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Original Paper

The Bone Isoenzyme of Alkaline Phosphatase in Hypercalcaemic Cancer Patients

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Alkaline phosphatase (AP) is the classic marker of bone formation, especially in cancer patients, but the interpretation of its measurement is complicated by the existence of various circulating isoenzymes, especially of liver origin. The introduction of a mass measurement of the bone isoenzyme of AP (BAP) by an immunoradiometric assay has markedly improved the sensitivity and the specificity of the determination. We measured BAP and other markers of bone turnover in 46 patients with tumour-induced hypercalcaemia (TIH), which is an interesting model for evaluating markers of bone formation because of the uncoupling between bone formation and bone resorption found by histomorphometric techniques. The extent of bone metastatic involvement was evaluated by planimetry on bone scintigraphy. Mean (±S.D.) BAP concentrations were slightly higher in patients with TIH than in healthy subjects, 15.5 ± 8.5 versus 12.4 ± 3.5 μg/L ($P < 0.05$). However, the scatter of the data in TIH patients was quite marked. Increased values (10/46 patients, 22%) occurred only in patients with bone metastases. Total AP, γGT and BGP levels, as well as markers of bone resorption, were not significantly different between patients with or without bone metastases. BAP levels were significantly correlated with AP ($r_s = 0.63$; $P < 0.01$) but not with BGP levels nor with markers of bone resorption. BAP levels were also correlated with the extent of bone uptake at scintigraphy ($r_s = 0.54$; $P < 0.01$), but this was not the case for total AP or BGP. In the 36 patients re-evaluated when normocalcemic after pamidronate therapy, BAP levels increased from 16.3 ± 9.2 to 22.2 ± 21.3 μg/L ($P < 0.05$) but there were no significant changes in AP or BGP concentrations. In summary, our data confirm the existence of an uncoupling in bone turnover in TIH and indicate that cancer hypercalcaemia is another pathological condition characterised by a discordance between BAP and BGP concentrations. BAP levels appear to be a better reflection of bone metastatic involvement than total AP or BGP and their short-term increase after pamidronate therapy could reflect the recently described effects of bisphosphonates on osteoblasts. © **1997 Elsevier Science Ltd.**

Key words: alkaline phosphatase, bone, bone metastases, hypercalcaemia, immunoassay
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INTRODUCTION

BIOCHEMICAL MARKERS allow non-invasive and sequential assessment of bone turnover [1]. These could be especially helpful in oncology for the diagnosis and assessment of bone metastases, the evaluation of which remains a constant challenge for the practicing oncologist [2]. However, the biochemical evaluation of bone formation remains difficult, notably because of the coupling between bone formation and bone resorption. Alkaline phosphatase (AP) is the classic marker of bone formation, especially in cancer patients, but the interpretation of its measurement is even more complicated by the existence of various circulating isoenzymes, particularly of liver origin, but also of tumour origin [1–3]. Determination of the bone isoenzyme of AP has been per-

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formed by several methods, but they suffer from a lack of specificity and are often quite tedious [4, 5]. The recent introduction of a mass measurement of the bone isoenzyme by an immunoradiometric assay (IRMA) for bone AP (BAP) appears to markedly improve the sensitivity and the specificity of the determination in various benign conditions, notably because the assay has only a 15% cross-reactivity with the liver isoenzyme of AP [6].

The measurement of total AP remains the 'gold standard' in oncology for the biochemical diagnosis and monitoring of bone metastases and there are few data about the bone isoenzyme of AP in cancer patients. Tumour-induced hypercalcaemia (TIH) is an interesting model for evaluating markers of bone formation because of the uncoupling between bone formation and bone resorption, as shown by histomorphometric studies [8]. We measured circulating BAP concentrations before and after therapy of TIH by the bisphosphonate pamidronate. To unravel further the pathophysiology of TIH, we compared BAP levels to the concentrations of osteocalcin (bone-GLA protein, BGP), which is a protein specifically made by the osteoblasts and to classical markers of bone resorption [1, 9]. They were also related to the presence and the extent of metastatic bone disease to expand the available information on the potential interest of BAP measurement in oncology.

PATIENTS AND METHODS

Patients

We studied 46 hypercalcaemic cancer patients before any hypocalcaemic therapy apart from intravenous rehydration. There were 27 females and 19 males, with a median age of 53 (range 24–77) years. Primary tumour sites consisted of 16 breast cancers, 13 head and neck tumours, 5 lung and 12 miscellaneous tumours. Twenty-four patients had definite bone metastases, 20 of them with extensive skeletal involvement; 18 were considered negative by scintigraphy and X-rays, and 4 cases remained doubtful after these investigations. Median (range) baseline calcium (Ca) concentrations were 12.4 (10.4–17.4) mg/dl, and intact PTH levels were adequately suppressed at 7.4 (2.2–13.5) pg/ml. Thirty-six patients were re-evaluated when normocalcaemic after pamidronate therapy given at a median dose of 1 mg/kg [10].

Assays

Serum BAP concentrations were measured by the IRMA Tandem-R Ostase (Hybritech Inc.). Briefly, the assay consists of a two-site IRMA using two murine monoclonal antibodies directed against different antigenic sities on BAP which was obtained from the human osteosarcoma cell line SAOS-2. The assay is calibrated with a six-point curve constructed from dimeric BAP extracted from SAOS-2 cells. Reference values were determined in 44 healthy subjects, 24 men, 32 (20–48) years old and 20 women, 40 (21–59) years old. The reference range, defined as the 2.5–97.5th percentiles, was 8.8–20.0 µg/l. All samples had been collected on ice, frozen at −20°C, kept for less than 6 months and never thawed before the assay. The functional detection limit of the assay was 0.5 µg/l. The intra- and interassay coefficients of variation were 4.5 and 6.7%, respectively.

As previously described [10–12], other measurements included total serum Ca (normal values, NI, 8.5–10.3 mg/dl), Ca corrected for protein levels (NI, 8.5–10.5 mg/dl), ionised Ca (measured by the Ciba-Corning electrode; NI, 4.2–5.1 mg/dl), inorganic phosphate (Pi; NI, 2.2–4.5 mg/dl), total AP (measured by the kinetic colour test AU 5000 of Merck Diagnostica; NI <110 U/l); intact PTH (NI, 10–50 pg/ml), $1,25(OH)_2vit.D_3$ (NI, 15–42 pg/ml) and osteocalcin (BGP; NI, 0.8–5.6 ng/l; all assays from INCSTAR, Stillwater, Minnesota, U.S.A.). The functional detection limit of this last assay was 0.7 ng/ml [13]. Urine measure-

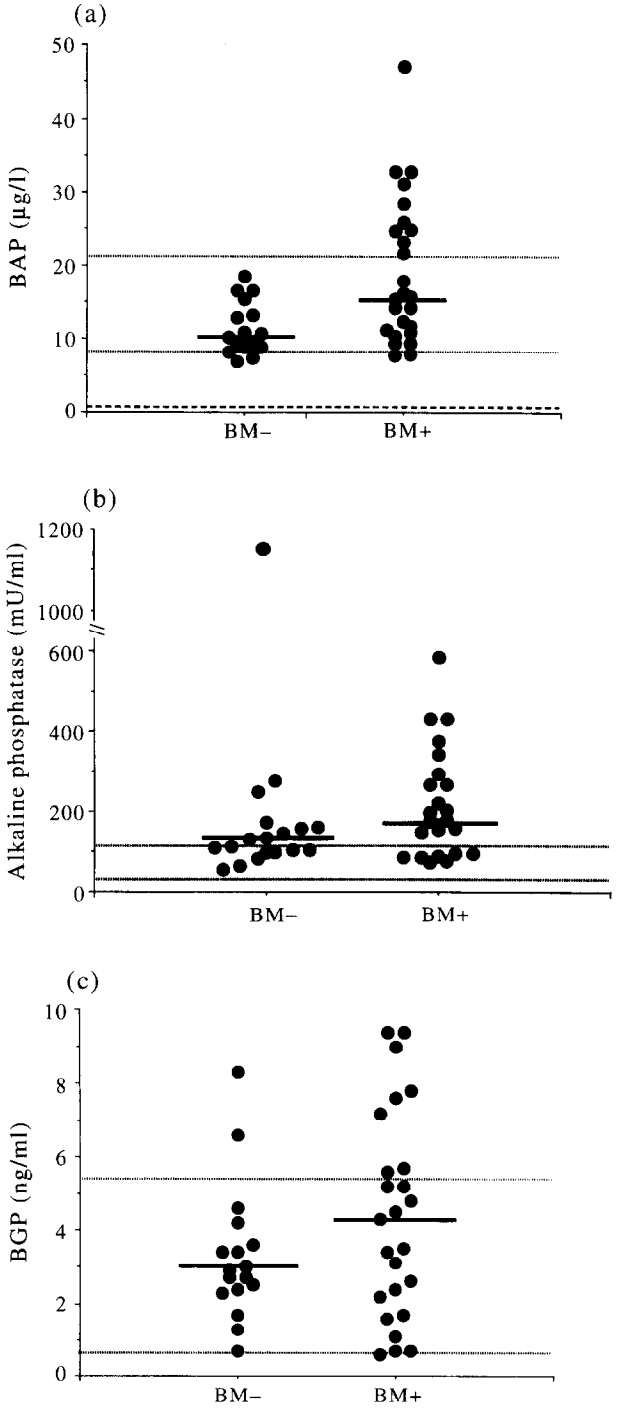

Figure 1. Parameters of bone formation in 46 hypercalcaemic cancer patients divided according to the presence (BM+) or absence (BM−) of bone metastases (bars represent the median values). (a) Bone alkaline phosphatase (BAP) concentrations. (b) Total AP concentrations. (c) Osteocalcin (BGP) concentrations.

Table 1. Mean (+S.D.) concentrations of various parameters of bone metabolism in 42 hypercalcaemic cancer patients, 18 without bone metastases (BM–) and 24 with bone metastases (BM+)

	Normal values	BM–	BM+	*P* value
Serum Ca(mg/dl)	8.5–10.3	12.1 ± 1.0	13.4 ± 2.3	
Ca, Correlated for protein levels (mg/dl)	8.5–10.5	13.1 ± 1.2	14.3 ± 1.2	
Ionised Ca (mg/dl)	4.2–5.1	6.6 ± 0.7	6.9 ± 1.1	
Pi (mg/dl)	2.2–4.5	2.5 ± 0.4	3.2 ± 0.6	0.0003
PTH (pg/ml)	10–50	7.4 ± 2.7	7.4 ± 2.2	
1,25 $(OH)_2$vit.D_3 pg/ml	15–42	19.3 ± 16	11.5 ± 10.3	0.02
cAMP (nmol/dl GFR)	1.4–6.3	7.6 ± 3.0	5.3 ± 2.4	0.01
BGP (ng/ml)	0.8–5.6	3.3 ± 1.8	4.4 ± 2.9	
AP (U/L)	<110	187 ± 240	217 ± 315	
γGT(U/L)	6–28	45 ± 12	126 ± 41	
BAP (μg/l)	8.8–20	11.2 ± 3.5	19.5 ± 9.9	0.001
Ca/Creat (mg/mg)	<0.21	0.592 ± 0.273	0.729 ± 0.338	
Hydroxyproline (mg × 100/mg Creat)	<4.7	6.3 ± 2.5	8.6 ± 4.9	

ments in 2 h morning fasting samples included Ca, creatinine (Ca/Creat; NI, <0.21 mg/mg), hydroxyproline (NI, <4.7 mg × 100/mg Creat) and cyclic AMP (cAMP; NI, 1.4–6.3 nmol/dl GFR) [10–13].

Determination of the extent of bone metastatic involvement

Bone metastases were diagnosed by bone scintigraphy and X-rays, and confirmed by computer tomography (CT) scan when necessary. We determined the extent of bone metastatic involvement by planimetry on the posterior view of a bone scintigraphy that was always performed within 3 months of the blood sampling; 25 patients were evaluable.

Statistical analysis

Data are expressed as the mean ± standard deviation of the mean (S.D.) and/or by the median (range) when indicated. We performed classical statistical tests, parametric (ANOVA and *t*-tests) and non-parametric tests (Mann–Witney, paired signed test and Spearman correlation). We used the Statistica™ program version 4 (Statsoft Inc., Tulsa, Oklahoma, U.S.A.) for an analysis of covariance (ANCOVA, with the pretreatment value as the covariable) to study the effects of bisphosphonates on BAP levels as a function of bone metastases.

RESULTS

Mean BAP concentrations were slightly, but significantly, higher in patients with TIH than in healthy subjects, 15.5 ± 8.5 versus 12.4 ± 3.5 μg/l ($P < 0.05$). However, the scatter of the data was quite marked in the TIH group, as 6 patients (13%) had subnormal values and 10 patients (22%) had elevated values. Total AP and parameters of bone resorption were also higher than normal values, but this was not the case for BGP. As shown in Figure 1(a), all patients with elevated BAP concentrations had bone metastases. In contrast, total AP and BGP levels were not significantly different between patients with or without BM (Figure 1(b) and (c), Table 1). Patients without BM also had lower Pi and higher urinary cAMP levels than patients with BM (Table 1). Liver function tests were not significantly different between patients with or without BM. γGT levels were slightly higher (Table 1) but this was due to a few high values. In patients with BM, the extent of bone metastatic involvement (determined by planimetry; see Materials and Methods) were higher in patients with elevated BAP than in patients with normal BAP levels. 19.0 ± 15.5% versus 7.5 ± 14.9%, respectively ($P < 0.05$).

As summarised in Table 2, BAP levels were significantly correlated with AP ($r_s = 0.63$; $P < 0.01$) but not with BGP levels nor with markers of bone resorption. BAP levels were also significantly correlated with the extent of bone uptake at scintigraphy ($r_s = 0.54$; $P < 0.01$), but this was not the case for AP or BGP. None of the correlations with BGP were significant, whereas the two markers of bone resorption correlated with each other ($r_s = 0.50$; $P < 0.01$).

In the 36 patients who were re-evaluation when normocalcaemic after pamidronate therapy, BAP levels increased from 16.3 ± 9.2 to 22.2 ± 21.3 μg/l ($P < 0.05$). The individual values are shown in Figure 2. The median (range) values were 12.3 (6.9–47.1) and 12.7 (7.1–116.0) μg/l before and after therapy, respectively ($P < 0.05$). There was, however, no significant change in total AP levels, from a median value of 158 (54–1119) before therapy to 145 (54–1148) U/l after therapy and the changes were not significant either for BGP levels, which fell from a median value of 3.4 (0.7–9.4) ng/ml to 2.6 (0.7–14.5) ng/ml after therapy. The increase in BAP levels was concomitant with an increase in PTH levels from 7.5 ± 2.4 to 28.4 ± 30.6 pg/ml ($P < 0.01$),

Table 2. Correlations (r_s) between markers of bone formation (BGP, AP, BAP) and bone resorption (Ca/Creat, hydroxyproline) and extent of bone metastases in 46 hypercalcaemic cancer patients

	BGP	AP	BAP	Ca/Creat	Hydroxyproline
AP	−0.14				
BAP	0.05	0.63*			
Ca/Creat	0.07	−0.12	0.22		
Hydroxyproline	0.07	0.13	0.21	0.50*	
Extent of BM (% uptake at scintigraphy: $n = 25$)	−0.10	0.36	0.54*	0.26	0.163

*$P < 0.05$

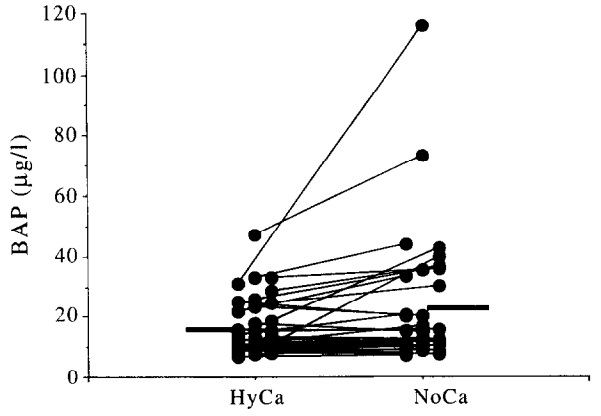

Figure 2. BAP concentrations measured before (HyCa) and after normalisation of serum Ca (NoCa) by pamidronate therapy in 36 hypercalcemic cancer patients (bars represent the mean values).

but there was no correlation between the changes in both parameters. Moreover, this increase in BAP levels did not depend on the presence of bone metastases.

DISCUSSION

Our data confirm the existence of an uncoupling in bone turnover in cancer hypercalcaemia. We found clearcut increases in parameters of bone resorption as previously reported in other studies [10, 12, 14], but the levels of BGP and BAP were highly variable. Moreover, there was no correlation with the parameters of bone resorption. We measured routinely available parameters of bone resorption, namely Ca/Creat and hydroxyproline. The determination of collagen cross-links and derived assays has been extensively evaluated [1], but we have shown that hydroxyproline gives comparable information to collagen cross-link determination in patients with TIH [12]. The meaning of an increase in total AP levels is often doubtful in patients with advanced cancer, since AP can be of bone, liver or tumoral origin [1–6], whereas the IRMA used for measuring BAP has only a 15% cross-reactivity with the liver isoenzyme of AP [6]. In our study, BAP levels were only increased in patients with bone metastases, and liver function tests were not significantly different between patients with or without BM. Th percentage of bone uptake at scintigraphy only correlated witih BAP, and not with BGP or with the tested markers of bone resorption. This percentage of uptake at bone scintigraphy is an indirect measurement of the bone metastatic involvement and our data suggest that it essentially reflects the osteoblastic reaction.

The measurement of BAP levels could thus be a valuable marker of the presence and the extent of bone metastases, but recent studies do not allow definite conclusions to be drawn. BAP determination has a better sensitivity than total AP patients with prostate cancer metastatic to the skeleton, especially when total AP is in the range of normal to twice-normal [15]. Moreover, BAP levels appear to provide complementary information to PSA determination in the diagnosis of bone metastases from prostate cancer [16]. However, in normocalcaemic patients with breast cancer, the sensitivity of BAP determinations, whether by lectin precipation, by the IRMA used in this study, or by a recently described enzyme immunoassay [17], does not appear to be better than AP measurement [18, 19]. The cross-reactivity with the liver isoenzyme of AP, even if it is only 15% with this assay, should also be taken into account. The clinical usefulness of these assays for the diagnosis and monitoring of bone metastases should thus be further investigated.

The meaning of BGP values in cancer patients is still unclear. As previously reported [13, 20], we could not find any relationship with the presence of bone metastases or the type of cancer hypercalcaemia. There was also no correlation with other markers of bone turnover. Besides glucocorticoid-induced osteoporosis and Paget's disease of bone [7, 21, 22], our data indicate that TIH is another clinical condition characterised by a dissociation between BGP and BAP levels. The reasons for these discrepancies are unknown, but our data further establish that these markers apear to reflect different osteoblast characteristics and functional properties.

Interestingly, we found a significant increase in BAP levels shortly after bisophosphonate administration, namely when the patients became normocalcaemic. This contrasted with the marked fall in parameters of bone resorption and the slight decline in BGP levels that we had previously found [10, 12, 13, 20]. This slight increase did not appear to be due to the PTH surge and literature data argue against this hypothesis. PTH has no direct effect on AP activity in osteoblast-like cells *in vitro* [23] and it even inhibits dexamethasone-induced AP activity in ROS cells [24]. Similarly, PTH infusion in man causes a decrease in all bone formation markers, whereas daily subcutaneous PTH injections cause a transient decrease followed by a late increase in AP levels [25]. The situation we are dealing with is probably closer to a PTH infusion rather than repeated PTH injections and, in all likelihood, the increase in BAP concentrations after bisphosphonate therapy is not due to the PTH surge. It more probably reflects the recently described effects of bisphosphonates on osteoblast-like cells [26]. Osteoblasts appear to be essential target cells for the anti-osteolytic action of bisphosphonates and may also be involved in the process of tumour-induced osteolysis itself [27]. Further research is needed to relate this increase in BAP levels to bisphosphonate activity.

In summary, this first report of BAP levels in cancer hypercalcaemia indicates that TIH is indeed characterised by an uncoupling in bone turnover and constitutes another pathological condition where there is a marked discordance between BAP and BGP concentrations. The slight increase in BAP levels after bisphosphonate therapy could be relevant to the mode of action of these drugs. More importantly from a clinical point of view, BAP levels appear to be a better reflection of bone metastatic involvement, or at least the osteoblastic reaction to the presence of tumour cells in bone, than total AP or BGP. Further research on the usefulness of BAP determination for the diagnosis, and even the monitoring of bone metastases, is certainly worthwhile.

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