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CLINICAL INVESTIGATION

Bone GLA protein in predialysis chronic renal failure. Effects of $1,25(OH)_2D_3$ administration in a long-term follow-up

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Bone GLA protein in predialysis chronic renal failure. Effects of $1,25(OH)_2D_3$ administration in a long-term follow-up. Serum bone GLA protein (BGP) was measured by radioimmunoassay in 42 patients (age, 47.5 ± 16.6 years; serum creatinine, 4.32 ± 1.9 mg/dl) with predialysis chronic renal failure (CRF). Nineteen patients were studied within a short period of time, while 23 were followed with repeated measurements of serum BGP, creatinine, iPTH, and alkaline phosphatase (AP) for a mean period of 17.1 ± 8.1 months. Eleven of these patients were treated with $1,25(OH)_2D_3$ for a mean of 16.8 ± 6.4 months. In 23 patients at various stages of CRF, a transiliac bone biopsy was performed for histomorphometric evaluation. In the untreated patients, serum BGP was higher than normal and showed a positive correlation with creatinine levels ($P < 0.001$). Serum BGP was also positively correlated with iPTH, AP, serum phosphate, active resorption surface, active osteoblastic surface, osteoid surface, and volume. During treatment with $1,25(OH)_2D_3$, BGP, iPTH, and AP were significantly lower than in the untreated patients. The reduction in iPTH and BGP was proportional, while BGP and AP no longer correlated. Repeated measurements of BGP during the long-term follow-up showed a progressive rise in the untreated patients and a downward course of BGP levels during treatment. In conclusion, serum BGP increases progressively in CRF, rising with advancing renal damage in close correlation with iPTH, AP, and the severity of renal osteodystrophy. Treatment with $1,25(OH)_2D_3$ causes a parallel decline in BGP and iPTH levels and dissociation between BGP and AP can be observed. Compared to AP, BGP seems to be a more reliable index of secondary hyperparathyroidism and potentially more useful in the long-term monitoring of treatment with $1,25(OH)_2D_3$.

GLA protéine osseuse au cours de l'insuffisance rénale chronique prédialytique. Effets de l'administration de $1,25(OH)_2D_3$ au cours d'une étude à long terme. La GLA protéine osseuse sérique (BGP) a été mesurée par dosage radioimmunologique chez 42 malades (âge 47,5 ± 16,6 ans; créatininémie 4,32 ± 1,9 mg/dl) en insuffisance rénale chronique prédialytique (CRF). Dix-neuf malades ont été étudiés pendant une courte période de temps, tandis que 23 autres ont été suivis avec des mesures répétées de BGP, de créatinine, d'iPTH et de phosphatase alcaline (AP) sériques pendant une période moyenne de 17,1 ± 8,1 mois. Onze de ces malades ont été traités avec du $1,25(OH)_2D_3$ pendant 16,8 ± 6,4 mois en moyenne. Chez 23 malades à des degrés divers de CRF, une biopsie osseuse transiliaque a été effectuée en vue d'une évaluation histomorphométrique. Chez les malades non traités, la BGP sérique était plus élevée que chez lesnormaux, et était positivement corrélée avec les niveaux de créatinine ($P < 0,001$). La BGP sérique était également positivement corrélée avec iPTH, AP, la phosphatémie, la surface de résorption active, la surface ostéoblastique active, la surface ostéoïde et le volume. Pendant le traitement avec $1,25(OH)_2D_3$, BGP, iPTH et AP étaient significativement plus faibles que chez les malades non traités. La réduction d' iPTH et de BGP était proportionnelle, alors que BGP et AP ne restaient plus corrélés. Des mesures répétées de BGP pendant le suivi à long terme ont révélé une augmentation progressive chez les malades non traités, et un abaissement des niveaux de BGP au cours du traitement. En conclusion, la BGP sérique s'élève progressivement au cours de CRF, augmentant avec la progression des lésions rénales de façon étroitement corrélée avec iPTH, AP et la sévérité de l'ostéodystrophie rénale. Le traitement par $1,25(OH)_2D_3$ entraîne une diminution parallèle de BGP et des niveaux d' iPTH, et une dissociation entre BGP et AP peut être observée. Par rapport à AP, BGP semble un index plus fiable d'hyperparathyroïdisme secondaire, et potentiellement plus utile pour la surveillance à long terme du traitement par $1,25(OH)_2D_3$.

Osteocalcin, or bone GLA protein (BGP), is a vitamin K-dependent protein of bone with 49 aminoacids that contains three γ-carboxyglutamic acid residues. BGP is the most abundant (25%) non-collagenous protein of bone [1]. Small concentrations of this protein are found also in the serum and are detectable by radioimmunoassay [2]. In the last few years, BGP has been found to be endowed with a rather selective binding affinity for insoluble Ca^{++} salts, especially hydroxyapatite crystals, presumably through the GLA residues [3]. In addition, BGP can be detected in embryonic bone tissue from the beginning of the mineralization process [4]. Therefore, BGP might be involved in the mechanism of mineralization of the osteoid matrix. There is experimental evidence that BGP is synthesized by the osteoblasts [5], and the circulating levels of BGP probably reflect the osteoblastic activity in protein synthesis and the rate of bone formation. BGP production by the osteoblasts seems to be strictly dependent on the levels of $1,25(OH)_2D_3$ in vitro [6]. Nonetheless, in predialytic chronic renal failure (CRF), in spite of the known defect in $1,25(OH)_2D_3$ synthesis [7], serum levels of BGP are elevated and closely correlated to serum iPTH levels [8]. As the increment in BGP does not seem to be explained by decreased renal filtration alone [9], other factors besides the vitamin D metabolites may possibly regulate the synthesis of BGP.

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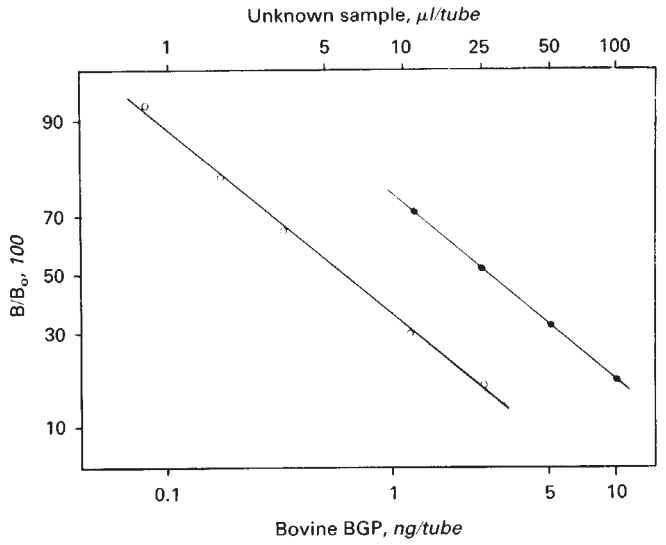

Fig. 1. *Radioimmunoassay of BGP showing similar reactivities of serial concentrations of bovine BGP standard (○—○) and dilutions of unknown serum sample (●—●) from a patient with CRF (log-logit).*

Another important issue is whether in CRF, BGP, and bone alkaline phosphatase (AP) serum measurements provide equivalent indication of osteoblastic activity.

The aim of this study was to explore the natural evolution of serum BGP levels in predialytic CRF, to examine the relationship between BGP and other biochemical and bone histomorphometric parameters, and to establish the role of serial measurements of serum BGP in the long-term treatment of predialysis CRF with $1,25(OH)_2D_3$.

Methods

Patients

A total of 42 patients with CRF from the outpatient renal clinic of the Division of Nephrology, Policlinico Umberto I of Rome, was studied. There were 25 males and 17 females, with a mean age of 47.5 ± 16.6 (M, SD) years (range, 12 to 73). All the patients showed a slow decline of renal function while being followed in the renal clinic and/or in the retrospective analysis of serial serum creatinine values. The presumed clinical diagnoses were: 23 patients with chronic glomerulonephritis, 12 with chronic pyelonephritis, three with polycystic kidney disease, one with medullary cystic disease (the only patient of adolescent age), and three patients with unknown causes of CRF. Serum creatinine levels ranged from 1.5 to 9 mg/dl (4.32 ± 1.9 mg/dl). Nineteen patients were studied within a short period of time, while 23 patients (44.8 ± 18.9 years; serum creatinine, 4.25 ± 1.5 mg/dl) were followed with repeated measurements of serum creatinine, iPTH, AP, and BGP in the outpatient clinic for a mean time of 17.1 ± 8.1 months. Eleven of these patients (54.1 ± 15.3 years; serum creatinine, 4.26 ± 1.3 mg/dl), after a short period of study for the evaluation of basal humoral parameters, were treated with daily doses of 0.25 μg of $1,25(OH)_2D_3$ for a mean of 16.8 ± 6.4 months. The dose of $1,25(OH)_2D_3$ was selected with the aim of avoiding hypercalcemia and renal damage [10] and at the same time effectively controlling secondary hyperparathyroidism. In a long-term study carried out on patients with CRF [11], the dose of 0.25 μg daily or an equivalent dose of $1\alpha OHD_3$, was able to fulfill these aims, at least for most of the time course of decline toward terminal renal insufficiency. However, our data do not exclude that higher doses might be equally safe and more effective.

Table 1. Serum biochemical measurements in 42 patients with chronic renal failure

		Normal ranges
Cr, *mg/dl*	4.34 ± 1.96	0.7 to 1.1
Ca, *mg/dl*	9.12 ± 0.72	9.0 to 10.4
P, *mg/dl*	4.10 ± 0.95	2.8 to 4.5
iPTH, *ng/ml*	1.94 ± 2.09	0.1 to 0.7
AP, *mμ/ml*	183.21 ± 115.95	70.0 to 160.0
BGP, *ng/ml*	17.35 ± 21.44	1.8 to 8.4

Mean ± SD.

None of the patients was receiving corticosteroid, anticoagulant, or anticonvulsant medication. They were following a diet regimen that, compared to the average Italian diet, was moderately restricted in protein (0.8 g/kg body wt) and phosphorus (12 mg/kg body wt) and received calcium p.o., 500 mg daily, as organic salts. None of the patients required at any time aluminum hydroxide administration to control serum phosphate levels.

In 23 patients at various stages of renal damage (serum creatinine 6.4 ± 2.25 mg/dl, range 2.2 to 9.8) who did not receive $1,25(OH)_2D_3$, a transiliac bone biopsy was performed for histomorphometric evaluation.

Assays

Serum BGP was measured by radioimmunoassay based on the method of Price and Nishimoto [2]. Purified calf BGP was used as standard. This protein is immunologically identical to human BGP [12]. The antiserum was a rabbit anti-BGP antibody. Phase separation was accomplished with a goat antiserum to rabbit γ-globulin and polyethylene glycol. The incubation mixture, 450 μl, was left overnight at 4°C in borosilicate tubes. Following the addition of 500 μl of the precipitating complex, centrifugation, and after discarding the supernatant, the pellet containing ^{125}I-labeled BGP bound to rabbit antibody was then assessed for radioactivity in a γ-counter (Packard Autogamma 500, Hewlett-Packard, Elkhart, Indiana, USA) for a time sufficient to achieve a counting accuracy of < 3%. Non-specific binding of radioactivity to the precipitate and to the reaction tube was measured by incubating ^{125}I-labeled BGP and unknown sera without specific antiserum, and precipitating with the usual second antibody technique. Serial dilutions of unknown samples showed a decline in value parallel to the standard curve (Fig. 1). The limit of detectability was < 0.3 ng/ml. The intraassay and interassay variations were respectively lower than 5 and 8%. Since a circadian variation of serum BGP levels with a morning fall in values is a known phenomenon [13], blood samples were drawn from ambulatory patients, at the same time of day, between 10 and 11 A.M. The samples were stored at −30°C until the assay. The results obtained in 25 adult normal subjects (13 male and 12 female) gave an average value of 3.89 ± 1.45 ng/ml. These values are slightly lower than those reported by other investigators [2, 9]. Circadian variation and sampling time differences may account for the discrepancy.

Table 2. Biochemical and histomorphometric data in 23 patients with predialytic chronic renal failure

Patient			Serum chemistry						Histomorphometry[a]							
Number	Age *years*	Sex	Cr *mg/dl*	Ca *mg/dl*	P *mg/dl*	AP *mU/ml*	PTH *ng/ml*	BGP *ng/ml*	TBV %	OV %	OS %	AOS %	RS %	ARS %	TIO	OI *number/mm²*
1	23	M	2.2	9.7	4.2	93	0.4	2.9	24.41	2.23	14.63	1.91	0.68	0.15	15.24	0.11
2	52	F	2.5	10.0	3.9	87	0.7	9.4	29.84	1.80	16.73	1.38	0.99	0.19	10.75	0.22
3	33	M	2.9	10.0	3.6	161	0.9	9.0	23.66	1.74	15.86	0.27	3.91	0.10	10.97	0.11
4	18	M	4.0	9.0	3.7	126	0.9	14.4	29.02	5.49	18.00	1.26	0.34	0.13	30.50	0.11
5	29	M	4.3	8.7	5.2	168	0.6	7.6	19.60	1.43	11.17	0.00	0.58	0.58	7.81	0.67
6	51	F	4.4	8.4	4.3	211	1.7	9.0	25.26	19.77	88.55	1.40	5.43	0.62	22.32	0.65
7	73	F	4.6	9.3	3.9	118	0.8	15.6	16.19	3.06	20.51	0.00	3.04	0.00	14.91	0.00
8	20	M	5.4	10.0	5.0	188	1.6	10.0	23.25	10.50	47.25	11.44	8.74	2.42	22.22	2.15
9	23	M	5.8	9.4	4.7	160	2.1	31.0	18.14	5.47	32.49	2.68	7.12	1.20	16.83	0.90
10	49	M	6.5	9.1	4.2	136	2.6	23.0	22.42	8.60	53.12	6.01	7.72	1.08	16.18	1.13
11	48	M	6.5	8.5	3.8	166	4.2	54.0	29.88	11.49	69.04	8.96	11.04	1.03	16.64	1.24
12	31	F	6.8	8.4	5.1	67	1.9	4.4	24.23	0.90	10.69	1.28	2.17	0.38	8.41	0.32
13	56	M	7.0	7.9	5.8	99	2.2	10.8	14.58	1.61	19.74	0.00	1.42	0.43	8.11	0.48
14	56	F	7.1	8.2	4.5	262	6.8	32.0	20.88	10.81	69.74	4.00	6.08	1.51	15.50	1.40
15	60	M	7.3	8.6	5.1	117	1.8	26.0	21.85	2.50	19.86	0.00	1.77	0.54	12.58	0.45
16	59	M	7.5	9.0	3.1	204	3.9	49.5	23.14	23.21	77.08	4.43	12.28	2.02	30.11	2.27
17	21	M	8.2	9.1	7.9	113	4.5	30.0	26.60	1.09	11.24	2.86	16.46	2.75	9.69	3.39
18	60	M	8.4	8.4	5.0	131	4.0	32.0	19.83	10.03	60.84	7.20	8.67	0.77	16.48	0.79
19	28	M	8.5	9.3	4.1	70	1.8	9.0	23.66	1.16	10.98	0.18	0.56	0.08	10.56	0.10
20	48	F	8.6	9.3	5.7	670	8.0	132.0	26.34	18.36	65.14	10.29	15.56	5.54	28.18	6.28
21	48	F	9.5	7.7	6.9	300	10.0	25.6	24.53	3.61	27.37	6.83	8.49	1.49	13.18	1.73
22	44	M	9.8	10.0	6.3	109	5.5	62.0	23.43	3.55	21.71	2.55	3.46	1.55	16.35	1.35
23	72	F	9.8	8.4	4.4	120	2.7	7.8	19.96	5.11	50.98	5.56	4.70	0.50	10.02	0.54
Mean ±	43.5		6.4	8.9	4.8	168	3.0	26.0	23.03	6.67	36.20	3.49	5.70	1.08	15.76	1.14
SD	16.8		2.2	0.6	1.1	123	2.5	28.5	3.96	6.47	25.13	3.49	4.80	1.23	6.75	1.40
Normals[b] Mean ±									20.33	1.44	9.47	0.27	1.77	0.19	13.50	0.20
(N = 57) SD									4.67	1.24	7.30	0.64	1.50	0.30	4.93	0.22
Range									18.98 to 30.39	0.03 to 5.11	0.59 to 31.25	0.00 to 3.01	0.20 to 6.78	0.00 to 2.07	4.68 to 23.81	0.00 to 0.86

[a] For abbreviations, see **Methods.**
[b] Normal values for the biochemical parameters are reported in Table 1.

In five idiopathic and surgical hypoparathyroid patients and in seven patients with primary hyperparathyroidism assessed by surgery, serum BGP levels were 1.14 ± 0.86 and 14.06 ± 8.1 ng/ml, respectively.

Serum iPTH levels were measured by radioimmunoassay based on a chicken antihuman parathyroid hormone serum, directed against the 65-84 portion of the molecule. The standard was a human synthetic PTH peptide (65-84). Phase separation was accomplished with a rabbit antichicken γ-globulin and polyethylene glycol, following 24-hr incubation at 4°C. This method is useful in patients with secondary hyperparathyroidism of CRF. Serial dilutions of the sera with high levels of the hormone give a linear decline parallel to the standard curve. Standards and unknown sera were run in triplicate with two dilution levels of the unknowns when iPTH levels were higher than 3 ng/ml. Intraassay and interassay variations were respectively 6 and 13.3%. Normal values expressed in terms of mass of the intact human molecule are 0.34 ± 0.19 ng/ml.

Serum $1,25(OH)_2D_3$ was measured by the receptorial method with the cytosol receptor from chicken duodenal mucosa as described by Eisman et al [14]. Preliminary purification of the sample following an extraction with acetonitrile was accomplished on Sep-pak C18 columns. After elution with acetonitrile, desiccation, and dissolution in hexane:isopropanol, the sample was further purified on silica Sep-pak cartridges and on direct phase silica HPLC columns (μPorasil, 10 μ, Waters Assoc. Inc., Milford, Massachusetts, USA) with hexane: isopropanol as the mobile phase [15]. Normal serum levels are 34.8 ± 10.2 pg/ml (range, 16 to 62). Intra- and interassay variations were respectively 10 and 14.2%.

AP measurements were performed spectrophotometrically using p-nitrophenyl phosphate as the substrate (Boehringer-Biochemia, Mannheim, Federal Republic of Germany). The normal range for the adult population is 70 to 160 mU/ml. Serum levels of calcium were measured by atomic absorption spectrophotometry (Perkin Elmer, Model 300, Norwalk, Connecticut USA). Serum creatinine and serum and urine phosphorus were measured by autoanalyzer (Technicon Autoanalyzer RA 1000, Tarrytown, New York USA). The normal range for serum calcium, creatinine, and phosphorus are reported in Table 1.

Transiliac bone biopsies were performed under local anesthesia with a Bordier trocar with an internal diameter of 5 mm. The sample was fixed in phosphate buffered (pH 7.2) 4% paraformaldehyde. Other details of the histological procedure have been reported previously [16]. Histomorphometric evaluation was performed with a semiautomatic image analyzer (Videoplan Kontron, Munich, Federal Republic of Germany).

The following parameters were evaluated: trabecular bone volume (TBV), the percentage of the cancellous bone space occupied by bone; osteoid volume (OV), the percentage of the trabecular bone made of uncalcified bone matrix; osteoid surface (OS), the percentage of trabecular surface covered by osteoid; active osteoblastic surface (AOS), the percentage of trabecular surface covered by osteoid lined by active

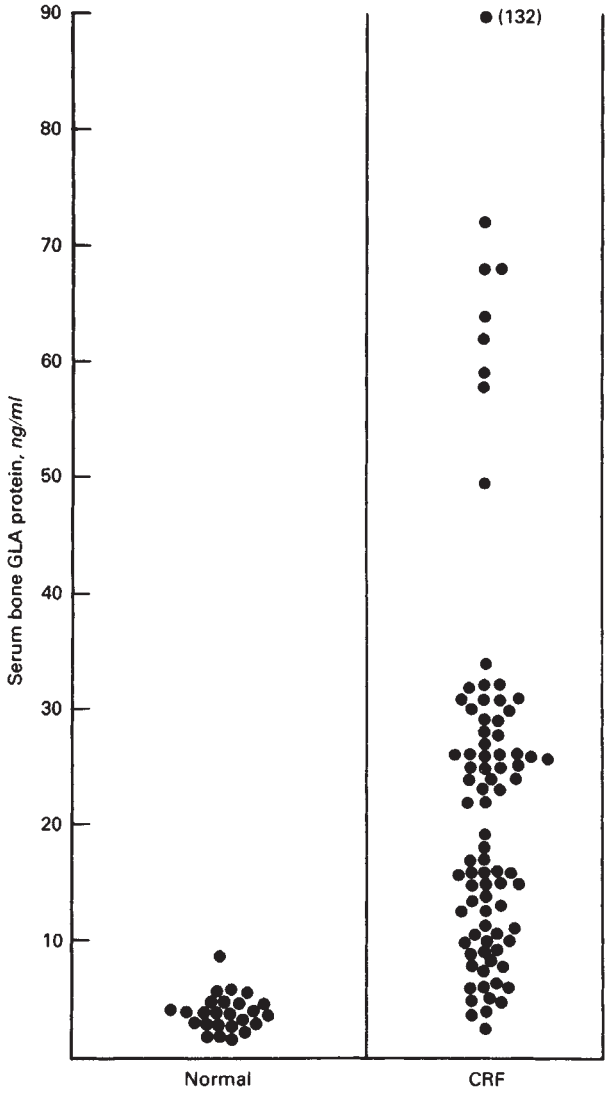

Fig. 2. *Serum BGP measurements in normal subjects and in patients with CRF.*

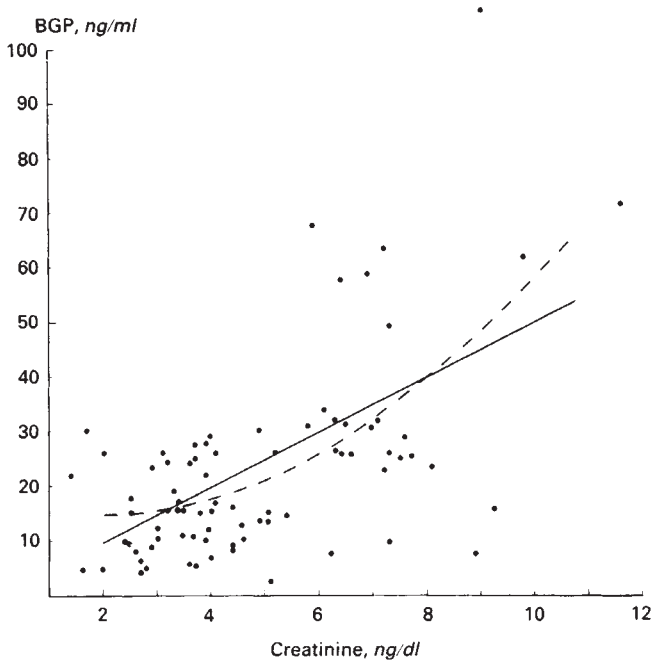

Fig. 3. *Serum BGP levels as a function of serum creatinine.* The non-linear correlation is a parabolic function.

osteoblasts; resorption surface (RS), the percentage of the total surface consisting of Howship's lacunae; active resorption surface (ARS), the percentage of the total bone surface undergoing resorption with visible osteoclasts; osteoclastic index (OI), the number of osteoclasts counted in a mm^2 surface of the bone section; thickness index of osteoid (TIO), the ratio between osteoid volume and osteoid surface. The normal values for the parameters are reported in Table 2.

Statistical analysis

The data were statistically evaluated with programs of the BMDP package from the University of Los Angeles [17] on an IBM 4341 computer at the Istituto Superiore di Sanità.

Results

Basal biochemical measurements of the patients are summarized in Table 1. Serum BGP levels in 25 normal subjects and in 42 patients with CRF are reported in Figure 2. Multiple measurements of BGP in some patients were carried out at different stages of renal insufficiency. Serum BGP levels in CRF are generally higher than in normal controls, with the higher levels being found in the more advanced stages of renal failure. This finding is illustrated by Figure 3, where BGP levels of patients who did not receive $1,25(OH)_2D_3$ are reported against serum creatinine levels. The changes in serum BGP as a function of serum creatinine were studied with different models. The data were satisfactorily fitted both by a linear ($y = 4.907 \times -0.051$; $r = 0.52$, $P < 0.001$) and a parabolic function ($y = 16.7 - 2.2 \times +0.632x^2$; $r = 0.54$, $P < 0.001$).

The humoral and histomorphometric data of the 23 patients submitted to bone biopsy are reported in Table 2. Sixteen of these patients had a serum creatinine level > 5 mg/dl. Serum creatinine correlated positively with BGP ($P < 0.05$) and OI ($P < 0.05$). But the positive correlations between BGP and OI ($P < 0.001$) and AOS ($P < 0.01$) were stronger (Table 3).

As for the other correlations among humoral and histomorphometric parameters (Table 3), OI and ARS correlated positively with iPTH and AP. Likewise, AOS correlated positively with iPTH and AP. OV was correlated positively with AP and BGP.

Stepwise multiple regression analysis was performed to correlate serum BGP as a dependent variable with the other biochemical and histomorphometric parameters. The resultant multiple correlation coefficient (r_m) was 0.836 ($P < 0.0001$). BGP serum levels were determined by ARS (serum BGP = 5.06 + 19.25 ARS), while contributions from the other variables were not statistically significant.

The correlations among biochemical measurements in all patients are reported in Table 4. Serum levels of BGP correlated significantly with serum iPTH, AP, and phosphate.

Serum $1,25(OH)_2D_3$ was measured in 11 patients before and 1 to 3 months after beginning of treatment with the D metabolite. From an average level of 17.4 ± 10.3 pg/ml, serum $1,25(OH)_2D_3$

Table 3. Correlations among biochemical and histomorphometric data in 23 patients with predialytic chronic renal failure

	Cr	Ca	P	AP	PTH	BGP	TBV	OV	OS	AOS	RS	ARS	TIO	OI
Cr	1.000													
Ca	−.418[a]	1.000												
P	.522[a]	−.227	1.000											
AP	.238	−.079	.183	1.000										
PTH	.702[c]	−.378	.530[b]	.644[c]	1.000									
BGP	.466[a]	.051	.239	.786[c]	.662[c]	1.000								
TBV	−.167	.257	−.064	.161	.130	.212	1.000							
OV	.134	−.109	−.286	.575[b]	.324	.531[b]	.172	1.000						
OS	.217	−.272	−.272	.473[a]	.366	.423[a]	.074	.917[c]	1.000					
AOS	.375	−.031	.133	.558[b]	.552[b]	.537[b]	.247	.537[b]	.597[b]	1.000				
RS	.433[a]	−.061	.343	.574[b]	.618[b]	.649[c]	.222	.577[b]	.544[b]	.705[c]	1.000			
ARS	.427[a]	.089	.444[a]	.823[c]	.651[c]	.836[c]	.204	.521[a]	.392	.664[c]	.808[c]	1.000		
TIO	−.037	.169	−.312	.497[a]	.185	.502[a]	.320	.777[c]	.570[b]	.425[a]	.379	.456[a]	1.000	
OI	.426[a]	.049	.455[a]	.826[c]	.657[c]	.835[c]	.239	.507[a]	.374	.634[b]	.827[c]	.990[c]	.427[a]	1.000

See **Methods** for histomorphometric abbreviations.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

Table 4. Correlations among age and the serum biochemical measurements made in 42 patients with CRF

		Without $1,25(OH)_2D_3$ treatment							
		AGE	BGP	PTH	AP	Cr	1/Cr	Ca	P
During $1,25(OH)_2D_3$ treatment	AGE		.0515 NS	.1560 NS	−.0780 NS	.2210 < .05	−.2550 < .05	−.1440 NS	−.2240 NS
	BGP	.2430 NS		.6410 < .001	.5800 < .001	.5210 < .001	−.3610 < .001	.1580 NS	.4090 < 0.001
	PTH	−.0256 NS	.6769 < .001		.2801 < .05	.6530 < .001	−.4560 < .001	−.0770 NS	.3290 < 0.01
	AP	−.2610 NS	.1654 NS	.3068 < .05		.0490 NS	.0104 NS	−.0230 NS	.3338 < 0.01
	Cr	−.1992 NS	.3930 < .01	.7790 < .001	.0990 NS		−.8580 < .001	−.0637 NS	.3500 < 0.01
	1/Cr	.1479 NS	.3390 < .05	−.6670 < .001	−.1894 NS	.9330 < .001		.0988 NS	−.2160 NS
	Ca	−.1000 NS	−.2778 NS	−.3409 < .05	.0059 NS	−.2077 NS	.1360 NS		−.1208 NS
	P	−.1390 NS	.0650 NS	.2580 NS	.2270 NS	.3078 < .05	−.3900 < .01	.1869 NS	

increased to 32.6 ± 9.7 pg/ml during treatment. Average values of blood calcium and phosphate levels before and after commencement of treatment with $1,25(OH)_2D_3$ were 9.00 ± 0.33 and 9.34 ± 0.50 mg/dl ($P < 0.005$) for serum calcium and 3.88 ± 0.28 and 4.34 ± 0.71 mg/dl ($P < 0.025$) for serum phosphate, respectively. Mean values for serum iPTH, AP, and BGP after starting $1,25(OH)_2D_3$ treatment showed a highly significant fall (serum iPTH, from 2.65 ± 2.9 to 1.34 ± 0.96 ng/ml, $P < 0.005$; AP, from 192.8 ± 110.5 to 123.1 ± 62.9 mU/ml, $P < 0.001$; serum BGP, from 23.5 ± 19.8 to 11.22 ± 8.65 ng/ml, $P < 0.0001$), while serum creatinine levels did not change significantly (4.78 ± 2.11 and 5.20 ± 1.80 mg/dl, respectively; NS).

A positive significant correlation between serum BGP and creatinine levels was also found during treatment with $1,25(OH)_2D_3$ (Fig. 4, Table 4). However, the slope was significantly lower ($P < 0.0001$) than in the untreated state. Serum BGP levels over the entire range of serum creatinine explored were lower in the patients treated with $1,25(OH)_2D_3$. Figure 5 shows a highly significant correlation ($P < 0.001$) between BGP and iPTH during treatment. The slopes of the regression lines of serum iPTH and BGP values obtained before and during treatment with $1,25(OH)_2D_3$ are very close, and we conclude that treatment affects both parameters proportionally. As for BGP and AP, in spite of a significant decrease during treatment, they no longer correlated (Table 4).

Figures 6 and 7 show the time course of serum BGP in patients without treatment and during $1,25(OH)_2D_3$ administration. The correlations of BGP versus time were strongly positive in ten of 12 untreated patients and negative in ten of 11 patients treated with the vitamin D metabolite. The Mann-Whitney U test applied to the slopes of treated and untreated patients showed a highly significant difference ($P < 0.001$). Eventual BGP rises (Fig. 7) observed in a few cases were coincident with terminal renal failure.

Discussion

In recent years, several vitamin K-dependent proteins containing γ-carboxyglutamic acid residues have been identified [18]. The bone GLA protein, which is also measurable in blood, is an important component of the non-collagen proteins of bone,

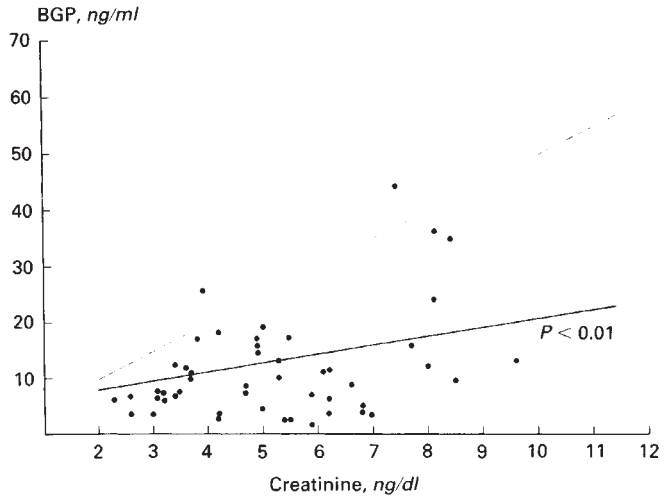

Fig. 4. *Correlation between serum BGP and creatinine during $1,25(OH)_2D_3$ treatment* (—). The correlation between parameters in patients without treatment is represented by (---).

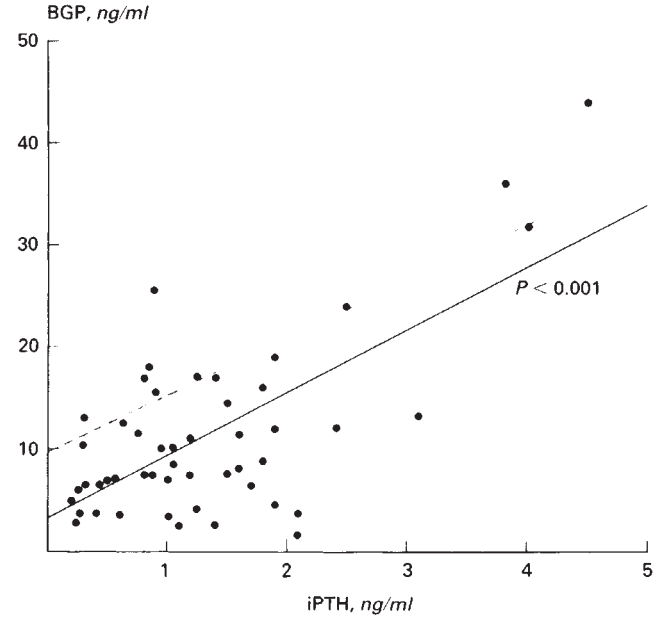

Fig. 5. *Correlation between serum BGP and iPTH during $1,25(OH)_2D_3$ treatment* (—). The correlation between parameters in untreated patients is also reported (---).

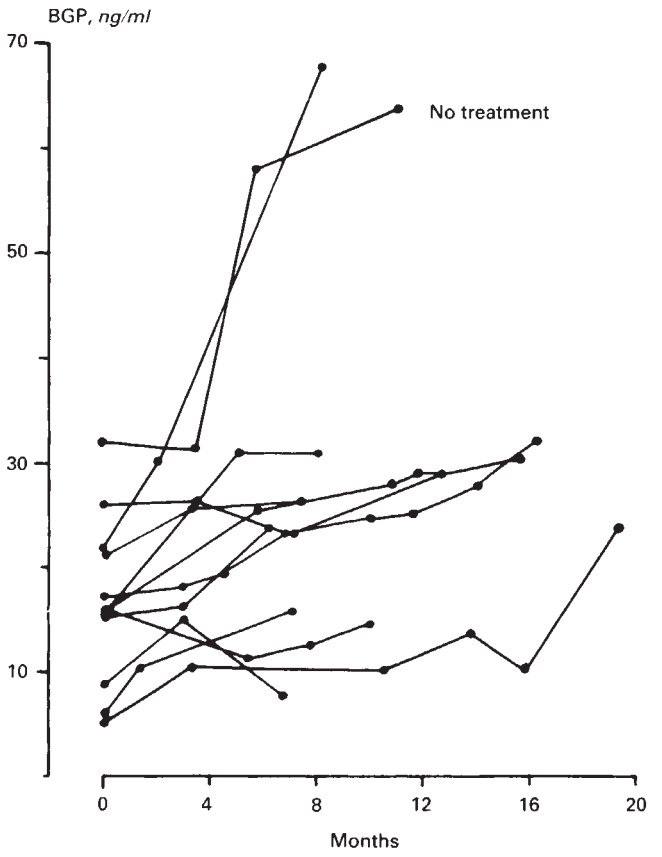

Fig. 6. *Time course of serum BGP levels in 12 patients with CRF who did not receive $1,25(OH)_2D_3$.*

and is probably connected with the process of osteoid mineralization. Several studies have supported the hypothesis that circulating BGP originates from new synthesis, presumably by the osteoblasts [5], and denied its derivation from the osteoclastic resorption of bone proteins [19, 20]. Riggs, Tsai, and Mann [20] have shown that 24-hr i.v. administration of the synthetic 1-34 fragment of PTH in normal women raised urine hydroxyproline excretion without any associated change in serum BGP. Other reports that BGP significantly correlated with bone mineralization rate, but not with the resorption parameters [21–23], favor the hypothesis that serum BGP is connected with the bone formation process. In addition, Price, Williamson, and Baukol [6] have underlined the relative dependence of BGP synthesis on $1,25(OH)_2D_3$ levels. However, a basal production of the protein was observed even without the vitamin D metabolite. Increased serum BGP levels have been reported in primary hyperparathyroidism, as well as in secondary hyperparathyroidism of CRF [6, 9, 12]. CRF, at least starting from a GFR below 30 ml/min [24], is associated with a deficit of $1,25(OH)_2D_3$ [7]. Therefore, serum BGP levels may be increased in spite of low levels of serum $1,25(OH)_2D_3$. On the other hand, in predialytic [8] and dialytic [23] CRF, no correlation was observed between serum $1,25(OH)_2D_3$ and BGP levels.

Our data confirm the finding of increased serum BGP levels in CRF [9, 25]. The serum levels of BGP are significantly correlated with serum creatinine. The data were satisfactorily fitted both by a linear and a parabolic function (Fig. 3). Direct inspection of the experimental values suggests that the best fit is given by the non-linear function. From a pathophysiological point of view, it cannot be excluded that, in the terminal stages of CRF, serum retention of BGP is faster than that of creatinine. However, the wide scatter of BGP values for elevated serum creatinine levels suggests that bone disease may be a more important determinant of increased BGP levels than the extent of renal damage per se. This hypothesis seems to be confirmed by analysis of humoral and histomorphometric data of the 23 patients submitted to bone biopsy.

The increase in BGP is proportional to the increase in serum iPTH, AP, and phosphate. The progressive increase in iPTH

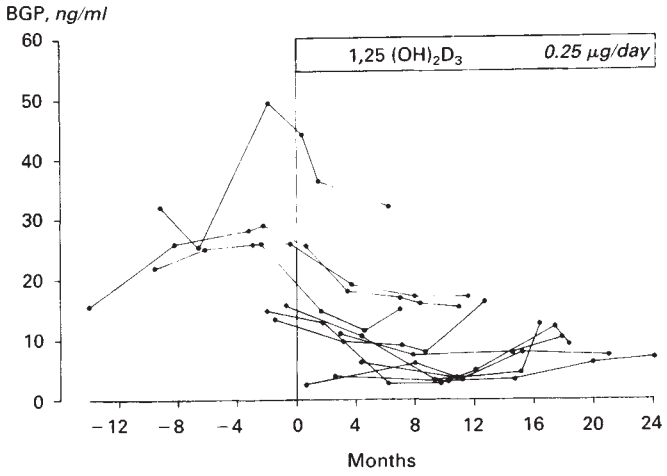

Fig. 7. *Time course of serum BGP levels during long-term treatment with 1,25(OH)$_2$D$_3$ in 11 patients with CRF.*

levels is clearly the cause of the increased serum BGP. The results of treatment with 1,25(OH)$_2$D$_3$ favor this conclusion. Administration of the D metabolite and the resultant normalization of serum levels induces a fall in serum BGP in the long term, which is parallel to the reduction in iPTH values. Moreover, it seems unreasonable to attribute the proportional increments in BGP and iPTH levels observed in advancing CRF to mere decreased renal disposal of these proteins, since a highly significant positive correlation was found to link these to the histologic parameters. Therefore, serum BGP reflects the osteodystrophic bone lesion and bone turnover and is unrelated to the existent serum levels of 1,25(OH)$_2$D$_3$, which are known to be decreased in CRF.

Theoretically, the decrease in serum BGP with 1,25(OH)$_2$D$_3$ administration might be attributed to aluminum accumulation in bone due to CRF, as suggested by the finding in dialysed uremic patients [26] of relatively lower levels of BGP associated with aluminum bone disease. However, in predialysis CRF, aluminum intoxication has rarely been reported and then mainly in young patients subjected to intensive and prolonged treatment with aluminum hydroxide [27], a medication never administered to our patients.

As for serum BGP and AP, the results indicate a more complex relationship. Deftos, Parthemore, and Price [25] conclude that in renal osteodystrophy, BGP and AP probably do not reflect the same aspects of bone metabolism. Our data, even if based on AP measurements non-selective for the bone isoenzyme, confirm that in some conditions there is no correlation between BGP and AP. Actually, the highly significant positive correlation observed without 1,25(OH)$_2$D$_3$ treatment is no longer found during the vitamin D metabolite administration. Therefore, it appears that BGP and AP may indicate different aspects of osteoblastic activity.

In conclusion, in predialytic CRF, serum BGP is a good index of hyperparathyroidism. Serum BGP falls with iPTH levels during treatment with 1,25(OH)$_2$D$_3$. Serial measurements of BGP in the long-term follow-up of patients with CRF not requiring dialysis is a reliable index of bone turnover and osteoblastic activity. However, these results do not imply that serum BGP measurements can replace iPTH in the assessment of hyperparathyroid bone disease.

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