

# Procollagen Type I Carboxy-Terminal Propeptide as a Marker of Osteoblastic Bone Metastases<sup>1</sup>

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## Abstract

**Collagen type I is the sole collagen type found in bones and tendons. Carboxyterminal propeptide, deriving and cleaved from procollagen type I (PICP) during collagen synthesis, is delivered into the blood, where it can be measured.**

**According to current knowledge, PICP correlates with bone collagen synthesis and bone formation rate. Elevated serum levels of PICP in patients with Paget's disease, compared with normal subjects and correlated with serum alkaline phosphatase (Alk.Ph.), have been previously described. Thus, PICP may be a valuable marker of bone formation.**

**PICP, serum Alk.Ph., serum bone Gla protein and 24-h urinary hydroxyproline:creatinine ratio have been measured in 47 cancer patients: 27 with predominantly osteolytic lesions (5 myeloma, 15 breast, 3 lung, 2 kidney, 1 bladder, 1 thyroid) and 20 with predominantly osteoblastic lesions (18 prostate and 2 breast).**

**The higher levels of PICP were noted in patients with osteoblastic or mixed metastases. In the entire group of patients, a statistically significant correlation between PICP and bone Gla protein ( $r = 0.57$ ;  $P < 0.001$ ), PICP and Alk.Ph. ( $r = 0.80$ ;  $P < 0.001$ ), and bone Gla protein and Alk.Ph. ( $r = 0.44$ ;  $P < 0.01$ ) was noted. In those patients with osteoblastic metastases we observed a significant correlation only between PICP and Alk.Ph. ( $r = 0.62$ ;  $P < 0.003$ ).**

**During chemotherapy, 13 of 20 patients with osteoblastic metastases who achieved objective response or stable disease showed a more rapid and significant decrease in PICP with respect to the other bone markers.**

**Serum PICP level could be considered a good marker of osteoblastic activity.**

## Introduction

Collagen type I is the most abundant collagenic protein in the organism. Not only is it an important constituent

of soft connective tissue, but it also comprises most of the organic matrix of bone (1-3). Procollagen type I is the biosynthetic precursor of collagen type I. It is larger in size and contains additional aminoacidic sequences at both ends with respect to its final product. These aminoacid fragments are cleaved by specific proteases before the final collagen molecule is assembled (4). One of these fragments is the carboxyterminal propeptide of PICP.<sup>3</sup> Its level in the serum can be evaluated.

A radioimmunoassay for PICP was described about 20 years ago, but only recently, a relatively low-cost reproducible assay method has been developed which allows its determination in various metabolic bone diseases (5, 6).

The amount of PICP released into the blood seems to be directly related to the number of collagen molecules formed; thus, PICP determination quantifies collagen type I synthesis in the same manner as C peptide of proinsulin is proportional to the endogenous insulin production (6-8). The PICP molecule, because of its size, cannot be filtered by the renal glomeruli and is eliminated by the hepatic endothelial cells; therefore, it accumulates in the blood in the presence of liver diseases (9-11).

Early studies showed that PICP concentrations varied with age and in the presence of metabolic bone diseases (12). More recently, a positive correlation between PICP and the histomorphometric parameters of new bone formation has been observed (13).

High concentrations of PICP have also been found in patients with Paget's bone disease in the exuberant and abnormal stage of new bone formation (14, 15).

Studies on women with osteoporosis confirmed the positive correlation of PICP levels with the histomorphometric and biochemical markers of new bone formation (16). Furthermore, significant increases of PICP as well as BGP have been reported in osteoporotic patients on treatment with activators of new bone formation, such as androgens and parathyroid hormone 1-34 (17, 18). On the contrary, the serum levels of PICP decreased after the drugs which decrease bone resorption (18).

Some authors report high concentrations of the procollagen type III N-peptide in patients with bone metastases from breast cancer. Procollagen has therefore been proposed in the early diagnosis and follow-up of bone metastases. The results of this, however, appear fragmentary and uncertain (19, 20).

It is known that sclerotic bone metastases are commonly associated with increased serum Alk.Ph. and BGP,

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<sup>3</sup> The abbreviations used are: PICP, procollagen type I; Alk.Ph., alkaline phosphatase; BGP, bone Gla protein; UHOP:Cr, urinary hydroxyproline:creatinine ratio.

which mainly reflect the activity of osteoblasts (21). Moreover, osteolytic bone metastases are commonly associated with an increased UHOP:Cr, which is considered a sufficient measure of bone resorption. Recently, a high correlation of the above index with pyridinoline crosslinks as a measure of bone collagen breakdown has been observed (22).

The aim of the present study is to evaluate the diagnostic value of PICP determination in bone metastases and its possible use in monitoring patients with this disease. Furthermore, the correlations between PICP and BGP, and Alk.Ph. and UHOP:Cr, have been studied.

### Materials and Methods

Forty-seven patients with secondary bone lesions (25 males and 22 females between 36 and 80 years of age) have been studied. Bone lesions were predominantly osteolytic in 27 cases (5 myeloma, 15 breast, 3 lung, 2 kidney, 1 bladder, 1 thyroid) and osteoblastic in 20 (18 prostate and 2 breast).

Patients with malignant disease without overt bone metastases, as well as patients with hypercalcemia, liver and/or kidney disease, and under treatment with drugs that influence bone metabolism were not included.

Morning venous blood and 24-h urinary collections were taken in all patients for the evaluation of PICP, BGP, Alk.Ph. and UHOP:Cr.

After the basal check-up, antineoplastic chemotherapy was administered to all patients, and the above markers were evaluated after 3, 6, 9, and 12 cycles of treatment.

PICP was assayed by radioimmunoassay (Orion Corporation, Farnos Diagnostica, Turku, Finland).

The radioimmunoassay for analyzing the concentration of the carboxyterminal propeptide of PICP was established by isolating type I procollagen from the medium of primary cultures of human skin fibroblasts and by digesting it with highly purified bacterial collagenase to liberate PICP. The radioimmunoassay was performed using polyclonal rabbit antibodies as described in detail by Melkko *et al.* (6). The procedure requires an initial 2-h incubation at 37°C of 100 µl of sample or standard with 200 µl of rabbit antiserum and 200 µl of radiolabeled tracer (<sup>125</sup>I), followed by the addition of 500 µl of separating reagent (a suspension of goat antirabbit globulins bound to solid particles). After another 30-min incubation period at room temperature, the test tubes are centrifuged, the supernatant aspirated, and the tubes counted with a gamma counter. The sensitivity of this method is 1.2 ng/ml. The intraassay coefficient of variation is around 6%. The corresponding interassay variation is around 8.5%. The reference interval of PICP in normal human serum ( $n = 50$ ) was found to vary between 70 and 160 ng/ml.

BGP, Alk.Ph., and UHOP:Cr were evaluated by previously described methods (23); the normal ranges in our laboratory were: BGP, 3–8 ng/ml; Alk.Ph., 4–13 King-Armstrong units; UHOP:Cr, 25 ± 7.2 mg/g.

### Results

Of the 27 patients with lytic-type lesions, 4 had high levels of PICP, 11 high levels of Alk.Ph., and 14 high levels of UHOP:Cr; in all cases BGP was within the normal range. The parameters were normal in all 5 patients with multiple myeloma. Of the 20 patients with

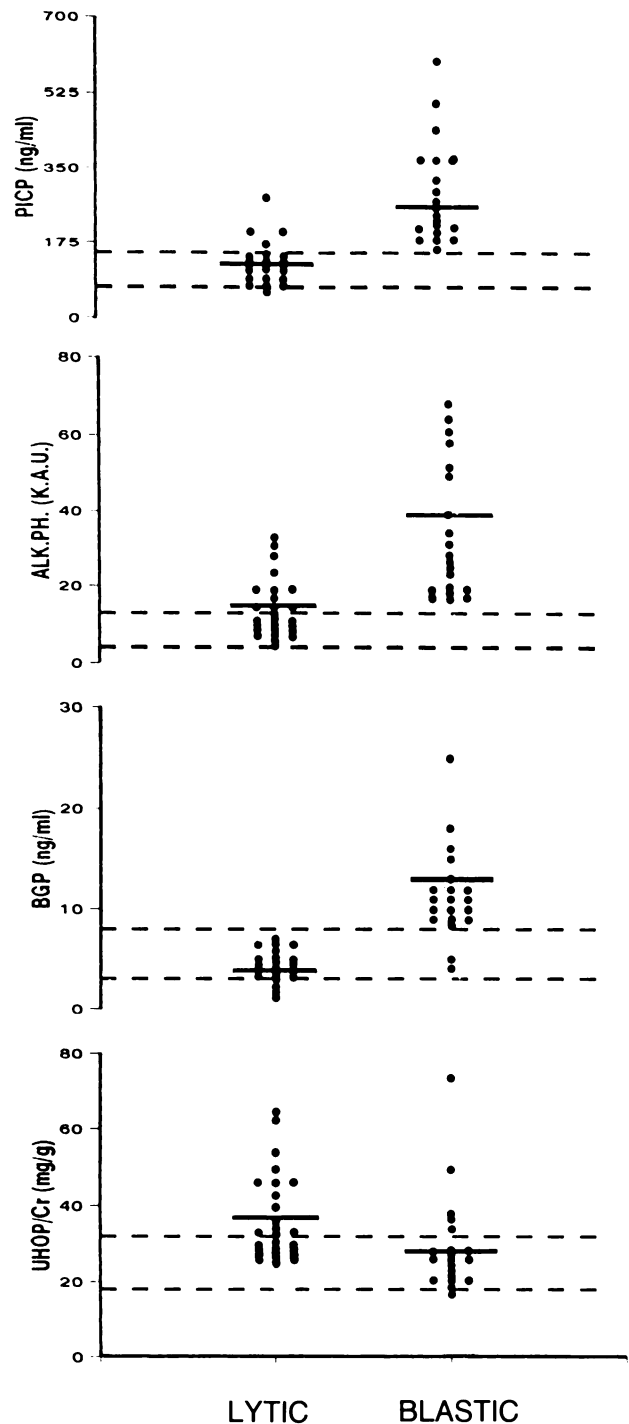


Fig. 1. PICP, Alk.Ph., BGP, and UHOP:Cr in 47 cancer patients: 27 with osteolytic lesions (LYTIC) and 20 with osteoblastic metastases (BLASTIC). —, mean; - - -, normal range.

osteoblastic lesions PICP and Alk.Ph. were high in all cases, BGP in 18 cases, and UHOP:Cr in 5 cases (Fig. 1).

In the group of patients with lytic and blastic lesions, a statistically significant relationship between PICP and BGP ( $r = 0.57$ ;  $P < 0.001$ ), PICP and Alk.Ph. ( $r = 0.80$ ;  $P < 0.001$ ), and BGP and Alk.Ph. ( $r = 0.44$ ;  $P < 0.01$ ) was

Table 1 Biochemical measurements (mean  $\pm$  SD) in 13 patients with widespread sclerotic bone metastases who achieved objective response or stable disease during antilastic treatment

Baseline values and values after 3, 6, 9, and 12 cycles of chemotherapy (epirubicin, 30 mg/m <sup>2</sup> weekly).	n.v. <sup>a</sup>	Baseline	3rd	6th	9th	12th
UHOP:Cr (mg/g)	25 $\pm$ 7.2	41.8 $\pm$ 10.4	39.2 $\pm$ 9.1	36.4 $\pm$ 8.4	35.3 $\pm$ 7.8	33.2 $\pm$ 8.8
Alk.Ph. (King-Armstrong units)	4–13	44.8 $\pm$ 18.6	39.6 $\pm$ 17.1	37.6 $\pm$ 16.8	35.4 $\pm$ 14.4	31.4 $\pm$ 11.3
BGP (ng/ml)	3–8	13.8 $\pm$ 6.6	12.2 $\pm$ 5.3	11.3 $\pm$ 4.8	9.4 $\pm$ 2.7	9.1 $\pm$ 2.5
PICP (ng/ml)	70–160	312.7 $\pm$ 151.6	232.5 $\pm$ 125.6	191.2 $\pm$ 106.4	121.8 $\pm$ 81.4	106.3 $\pm$ 65.6
			n.s.	n.s.	<i>P</i> < 0.05	<i>P</i> < 0.05
			n.s.	<i>P</i> < 0.05	<i>P</i> < 0.005	<i>P</i> < 0.001

<sup>a</sup> n.v., normal values; *P*, significant differences during treatment versus pretreatment values; n.s., not significant.

observed. Moreover, no relationship between PICP and UHOP:Cr, between BGP and UHOP:Cr, or between Alk.Ph. and UHOP:Cr was found.

In the 20 patients with predominant osteoblastic metastases a statistically significant correlation between PICP and Alk.Ph. ( $r = 0.62$ ;  $P < 0.003$ ) was seen, whereas no correlation between PICP and BGP or between BGP and Alk.Ph. was found.

Table 1 lists the results (mean  $\pm$  SD) in 13 of 20 patients with predominant osteoblastic metastases who achieved objective response or stable disease during chemotherapy; Alk.Ph., BGP, and UHOP:Cr decreased progressively, but they were still above normal values after 12 weekly cycles of chemotherapy. PICP significantly decreased at the sixth cycle of treatment. Its reduction was more rapid and striking as compared to the other two markers of bone formation (Alk.Ph. and BGP). Fig. 2 shows the individual PICP and BGP responses in these patients; as observed, PICP decreased more rapidly than BGP in each case.

Four cases with osteolytic metastases had high levels of PICP. In one of these, a remission of the bone lesions occurred with a striking reduction of UHOP:Cr without significant modifications of PICP levels, while no alteration of the osteolytic lesions was observed in the other 3 patients. Three subjects with normal PICP levels showed recalcification of the osteolytic lesions without significant changes in the PICP levels.

## Discussion

The main function of osteoblasts is to produce the organic matrix of bone which is mainly composed of collagen type I. During collagen type I formation, a COOH-terminal fragment (PICP) is cleaved from each molecule, and this fragment is rapidly released into the extracellular compartment. Serum PICP concentrations may therefore theoretically be used as a highly specific marker for osteoblast activity (13, 16).

Our results seem to support this hypothesis. Indeed, PICP levels were found to be in correlation with the traditional markers of new bone formation (Alk.Ph. and BGP); however, there was no correlation with UHOP:Cr, an index of bone resorption (Table 1) (22, 24, 25).

High values of PICP with respect to the other markers were found in 4 cases of osteolytic metastases (Fig. 1). This finding may be related to the high bone turnover in this type of metastasis. It is known that in osteolytic metastases a large osteoclastic activity is accompanied by a proportional osteoblastic activity (26, 27). Hence, in addition to PICP, Alk.Ph. and BGP may also be elevated in these cases (28, 29).

Myeloma patients showed normal to low values for PICP, BGP, and Alk.Ph. with respect to the other patients with osteolytic lesions, as recently described by Elomaa *et al.* (30). Indeed, myeloma is known to be a heterogeneous neoplasia for bone turnover, and most of the

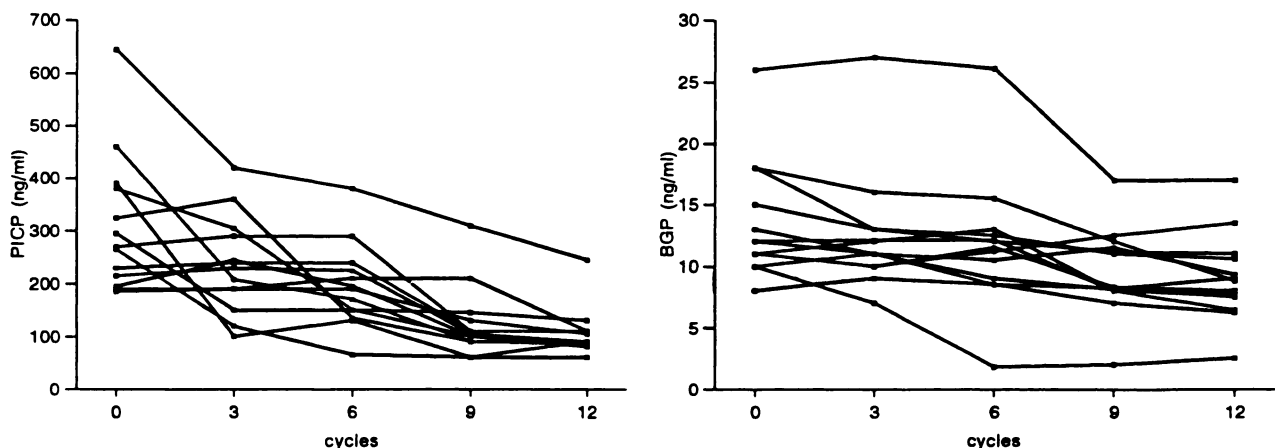


Fig. 2. Individual PICP and BGP responses of 13 patients with widespread sclerotic bone metastases who achieved objective response or stable disease during chemotherapy.

myeloma patients have low to normal BGP and Alk.Ph. levels. The bone lesions of myeloma are essentially osteolytic without an overt osteoblastic repair (31).

The highest levels of PICP, BGP, and Alk.Ph. were observed in patients with osteoblastic metastases (Fig. 1). In these patients the exuberant osteoblastic activity produces an abnormal quantity of organic matrix, which may later be transformed into new bone (31, 32).

It is well known that BGP is more frequently elevated with respect to Alk.Ph. in patients with osteoblastic or mixed-type metastases. Furthermore, BGP levels tend to reach normal ranges if the antitumor treatment is effective (21, 33). In patients with osteoblastic metastases that achieved an objective response or stable disease during chemotherapy, PICP decreased in a manner similar to that of BGP but unlike Alk.Ph. (Table 1). Furthermore, Alk.Ph. levels are significantly altered only for large metabolic variations in bone turnover. The individual PICP and BGP responses in these patients are illustrated in Fig. 2; as observed from the graph, PICP is more sensitive than BGP in each case.

It is possible to conclude that PICP may be a sensitive marker for osteoblastic activity in patients with bone metastases, which would be particularly useful in monitoring patients with osteoblastic and mixed-type metastases.

Our study excluded patients with liver metastases, since we aimed to investigate the difference in behavior between PICP and other bone markers in patients with only bone metastases. Indeed, it is known that only important diseases of the liver influence the circulating levels of PICP (10). Obviously, in patients with both liver disease and bone metastases PICP will appear to be less useful than in patients without liver disease. However, we observed a significant reduction of PICP levels in some patients with liver and bone metastases (excluded from this study) when a remission of the bone metastases occurred.

In basal conditions (*i.e.*, before therapeutic treatments) PICP levels correlate more strictly with Alk.Ph. than with BGP ( $r = 0.80$  versus  $0.57$ ), and this finding is more evident in osteoblastic metastases where no correlation between Alk.Ph. and BGP exists. Hence, PICP is an interesting indicator of bone matrix production in osteoblastic metastases.

According to these findings, one may think that bone matrix production is accompanied by an increase in Alk.Ph. and BGP; however, this seems to be primarily linked to a later stage of osteoblast activity (34, 35).

Thus, Alk.Ph., PICP, and BGP appear to be markers of different stages of osteoblast activity. Alk.Ph. and PICP are both present during collagen production. Alk.Ph. is an enzyme involved in the intrinsic activity of the osteoblasts when osteoblasts are activated to produce collagen in which the COOH-terminal fragment (PICP) is immediately released into the circulation. The significance of BGP is still unclear. It may be hypothesized to have a role in a later stage, closer to the mineralization stage of bone formation.

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