using standard methods for specific fracture types, with standard films and standard exposition. The films were processed automatically in the x-ray chamber.

The results were analyzed using the Mann-Whitney »U«-test for comparing quantitative data and the Wilcoxon rank-sum test for comparing data within two periods of time[16,17]. The values $p \leq 0.05$ were considered statistically significant.

Results

Levels of total ALP and BsALP were measured on day 1, 7, 14 and 21 in all patients. According to the final outcome of bone healing, the patients were divided into two groups:

1. the fast bone healing group
2. the slow bone healing group

TABLE 1
DESCRIPTIVE STATISTICS OF TOTAL ALP AND BsALP INA ALL PATIENTS ACCORDING TO THE FINAL OUTCOME AND DAY OF BONE HEALING

Outcome of bone healing		Total alkaline phosphatase (Total ALP)				Bone-specific alkaline phosphatase (BsALP)			
		Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
Fast	N	15	15	3		15	15	3	
	Arithmetic mean	139.60	121.73	113.00		56.47	19.67	17.67	
	Median	109.00	118.00	113.00		29.00	12.00	19.00	
	S.D.	91.41	36.74	6.00		88.34	18.80	15.04	
	Minimum level	73.00	74.00	107.00		7.00	2.00	2.00	
	Maximum level	426.00	211.00	119.00		368.00	74.00	32.00	
Slow	N	26	26	7	5	26	26	7	5
	Arithmetic mean	120.19	139.12	201.57	246.20	36.50	44.35	62.14	106.40
	Median	114.50	124.50	187.00	219.00	22.50	19.00	57.00	64.00
	S.D.	84.90	56.82	68.68	116.54	64.75	61.54	43.79	100.05
	Minimum level	41.00	59.00	135.00	145.00	2.00	2.00	14.00	30.00
	Maximum level	508.00	317.00	340.00	429.00	342.00	253.00	148.00	278.00

Table 1 shows measured parameters for both patient groups. The arithmetic mean of both measured parameters decreased gradually with time/days of healing in the fast healing group and in the slow healing group it increased gradually.

Table 2 presents testing of differences in the levels of total ALP dependent on time/days of bone healing.

The results of testing demonstrate a statistically significant difference in the level of total ALP between day 21 and day 7 after injury (Z=2.023; P=0.043). No statistically significant difference was found between other periods of time.

Testing of differences in the levels of BsALP dependent on time/days of healing is shown in Table 3.

The results of testing demonstrate a statistically significant difference in the level of BsALP between day 21 and day 7 after injury (Z=2.023; P=0.043) and between day 7 and day 1 (Z=1.997; P=0.046).

Testing of differences in the levels of total ALP and BsALP according to the final outcome of bone healing for all patients is shown in Table 4.

The results of testing show a statistically significant difference between the outcomes of healing for total ALP on day 14 (Z=2.393; P=0.017), whereas for other days and BsALP no statistically significant difference was established.

The results of our study and changes in the total ALP and BsALP activity according to days of measurement are presented in Figure 1 for all patients included in the study. It is obvious that changes in the total ALP and BsALP activity follow the same pattern at selected time points.

The subjects of our study were divided into two groups: the fast bone healing and slow bone healing group. Figure 2 shows changes in the total ALP activity and Figure 3 in the BsALP activity according to days and the rate of bone healing. It is evident that in the fast healing group a decline in the total ALP and BsALP activity occurs on day 7 and 14 as compared to the total ALP and BsALP measured on day1. In the slow healing group, an increase in the total ALP and BsALP activity occurs on day 7, 14 and 21 as compared to the levels measured on day 1.

Comparing the total ALP and BsALP levels measured in both patient groups at specified days of healing, the following conclusions may be drawn: the decrease in the total ALP and BsALP activity on day 7 and 14 after injury as compared to the levels measured on day 1 after

TABLE 2
TESTING OF DIFFERENCES IN THE LEVELS OF TOTAL ALP DEPENDENT ON DAYS OF HEALING

	Total alkaline phosphatase (total ALP)					
Wilcoxon rank-sum test	Day 7–Day 1	Day 14–Day 1	Day 21–Day 1	Day 14–Day 7	Day 21–Day 7	Day 21–Day 14
Z	1.438	0.765	1.214	1.682	2.023	1.826
P	0.150	0.444	0.225	0.093	0.043	0.068

Z – result of testing, P – level of significance

TABLE 3
TESTING OF DIFFERENCES IN THE LEVELS OF BsALP DEPENDENT ON DAYS OF HEALING

	Bone-specific alkaline phosphatase (BsALP)					
Wilcoxon Rank-sum test	Day 7–Day 1	Day 14–Day 1	Day 21–Day 1	Day 14–Day 7	Day 21–Day 7	Day 21–Day 14
Z	1.997	0.051	0.944	1.84	2.023	1.214
P	0.046	0.959	0.345	0.074	0.043	0.225

Z – result of testing, P – level of significance

TABLE 4
TESTING OF DIFFERENCES IN THE LEVELS OF TOTAL ALP AND BsALP IN RELATION TO THE FINAL OUTCOME OF BONE HEALING

	Total ALP			BsALP		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Mann-Whitney U test	175.00	147.00	0.00	134.50	159.50	3.00
Z	0.542	1.300	2.393	1.639	0.961	1.709
P	0.588	0.194	0.017	0.101	0.336	0.087
Outcome of healing: fast-slow						

Z – result of testing, P – level of significance

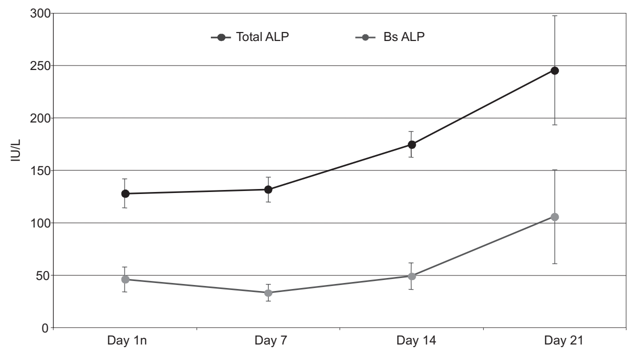

Fig. 1. Arithmetic mean ± S.E. of total ALP and BsALP according to days and independent of the outcome of healing.

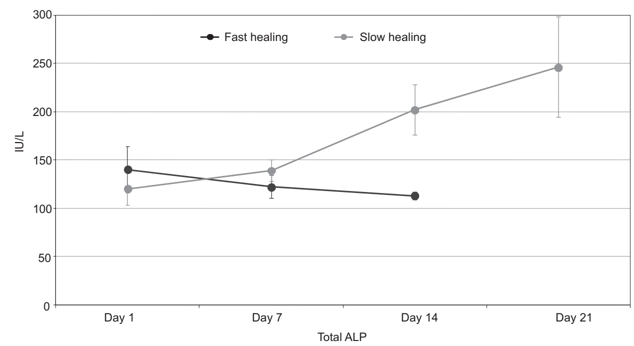

Fig. 2. Arithmetic mean ± S.E. of total ALP according to days and outcome of healing.

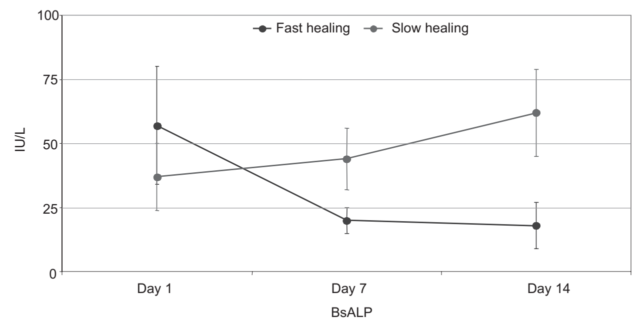

Fig. 3. Arithmetic mean ± S.E. of BsALP according to days and outcome of healing.

injury are observed in the fast bone healing group. The increase in the total ALP and BsALP activity on day 7, 14 and 21 after injury as compared to the levels measured on day 1 after injury indicates a slow healing of fractures.

Callus volume

The volume of callus after completed fracture treatment and successful bone healing in the operatively treated fractures was calculated for each patient included into the study. Callus volume according to the site of fracture (lower leg, femur, humerus) and outcome of bone healing is presented in Table 5 for both fast and slow healing.

Formation of a larger callus, which is typical for slow bone healing, is evident after a longer period of time, i.e. after completed healing of the fracture.

Discussion

For many years now, it has been known that the enzyme AP participates in the process of bone healing. This biochemical marker of bone formation is specific for bone tissue and is a better indicator of bone turnover in clinical practice. Our investigations showed that the increase in the total ALP activity was paralleled by the increase in the BsALP activity. Furthermore, the change of total ALP activity on day 7 and 14 as compared to day 1 after injury was coupled by the change of BsALP activity on the same day. In our study, the callus volume also followed the pattern of changes in the total ALP and BsALP activity with regard to the rate of bone healing.

Although a correlation between ALP and callus volume on day 7, 14 and 21 was found in our research, it was not confirmed statistically. A correlation between the BsALP activity and callus volume was observed on day 7, 14 and 21 but a statistically significant correlation was confirmed only for day 7. The correlation between the BsALP and callus volume has also been confirmed by other authors but based only upon comparisons[1,18]. However, in our research this correlation was confirmed by actual measurements of BsALP in patients.

Bone fracture causes enhanced bone turnover and an increase in biochemical indicators of bone turnover up to 50%. Due to the activity at the cellular level throughout the entire process of fracture healing, the level of these

TABLE 5
CALLUS VOLUME (cm^3) ACCORDING TO SITE OF FRACTURE (LOCALIZATION), OUTCOME OF HEALING AND OVERALL RESULT

			Callus volume (cm^3)				
Site of fracture	Outcome of bone healing	N	Arithmetic mean	Median	S.D.	Minimum level	Maximum level
Lower leg	fast	14	1.31	0.00	2.67	0.00	9.05
	slow	19	14.04	9.42	17.65	1.37	72.45
Femur	fast	1	0.00				
	slow	6	46.82	31.25	40.91	11.23	113.04
Humerus	slow	1	25.53				

biochemical indicators may remain enhanced during 6–12 months[19]. The increase in the BsALP level up to 50% was also recorded in a large number of our patients between day 1 and 14.

According to our results, ALP reaches the maximum level on day 14 post injury, which is different from the results of other studies, in which the maximum level of ALP is measured as late as on day 21[7,20]. The initial decline of total ALP reported by Bowles, Jingushi and Joerring[10,21,22] has not been observed in our study. The increase in total ALP already on day 1 has been recorded in our study, which corresponds to the results reported by Leung and Oni[12,13].

Reduced BsALP activity in the first days post injury might be associated with some other events in the posttraumatic period. Certain studies report on the so-called inflammatory response of the body to trauma[23]. It is well known that after trauma cytokines released by variety of activated inflammatory cells may cause an initial decrease in the BsALP activity directly or indirectly via prostaglandin activation[13]. It is also known that interleukin (IL-1) causes bone resorption and leads to the reduction of markers specific for bone formation[24]. In the next phase of the posttraumatic period, stimulation, replication and proliferation of osteoblasts occur, which is the prerequisite of bone healing[25].

Different results obtained by the aforementioned authors can be explained by the complexity of factors affecting the level of ALP in the blood. This includes the influence of daily rhythm of bone turnover, season of the year, patient's age, gender and race[26,27]. Daily oscillations in the level of biochemical parameters of bone turnover are observed from 2 a.m. to 8 a.m. (higher concentrations) and from 1 p.m. to 11 p.m. (lower concentrations). The size of these oscillations may amount up to 50%. Increased physical activity can also initiate changes in the level of biochemical markers of bone turnover (from 15–40%) in the period of 24–72 hrs after physical activity. In professional athletes, levels of bone turnover markers differ from those encountered in the general populations[28,29]. Various pathological conditions are associated with changes in bone metabolism.

The aforementioned factors and significant biological variations in the biochemical indicators of bone turnover account for differences in the results of research studies conducted in this field. Therefore, a thorough study of causes related to the conditions and the length of the follow-up of individual subjects or subject groups seems mandatory. In this way, it would be possible to avoid undesirable effects of factors that increase variations and consequently cover up clinical appearance or change(s) being researched in the study. Furthermore, this would make the results of studies more comparable.

Conclusion

Based on the results of our study, the following conclusions can be drawn:

Changes in the ALP activity show an almost identical pattern as the changes in the BsALp activity. The decrease in the ALP activity fully coincides (100%) with the decrease in the BsALP activity. The increase in the ALP activity mainly corresponds (88.9%) to the increase in the BsALP activity. This indicates an almost complete concordance of these two parameters.

Depending upon the outcome of bone healing, differences in the levels of total ALP and BsALP have been confirmed as early as day 7 after injury. In case of fast healing of fractures, both the total ALP and BsALP activity decrease already seven days after injury. In case of slow healing of fractures, a continuous increase in the level of ALP and BsALP is noted in the posttraumatic period.

The callus volume correlates with the level of total ALP and BsALP, but this correlation is rarely statistically significant.

This study has shown that the total ALP and BsALP activity may be prognostically relevant indicators of bone healing in the operative treatment of long-bone fractures since the difference between slow and fast healing was noted in our patients as early as seven days after injury.

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PROGNOSTIČKI ZNAČAJ KOŠTANOG IZOENZIMA U CIJELJENJU PRIJELOMA DUGIH KOSTIJU

S A Ž E T A K

Cilj rada je procjena povezanosti vrijednosti ukupne alkalne fosfataze (AF) i koštane alkalne fosfataze (KAF) s tijekom cijeljenja i ishodom operacijskog liječenja prijeloma dugih kostiju. Aktivnost ukupne alkalne fosfataze i koštane alkalne fosfataze mjerena je u 41 bolesnika (26 muškarca i 15 žena) s prijelomima dugih kostiju, koji su svi liječeni operacijski. Razina obaju parametara u serumu mjerena je 1., 7., 14. i 21. dan nakon ozljede. Bolesnici su također praćeni radiološki. S obzirom na ishod liječenja, ispitanici su podijeljeni u dvije skupine: skupina brzog cijeljenja i skupina sporog cijeljenje prijeloma. Razina ukupne i koštane alkalne fosfataze je pokazala usporedni trend tijekom ispitivanja. Zavisno od ishoda cijeljenja, sedmi dan nakon prijeloma zabilježen je porast vrijednosti oba parametra u skupini sa sporim cijeljenjem, a u skupini s brzim cijeljenjem pad vrijednosti oba parametra. S obzirom na mjesto prijeloma nije zabilježena razlika izmjerenih aktivnosti biokemijskih parametara. To je značajan rezultat koji ukazuje na prognostički značaj vrijednosti ukupne i koštane alkalne fosfataze u praćenju tijeka koštanog cijeljenja. Osim toga, rana promjena u razini ovih dvaju enzima ukazuje na moguću razinu uspješnosti izvršenog operacijskog liječenja.