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Osteocalcin: a Potential Marker of Metastatic Bone Disease and Response to Treatment

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Abstract—*Serum osteocalcin (BGP) is an osteoblast product that probably reflects the rate of bone formation. It is a potential marker of skeletal metastases and, to investigate this, BGP was measured by radioimmunoassay in the serum of normal subjects and patients with breast or prostate cancer. Significantly higher levels were found in patients with metastatic bone disease in comparison to both normal subjects* ($P < 0.001$) *and patients with non-metastatic cancer* ($P < 0.05$ *for breast cancer and* <0.001 *for prostate cancer). The range of values was wide. Levels were higher in sclerotic than lytic bone metastases* ($P < 0.01$) *and lower in patients with hypercalcaemia* ($P < 0.001$).

Serial measurements of BGP were made in 53 patients with skeletal metastases from breast cancer receiving systemic therapy. At 1 month BGP rose by >0.5 ng/ml *in 15/16 responding patients compared with 7/23 patients with progressive disease* ($P < 0.01$). *Responding patients also showed a rise in the bone isoenzyme of alkaline phosphatase and a paradoxical deterioration in the bone scan appearance, both reflecting a flare in osteoblast activity. The early increase in responding patients was followed by a gradual decrease over subsequent months as the osteoblast reaction induced by systemic therapy subsided.*

We conclude that BGP measurements reflect a wide variability of bone formation rates in metastatic bone disease. Bone formation was usually increased, particularly when metastases were sclerotic in appearance, but in patients with hypercalcaemia the low BGP levels suggest uncoupling of bone resorption and formation. Serial measurements of BGP may be useful in monitoring response to treatment.

INTRODUCTION

OSTEOCALCIN (bone GLA protein or BGP) is a small protein (molecular weight 5600 D), containing three residues of the vitamin K dependent amino acid α-carboxyglutamic acid. It is unique to bone and tooth dentine [1, 2]. BGP is synthesized in osteoblasts [3] and, once secreted, binds strongly to hydroxyapatite. The small fraction of newly synthesized protein which fails to bind to hydroxyapatite appears in the circulation [4] from which it is rapidly cleared by the kidney with a half-life of 4–5 min. Human BGP is immunologically indistinguishable from calf BGP [5] and measurement of serum levels is possible by radioimmunoassay using an antibody raised against bovine BGP [5, 6].

Serum levels probably reflect new bone formation [7] and have been shown to correlate with the bone mineralization rate as measured by histomorphometry [8]. Raised levels are seen in conditions associated with accelerated bone turnover such as Paget's disease [6], hyperparathyroidism [9] and some patients with osteoporosis [8] or metastatic bone disease [6, 10].

Bone metastases are a common clinical problem in breast and prostate cancer. Malignant cells secrete paracrine factors which stimulate osteoclasts to resorb bone resulting in areas of lytic bone destruction. Typically, in an attempt to repair these areas and reflecting the normal coupling between bone resorption and formation, an increase in osteoblast activity also occurs [11]. However in some cases of metastatic bone disease, particularly from multiple myeloma [12] or complicated by hypercalcaemia [9], low values are seen suggesting disruption of the normal coupling.

Following successful systemic therapy for bone metastases, a flare in osteoblast activity is seen characterized by a rise in alkaline phosphatase bone isoenzyme (ALP-BI) [13] and a paradoxical deterioration of the bone scan appearances during the first months of treatment [14]. This early healing phase may be detectable also by changes in BGP levels [12].

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Table 1. Characteristics of the patient population groups studied

Normal women (<75 years)	47
Women with early breast cancer (<75 years)	21
Women with breast cancer and progressing bone metastases*	70
Women with advanced breast cancer and hypercalcaemia	23
Normal men	13
Men with clinically localized prostate cancer	42
Men with prostate cancer and progressing bone metastases	38

*Serial measurements performed.

The aim of this study was to measure BGP in patients with metastatic bone disease, compare these values with normal controls, and determine whether changes in BGP induced by systemic treatment were useful in assessing the response to therapy.

MATERIALS AND METHODS

The subjects included in this study are shown in Table 1 and include normal controls, women with early and advanced breast cancer, men with localized and metastatic prostate cancer and women with hypercalcaemia secondary to advanced breast cancer. Serum for measurement of BGP was obtained from a morning blood sample following centrifugation. All samples were frozen within 6 h and stored at −20°C. Under these conditions BGP is stable for more than one year (Table 2). In addition ALP-BI activity was measured on these samples.

BGP was measured by radioimmunoassay using the Immuno Nuclear Osteocalcin RIA kit. Simultaneous addition of sample, rabbit anti-bovine BGP and [^{125}I]bovine BGP was followed by overnight incubation at 2–8°C. Phase separation was accomplished by the addition of a complex containing goat anti-rabbit serum, carrier rabbit serum and polyethylene glycol. After a further 2 h incubation the sample was centrifuged, decanted and the precipitate counted in a γ scintillation counter. All values for standards and samples were determined in duplicate. The inter- and intra-assay variations were 7% and 5% respectively. The sensitivity of the assay was 0.3 ng/ml.

Alkaline phosphatase is commonly measured in clinical medicine but is not specific to bone. Raised levels in patients with advanced breast cancer may result from disease in bone or liver and isoenzyme separation is preferable for monitoring therapy.

Table 2. Stability of osteocalcin in serum under different storage conditions

Storage conditions	No. of samples	Duration of storage	% of T_0
+20°C	20	4 h	85 (10)
+4°C	20	18 h	87 (13)
−20°C	13	1 year	99 (18)

T_0 is the value obtained from serum frozen within 30 min of sample collection.

Qualitative separation by electrophoresis is not difficult but separation is incomplete unless complex pretreatment of the serum is performed [15]. For quantification of the ALP-BI activity the heat-inactivation technique described by Moss and Whitby was used [16].

Bone resorption was indirectly assessed by serum calcium and urinary calcium excretion. A spot sample of urine was collected after an overnight fast for measurement of urinary calcium and creatinine. Hypercalcaemia was treated by intravenous saline, before serum for BGP was taken, followed by a single infusion of the diphosphonate APD [17].

Serial measurements of BGP were made in 70 women with progressive bone metastases from breast cancer. The median age of this group was 58 years (range 32–82). In 26/70 (31%) metastatic disease was clinically confined to the skeleton. Metastatic spread in the other 44 patients was to all common sites including liver and lung. No attempt was made to select patients on any particular therapy resulting in a diversity of systemic treatments as listed in Table 3.

BGP and ALP-BI were measured before and 1 month after starting treatment. Subsequent measurements of BGP and ALP-BI were made at monthly and 3 monthly intervals respectively until radiological evidence of progressive disease occurred. Radionuclide bone scans with technetium^{99m} labelled methylene diphosphonate (MDP) and plain radiographs of abnormal areas were performed before treatment and repeated 3 monthly.

The UICC criteria of response were used to assess the results of treatment [18]. Radiological evidence of healing with sclerosis of lytic metastases was necessary for a patient to be termed a 'responder'. In this study, the response category 'no change' denoted stabilization of disease for a minimum of 12 weeks. The changes in BGP seen in response to systemic therapy were correlated with the UICC response in bone.

Seventy patient-treatments are available for analysis. Seventeen are not assessable for response in bone and the reasons for exclusion are shown with the response data in Table 4. Sixteen patients achieved a partial response in bone, 14 showed no change and 23 had progressive disease. No patient had a complete response.

Table 3. Systemic treatments in patients with metastatic breast cancer

Treatment type	No. of patients
Tamoxifen	6 (6)
Tamoxifen and prednisolone	14 (12)
Ovarian ablation	1 (1)
Aminoglutethimide and hydrocortisone	8 (4)
Progestogens	4 (3)
Corticosteroids	2 (0)
Adriamycin	9 (7)
CMF (cyclophosphamide, methotrexate, 5-fluorouracil)	18 (16)
Mitomycin C and vinblastine	3 (1)
Prednimustine	4 (2)
4-Deoxydoxorubicin (esorubicin)	1 (1)
	70 (53)

Figures in brackets indicate number of treatments evaluable for UICC response in bone.

Table 4. Response to systemic treatment

53 patients
70 treatments
53 treatments evaluable for response in bone (UICC)

16 partial response
14 no change
23 progressive disease

17 treatments non-evaluable for response in bone

12 early death or early extra-skeletal progression.
1 lost to follow-up
2 treatment toxicity
2 radiotherapy to all evaluable sites of disease.

Standard statistical methods were used for analysis. The Mann–Whitney non-parametric test was used for the difference between patients and controls, linear correlation for relation between measurements and the chi-squared and paired Student's *t* tests for variation of BGP with time.

RESULTS

The BGP concentrations in the seven subject groups are summarized in Table 5. Our normal range for BGP in women was 1.4–3.8 ng/ml (mean ± 2 S.D.). These values are shown as in Fig. 1 compared with the groups of patients with early breast cancer, bone metastases and hypercalcaemia secondary to advanced breast cancer. BGP was above the normal range in 35 patients (50%) of women with bone metastases and the values significantly higher in this group compared to both normal women ($P < 0.001$) and women with early breast cancer ($P < 0.05$). However, a wide range (0.5–26.6 ng/ml) was seen in the women with skeletal metastases. Those with predominantly sclerotic metastases had higher values than those with lytic disease ($P < 0.01$) (Table 5). BGP levels were lower in patients with hypercalcaemia of malignancy than either normocalcaemic patients with bone metastases ($P < 0.001$) or those with early breast cancer ($P < 0.05$).

Our normal range in men was 1.7–4.9 ng/ml (mean ± 2 S.D.) and the individual values are shown in Fig. 2 in comparison with the groups of patients with localized prostate cancer and bone metastases from prostate cancer. BGP was above the normal range in 21/38 (53%) of men with bone metastases and the values significantly higher than those of normal men ($P < 0.001$) and those with localized disease ($P < 0.001$) (Table 5). Levels in metastatic prostate cancer were higher than in metastatic breast cancer ($P < 0.01$).

A significant correlation between BGP and ALP-BI was seen in both normocalcaemic patients with bone metastases ($r = 0.47$, $P < 0.001$) and hypercalcaemic patients with advanced breast cancer ($r = 0.66$, $P < 0.01$). There was no correlation between BGP and urinary calcium excretion or serum calcium. BGP levels were unchanged after control of hypercalcaemia with APD.

The serial changes in BGP during systemic therapy for breast cancer with bone metastases are

Table 5. Osteocalcin levels in the seven study groups

Group	Number	Median	Mean	S.D.
Female controls	47	2.6	2.6	0.6
Early breast cancer	21	3.1	3.3	1.3
Breast cancer and bone metastases				
(all patients)	70	3.8	5.0	4.3
(sclerotic disease)	19	7.1	7.1	4.1
(lytic disease)	51	3.5	4.1	4.0
Breast cancer and hypercalcaemia*	23	1.8	2.5	2.0
Male controls	13	2.5	2.8	1.1
Localized prostate cancer	42	2.7	3.2	1.7
Prostate cancer and bone metastases	38	5.3	5.9	3.5

*Measurements performed after intravenous rehydration for 24–48 h.

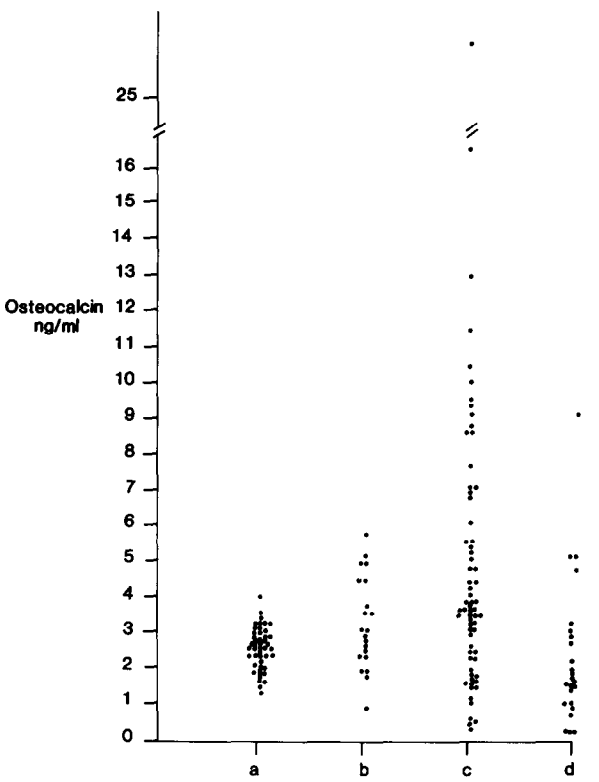

Fig. 1. BGP levels in women: (a) normal; (b) with early breast cancer; (c) with bone metastases from breast cancer; (d) hypercalcaemia secondary to advanced breast cancer.

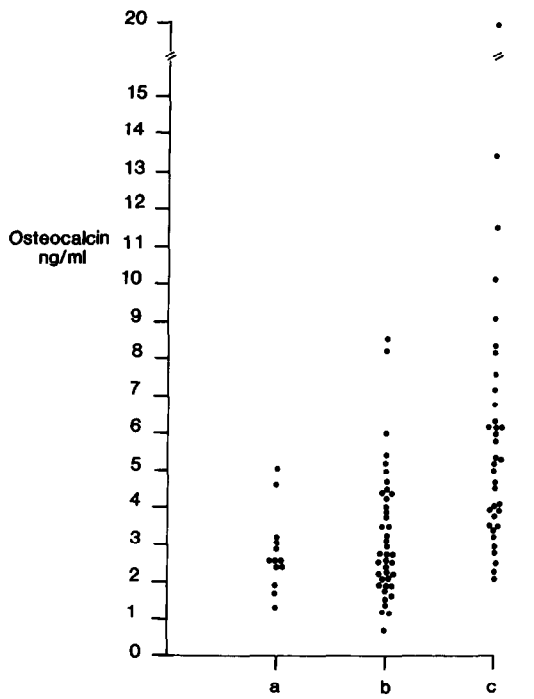

Fig. 2. BGP levels in men: (a) normal; (b) with localized prostate cancer; (c) with bone metastases from prostate cancer.

shown in Fig. 3. The percentage change from baseline of the median values at each month for the UICC response categories are plotted. BGP rose at 1 month by > 0.5 ng/ml in 15/16 responding patients (mean rise 2.1 ng/ml, median 2.3 ng/ml) compared with 7/23 patients with progressive disease (mean rise 0.04 ng/ml, median −0.1 ng/ml). Both the number of patients in each response group showing a rise and the individual changes from baseline were significantly different ($P < 0.01$). Patients in the no change category had a lesser increase (mean 1.94 ng/ml, median 1.35 ng/ml) than responding patients. The early increase in responding patients was followed by a gradual decrease over subsequent months as the osteoblastic reaction induced by successful therapy subsided.

Other manifestations of the increase in osteobast activity were a rise in ALP-BI and paradoxical deterioration in the bone scan appearances. Figure 4 shows the baseline and 1 month values of ALP-BI and BGP in the 16 responders. A mean increase in ALP-BI at 1 month of 233 i.u./l was seen in responding patients compared with only 42 i.u./l in patients with progressive disease ($P < 0.05$). The flare in ALP-BI was short-lived, returning to near pre-treatment levels at 3 months, whereas BGP levels remained higher for longer, often not falling to baseline levels for many months (Fig. 5). The osteoblast flare was visible also on the bone scan. In 12/16 responding patients a repeat bone scan

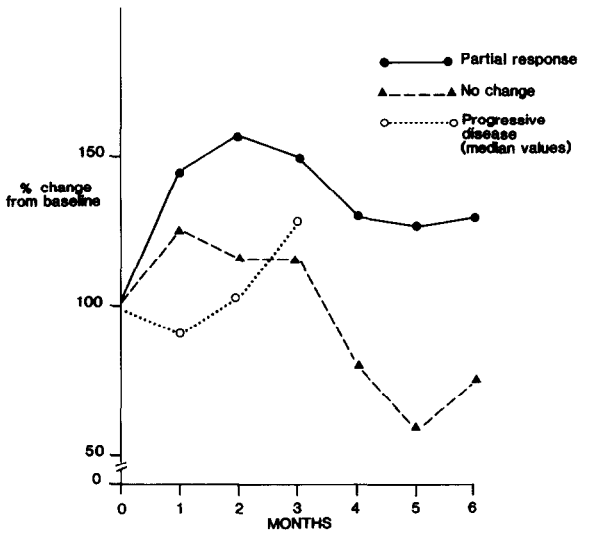

Fig. 3. Serial changes in BGP during systemic therapy of breast cancer with bone metastases. Data are presented as a percentage of the baseline values for each UICC response group. Points represent median values.

performed after 3 months treatment showed increased focal activity in existing lesions and the appearance of apparent new lesions. Bone scan appearances subsequently improved when repeated after 6 months. These results are reported in detail elsewhere [14].

DISCUSSION

We have shown that serum BGP is elevated in the majority of patients with metastatic bone disease from both breast and prostate cancer. A wide range of values were seen with the highest levels in patients with a large sclerotic component to their disease and low, sometimes subnormal, values in those with lytic destruction or hypercalcaemia. There was a significant correlation between BGP and ALP-BI activity and none with urinary calcium excretion or serum calcium indicating that BGP probably reflects bone formation rather than resorption. Indeed osteocalcin was unaffected by inhibition of bone resorption by APD for the treatment of hypercalcaemia.

The wide range of values in patients with the same tumour type illustrates the heterogeneity of bone formation rates in metastatic disease. As might be expected, levels were higher in patients with sclerotic bone metastases. Values were higher in advanced prostate cancer, where metastases are typically sclerotic, than breast cancer where metastases throughout the spectrum of lytic to sclerotic occur. Other studies have reported raised BGP serum levels in patients with bone metastases

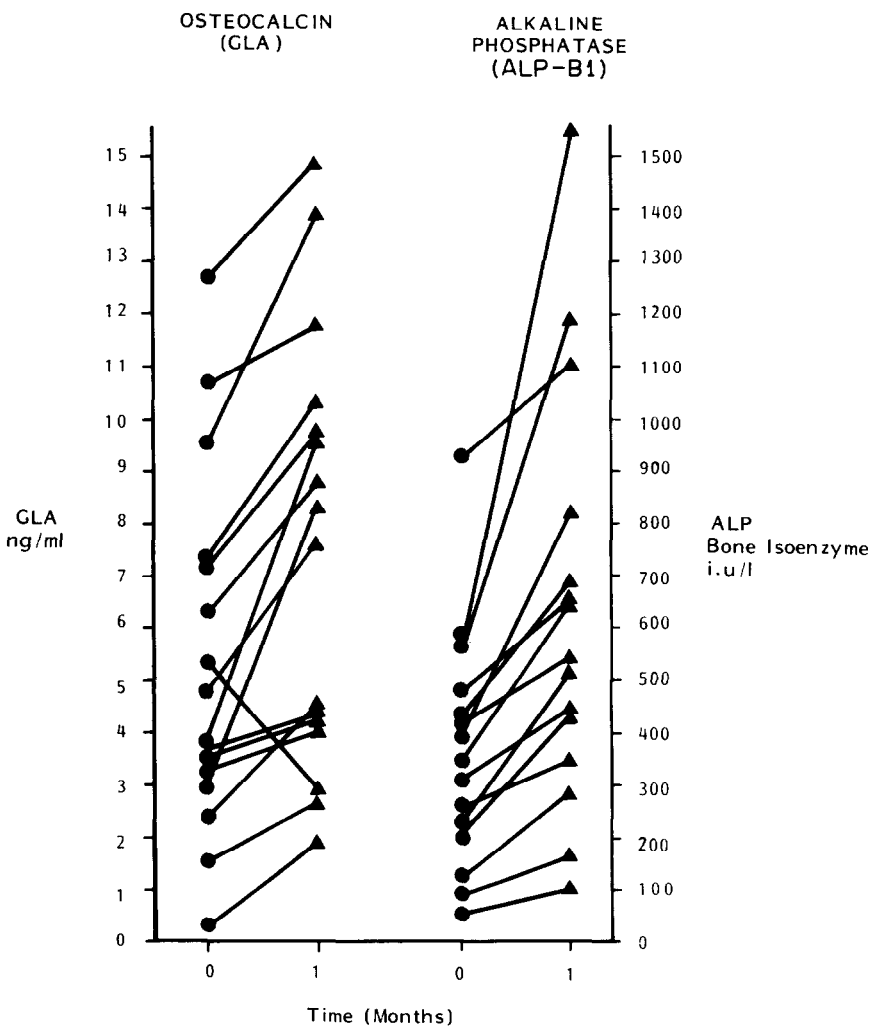

Fig. 4. BGP and ALP-BI levels before and 1 month after successful systemic therapy (individual values in responding patients).

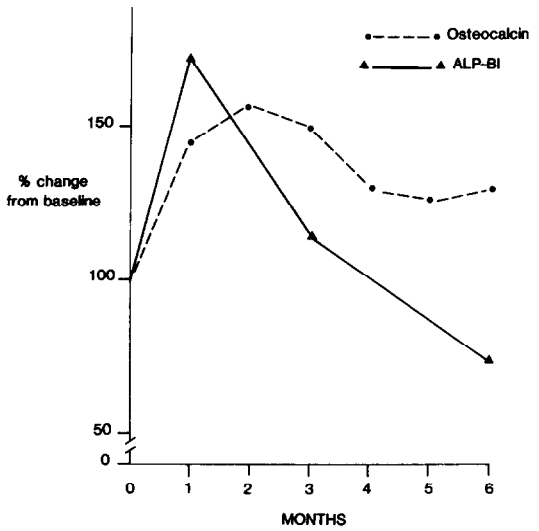

Fig. 5. Serial BGP and ALP-BI levels following successful systemic therapy (responders only). Points represent median values.

[6, 10] but Delmas reported normal values in nine patients with bone metastases [9]. Patient selection and the heterogeneous nature of metastatic disease probably account for this variation.

The low values in hypercalcaemia of malignancy have been noted previously [9] but are not invariable [19]. Subnormal levels of BGP indicate an uncoupling of the normal relationship between bone resorption and formation. Bone histology in hypercalcaemia of malignancy has confirmed the impaired bone formation with a decrease in the osteoid seam width in the presence of increased resorption [9]. It is unknown whether the uncoupling is brought about by local paracrine factors produced by the tumour cells or a systemic humoral factor. However, the histological appearances are found at non-metastatic sites and in patients with humoral hypercalcaemia without bone metastases suggesting that systemic factors probably contribute in many cases.

Unlike many other cancers which commonly spread to bone the clinical course of advanced breast cancer is relatively long and can be modified by successful systemic therapy [20]. Assessment of response in bone to systemic therapy is difficult and has relied on radiological evidence of healing (recalcification) within lytic disease [18]. This may not be apparent for many months and more sensitive response criteria are needed to monitor treatment and improve the reporting of clinical trials [11]. Response to systemic therapy is associated with an increase in osteoblast activity producing a transient rise in ALP-BI [13] and the flare response seen on bone scintigraphy [14, 21].

The serial BGP levels obtained during systemic therapy for breast cancer were useful in identifying responding patients. Levels rose at 1 month in 94% of responders with a concomitant rise in ALP-BI. BGP levels peaked at 2–3 months, declining gradually thereafter as the remission continued and remineralization took place. The flare in ALP-BI subsided more quickly than BGP, suggesting these two markers may reflect different aspects of osteoblast function. Similar findings are found in multiple myeloma, a disease characterized by impaired osteoblast activity [22]. Here response to therapy results in an increase in BGP from subnormal to normal as coupling is restored [12].

Patients with radiologically stable disease showed a less consistent pattern. Some patients showed an osteoblastic flare which was similar to that seen in responding patients. However, changes in BGP did not predict patients achieving a prolonged (>6 months) period of disease stabilization. These patients probably have a tumour response as their survival expectation is similar to that of responding patients. Nevertheless, it seems that on the evidence of both plain radiography and biochemical monitoring that the usual end-result of a tumour response, that is remineralization, does not occur in these patients. The rise in some patients with progressive disease may reflect an increase in sclerotic component of their disease.

In conclusion, we have found BGP to be an easily measured biochemical index of osteoblast function. Raised levels were common in metastatic bone disease but the range of values was large and it seems unlikely, although not tested in this study, that BGP could be a useful marker for detecting early metastatic involvement of the skeleton. BGP may be useful for investigating the pathogenesis of hypercalcaemia as low levels were found in hypercalcaemia of malignancy due to uncoupling of bone resorption and formation whereas in hyperparathyroidism BGP is raised [9, 23]. Our serial measurements suggest BGP may be a useful marker for monitoring therapy particularly when combined with other biochemical parameters such as ALP-BI and urinary calcium excretion [24].

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