Nephrol Dial Transplant (2002) 17: 233–238

Nephrology Dialysis Transplantation

Rapid Communication

Serum osteoprotegerin and renal osteodystrophy

Giorgio Coen[1], Paola Ballanti[2], Alessandro Balducci[3], Santo Calabria[1], Maria Stephanie Fischer[1], Ljiljana Jankovic[4], Micaela Manni[3], Massimo Morosetti[4], Eleonora Moscaritolo[1], Daniela Sardella[1] and Ermanno Bonucci[2]

[1]Department of Nephrology, Second Medical Faculty, [2]Department of Experimental Medicine and Pathology, First Medical Faculty, La Sapienza University, [3]Department of Nephrology and Dialysis, San Giovanni Hospital, Rome, and [4]GB Grassi Hospital, Ostia, Italy

Abstract

Background. Numerous growth factors and cytokines are known to modulate bone turnover. An important, recently discovered complex involved in osteoclastogenesis is the osteoprotegerin/osteoprotegerin-ligand (OPG/OPGL) cytokine complex, which is produced by osteoblasts. Many factors, including parathyroid hormone (PTH), appear to affect bone turnover through this pathway. In this disorder, the role of the OPG/OPGL system in the pathogenesis of renal osteodystrophy, a disease with either low or high bone turnover, has not been investigated so far.

Methods. Thirty-nine chronic haemodialysis patients had bone biopsies, including histomorphometric and histodynamic examinations. In addition, the following serum biochemistry parameters were measured: serum OPG, intact PTH, PTH 1–84, total PTH, osteocalcin, total and bone alkaline phosphatases, 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol.

Results. On average, serum OPG levels were above the normal range. They were lower in adynamic bone disease (ABD) patients, than in patients with predominant hyperparathyroidism (HP) or mixed osteodystrophy (MO). Significant negative correlations were found between serum OPG and PTH levels, and between serum OPG and parameters of bone resorption (ES/BS) and bone formation (ObS/BS and BFR/BS) in HP and MO patients with PTH values $\leqslant$1000 pg/ml. For intact PTH levels $\leqslant$300 pg/ml, serum OPG was significantly lower in the group with ABD than in those with HP or MO ($P < 0.05$).

Conclusion. In renal osteodystrophy the OPG/OPGL system is involved in the regulation of bone turnover induced by PTH. The determination of serum OPG levels could be of use in the diagnosis of low turnover bone disease, at least in association with PTH levels $\leqslant$300 pg/ml.

Keywords: bone resorption; bone turnover; osteoprotegerin; PTH; renal osteodystrophy

Introduction

Bone is a dynamic tissue, which is continuously renovating in a process called 'remodelling'. In pathologic conditions, such as renal osteodystrophy, this process may be either accelerated or downregulated, so both high- and low-turnover renal osteopathies can be found.

The pathogenesis of renal bone disease has so far mainly centred on perturbations related to the parathyroid and vitamin D system. It is well known that an excess of parathyroid hormone (PTH) can produce a condition of high turnover bone disease, while relatively low levels of PTH and/or bone resistance to the hormone are associated with low bone turnover states, like adynamic bone disease (ABD) or osteomalacia (OM). However, the many manifestations of renal osteodystrophy cannot be explained exclusively by PTH excess or defect. The levels of PTH correlate poorly with histodynamic parameters of bone turnover [1,2]. In addition there is no clear-cut evidence for a direct relationship between active vitamin D metabolite serum levels and the activity of bone turnover, except for a reduction in the rate of turnover following pharmacologic doses of calcitriol administration [3].

It is well known at present that a multitude of local factors, such as interleukins, growth factors and cytokines, are involved in the process of bone turnover and in mediating the effect of PTH [4,5]. The importance of some of these factors in chronic renal failure has already been reported [4,6,7]. In recent years new aspects of bone physiology have been disclosed, in

Correspondence and offprint requests: Prof. G. Coen, Renal Pathophysiology and Hypertension Unit, Policlinico Umberto I, Viale del Policlinico 155, I-00161 Rome, Italy. Email: coen.gf@flashnet.it

particular the findings of the cytokine system regulating osteoclastogenesis and of a new role for osteoblasts [5,8,9]. Osteoprotegerin (OPG) and osteoprotegerin-ligand (OPGL) constitute a complex mediator system involved in the regulation of the resorption process in bone, which is probably responsible for the homeostatic mechanism of bone turnover. Alterations in this system could form the basis of some metabolic bone diseases, like osteoporosis and osteopetrosis, as suggested by recent experimental evidence [10,11]. However, *in vivo* studies regarding the role of the OPG/OPGL system in metabolic bone diseases are still needed. A recent review [12] has highlighted the importance of this cytokine system of osteoblastic origin, which is able to control osteoclastic activity through the interplay of many factors, including PTH and calcitriol, that act mainly on the osteoblasts. It is known from experimental *in vivo* and *in vitro* studies that OPG, which is secreted by osteoblasts, is able to block the osteoclastogenesis induced by OPGL, which is also a product of the osteoblast. In addition, several studies have shown that PTH acts by enhancing the production of the osteoclastogenic factor OPGL and by inhibiting the synthesis of the soluble receptor OPG, which blocks the biological effect of OPGL [13–15]. We still do not know how this cytokine system is involved in renal bone disease.

The present study was carried out to evaluate the serum levels of OPG in different bone histological patterns of chronic renal failure, and to establish a possible relationship between its serum levels and those of PTH and several other biochemical markers, as well as histomorphometric and histodynamic parameters. The results obtained provide valuable information on the role of OPG in renal osteodystrophy.

Subjects and methods

This study has been conducted on 39 haemodialysis patients with a wide range of serum PTH levels, who volunteered to undergo a bone biopsy. The average age of the patients (28 males and 11 females) was 57.1 ± 11.5 years, while the time on dialysis was 60.9 ± 83.4 months. The causes of renal failure were the following: chronic glomerulonephritis in six patients, hypertension/ischaemic nephropathy in six, tubulointerstitial nephropathy in four, polycystic kidney disease in five, diabetic nephropathy in five, renal stone disease and obstructive nephropathy in three, renal malformations in two and unknown causes in eight patients. None of the patients were undergoing treatment with vitamin D or its active metabolites and analogues. The patients did not receive corticosteroids, NSAIDs, anticoagulants, antiepileptics, oestrogens or androgens. Phosphate chelating agents were mainly sevelamer and calcium salts. All the patients were treated with standard haemodialysis, 12 h per week, divided into three sessions.

All the patients were subjected to transiliac bone biopsy with a Bordier trocar, following a double labelling course with tetracycline per os, with a 12 day interval. The biopsy was performed 3–5 days after the end of tetracycline administration. At the same time a blood sample was drawn for the following assays: intact PTH, PTH1-84, total PTH, osteocalcin, bone alkaline phosphatase, total alkaline phosphatase, 25-hydroxycholecalciferol, 1,25-dihydroxycholecalciferol, calcium and phosphate.

Serum OPG was measured on undiluted samples with a sandwich enzyme immunoassay provided by Immundiagnostik (Bensheim, Germany), which uses two highly specific antibodies against human OPG. The detection antibody was a biotin-labelled polyclonal antihuman OPG antibody, derived from a goat immunized with rhOPG. The intra- and inter-assay variation coefficients were 8 and 12% respectively. Normal values for 22 normal subjects aged 29.8 ± 4.8 years, are 30.45 ± 12.1 pg/ml. Normal values on a vast number of normal subjects of different ages, obtained with the same assay method, have been reported recently [16].

Serum intact PTH was measured with a commercial (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) immunoradiometric assay based on a double antibody technique. The normal range of values is 10–65 pg/ml. PTH 1–84 and total PTH were measured with an assay provided by Scantibodies Laboratory, Inc. (Santee, CA, USA). Normal values for 22 normal subjects, 11 males and 11 females, are reported in Table 1.

Serum osteocalcin, or BGP, was measured by an IRMA assay (Nichols Institute Diagnostics). The intra- and inter-assay variations were less than 5.2 and 7.1%, respectively. The normal range is 6.8–32.2 ng/ml.

Serum 25-hydroxycholecalciferol was measured with a competitive protein-binding method, after purification on Sep-Pack C18 cartridges and extraction with acetonitrile. The normal range is 10–60 ng/ml.

Table 1. Mean ($\pm$SD) values and ranges of clinical, biochemical and histomorphometric and histodynamic parameters

		Normal values
Number	39	–
M/F	28/11	–
Age (years)	57.08 ± 11.48	–
HD time (months)	60.90 ± 63.43	–
PTH intact (pg/ml)	369.28 ± 474.42	15–55
PTH 1-84 (pg/ml)	227.44 ± 300.77	26.84 ± 9.56
PTH Total (pg/ml)	410.93 ± 569.44	30.94 ± 12.81
BGP (ng/ml)	178.85 ± 155.87	6.8–32.2
BALP (U/l)	49.31 ± 63.32	10–23
AP (U/l)	256.9 ± 194.83	60–170
25-OHD$_3$ (ng/ml)	25.06 ± 13.13	10–40
1-25(OH)$_2$D$_3$ (pg/ml)	10.93 ± 12.37	19.9–67
Ca (mg/dl)	10.33 ± 1.09	8–10
P (mg/dl)	5.29 ± 1.57	2.5–4.5
OPG (pg/ml)	90.91 ± 66.12	30.45 ± 12.12
Number	33	–
BV/TV (%)	21.33 ± 4.47	19.10 ± 4.5
OV/BV (%)	6.46 ± 7.59	1.39 ± 1.08
O.Th (μm)	14.03 ± 6.78	9.55 ± 3.32
Ob.S/BS (%)	11.29 ± 13.18	0.20 ± 0.49
ES/BS (%)	7.29 ± 5.44	1.52 ± 1.28
Oc.S/BS (%)	2.49 ± 2.03	0.18 ± 0.19
MAR (μm/day)	0.7255 ± 0.4768	0.64 ± 0.13
BFR/BS (μm^3/μm^2/day)	0.1483 ± 0.2184	0.066 ± 0.037
Aj.AR (μm/day)	0.4682 ± 0.4489	0.441 ± 0.123
Mlt (days)	291.60 ± 697.63	33.80 ± 10.18

BV/TV, bone volume; OV/BV, osteoid volume; O.Th, osteoid thickness; Ob.S/BS, osteoblast surface; ES/BS, eroded surface; Oc.S/BS, osteoclast surface; MAR, mineral apposition rate; BFR/BS, bone formation rate; Aj.AR, adjusted apposition rate; Mlt, mineralization lag-time.

1,25-dihydroxycholecalciferol was measured with a radioimmunoassay provided by Nichols Institute Diagnostic. The normal range of values is 19.9–67 pg/ml.

Bone alkaline phosphatase was measured with an immunoassay, using a monoclonal anti-bAP antibody, coated onto a microtiter strip to capture bAP in the sample, provided by Metra Biosystems (Mountain View, CA, USA). Normal range of values is 10–23 mU/ml.

Serum total calcium was determined by a spectrophotometric assay using cresolftaleine as substrate. Serum phosphate and alkaline phosphatase measurements were performed spectrophotometrically (DU-65 Beckman, Fullerton, CA, USA) using molybdate or *p*-nitrophenyl-phosphate as respective substrates. The normal range for the adult population is 3.0–4.5 mg/dl and 35–125 mU/ml respectively.

Bone specimens were processed as previously described [2,17]. Sections were stained using the Aluminon technique [18] for detection of aluminium, and with azure II-methylene blue for histomorphometric measurement of structural and static variables. Unstained sections were examined under UV light for histodynamic evaluation of tetracycline fluorescent labels. Histomorphometric and histodynamic measurements, which were carried out in 33 patients (in six patients the sample was not found to be suitable for statistical adequacy), were obtained using the Image Analysis System (IAS 2000, Delta Sistemi, Rome, Italy). All variables were measured according to the guidelines of the ASBMR Histomorphometry Nomenclature Committee [19], as previously described [2,17]. Normal values for the histomorphometric [20] and histodynamic [17] parameters were obtained in our own histomorphometric laboratory (Table 1).

Bone pathology was classified as OM, ABD, predominant hyperparathyroidism (HP) or mixed osteodystrophy (MO). The classifications were made on the basis of morphological criteria, as already reported [2,17,21]. The designation of HP implied a general increase in bone turnover rate and predominant OM was characterized by a decrease in bone turnover rate associated with an increase of both osteoid surface and thickness. MO included all the intermediate features between HP and OM [22] and ABD was characterized by reduced bone turnover associated with thin osteoid seams, bone cell paucity, and a decrease in tetracycline uptake.

Statistical evaluation was carried out with Statistical Package for the Social Sciences (SPSS) on a PC. Evaluations were based on correlation analyses and ANOVAs. Values are expressed as mean $\pm$ SD.

Results

The average values of biochemical assays, and of bone histomorphometric and histodynamic parameters are reported in Table 1, together with normal reference values. The average value for OPG was 90.9 ± 66 pg/ml, markedly higher than the normal range, but with a wide scatter of data.

The histological diagnosis identified 12 patients with HP, 17 with MO, eight with ABD and two with OM. Aluminum histochemistry was found to be positive in one case of MO and one case of ABD. The case of ABD with bone aluminum accumulation was excluded from statistical evaluation.

Pearson's correlation coefficients for the entire cohort of patients did not show statistical significance as regards OPG *vs* any of the other parameters, except for OPG *vs* total AP (0.467, $P < 0.01$) and OPG *vs* serum calcitriol (0.467, $P < 0.05$). As expected, intact PTH was strictly correlated with the parameters ES/BS (0.616, $P < 0.001$), ObS/BS (0.852, $P < 0.001$), and BFR/BS (0.872, $P < 0.001$). By leaving out the patients with low turnover osteodystrophy (ABD and OM), a negative correlation was observed between OPG serum levels and the resorption parameter ES/BS (NS). After selection of the patients with the histologic diagnosis of HP and MO and an intact PTH level $\leqslant 1000$ pg/ml ($n = 25$), several significant correlations were found (Table 2). Serum OPG was significantly inversely correlated to intact PTH (-0.399, $P < 0.05$) (Figure 1), PTH1 1–84 (-0.443, $P < 0.01$) and total PTH (-0.504, $P < 0.01$), and ES/BS (-0.450, $P < 0.05$) (Figure 2). Also, after the exclusion of a patient with outlying values, significant negative correlations were found between serum OPG and ObS/BS, and between serum OPG and BFR/BS (Table 2).

In addition, the patients were grouped by histological diagnosis. The only patient with ABD and positive aluminum staining, showed a relatively high level of serum OPG, in contrast to the other ABD cases. It was considered an out-lying case and excluded from group statistics. Comparison was made between the remaining seven patients with ABD, the 12 patients with HP and the 17 cases with MO. As shown in Table 3, average values of serum OPG was lower in the ABD group compared with the other groups, without reaching statistical significance ($P < 0.15$). Finally all the patients with serum intact PTH levels below 300 pg/ml were selected. Within this group of 22 patients, a significant difference ($P < 0.03$) in OPG serum levels was found between the seven patients with ABD and the 15 patients with either HP or MO (Figure 3).

Discussion

OPG serum levels were on average higher in renal osteodystrophy than in normal subjects. Among the histological classes, the group with lowest OPG levels is ABD. This finding may at first lead to the conclusion

Table 2. Correlation coefficients among OPG, PTH and selected histomorphometric and histodynamic parameters in HP + MO patients with intact PTH $\leqslant 1000$ pg/ml

	OPG	*P*
iPTH (pg/ml)	-0.399	< 0.05
PTH 1-84 (pg/ml)	-0.443	< 0.01
PTH Total (pg/ml)	-0.505	< 0.01
ES-BS (%)	-0.450	< 0.05
OcS-BS (%)	-0.332	< 0.1
ObS-BS (%)	-0.492	< 0.05
O.Th (μm)	-0.430	< 0.05
BV/TV (%)	-0.368	< 0.1
BFR/BS ($\mu m^3/\mu m^2$/day)	-0.455	< 0.05

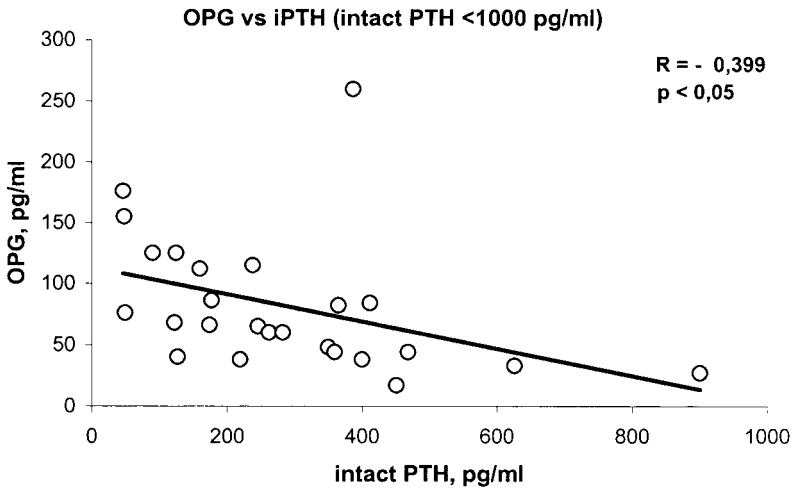

Fig. 1. Correlation between serum OPG and intact PTH in patients with HP or MO with intact PTH values $\leqslant$1000 pg/ml.

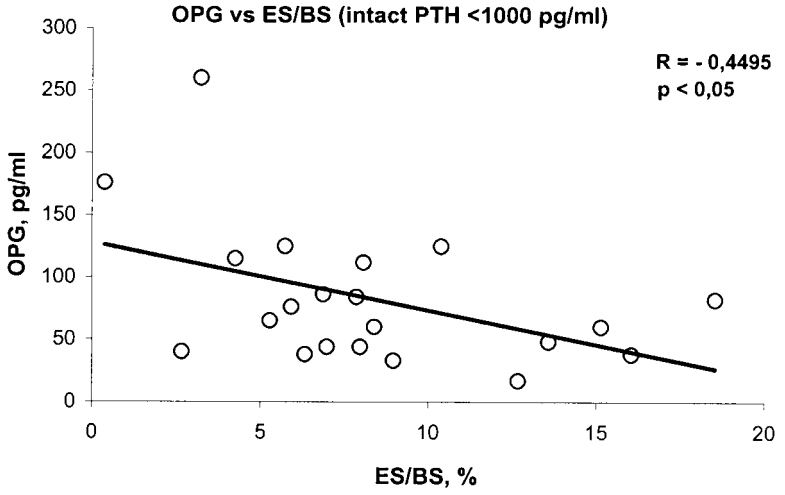

Fig. 2. Correlation between serum OPG and the histomorphometric parameter ES/BS in patients with intact PTH values $\leqslant$1000 pg/ml.

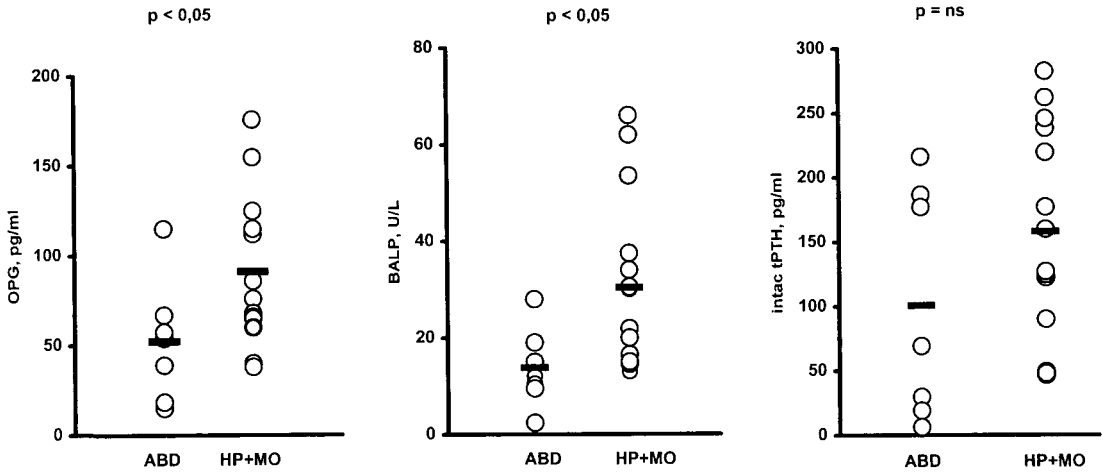

Fig. 3. OPG, Bone AP and intact PTH serum levels in the patients with PTH serum values $\leqslant$300 pg/ml, grouped according to histological diagnosis (ABD and HP + MO).

Table 3. Means (±SD) and *P* values of clinical, biochemical, histomorphometric and histodynamic parameters in the histological classes

	HP	MO	ABD	ANOVA
Number	12	17	7	
M/F	6/6	13/4	7/0	NS
Age (years)	57.17 ± 10.62	56.82 ± 12.49	60.14 ± 12.77	NS
HD time (months)	111.33 ± 81.79	36.47 ± 33.01	21.71 ± 10.03	NS
PTH intact (pg/ml)	776.33 ± 659.64	241.78 ± 209.84	101.16 ± 91.7	$P < 0.01$
PTH 1-84 (pg/ml)	434.41 ± 426.51	154.33 ± 131.44	58.31 ± 57.88	$P < 0.01$
PTH Total (pg/ml)	833.58 ± 801.79	240.34 ± 203.51	108.23 ± 98.95	$P < 0.01$
BGP (ng/ml)	332.17 ± 185.24	140.96 ± 63.8	42.67 ± 41.45	$P < 0.01$
BALP (U/l)	91.78 ± 96.64	30.21 ± 15.44	12.64 ± 6.99	$P < 0.01$
AP (U/l)	362.2 ± 247.25	189.85 ± 53.72	91.52 ± 35.52	NS
25-OHD$_3$ (ng/ml)	20.60 ± 9.12	27.79 ± 13.74	23.36 ± 12.54	NS
1-25(OH)$_2$D$_3$ (pg/ml)	13.40 ± 18.85	9.88 ± 10.28	8.09 ± 4.52	NS
Ca (mg/dl)	10.55 ± 1.27	10.01 ± 1.06	10.32 ± 0.63	NS
P (mg/dl)	5.93 ± 1.73	4.94 ± 1.57	5.24 ± 0.75	NS
BV-TV (%)	23.24 ± 4.71	19.92 ± 4.04	21.66 ± 4.80	NS
OV-BV (%)	8.06 ± 5.12	4.49 ± 2.66	2.01 ± 3.71	NS
O.Th (μm)	15.37 ± 3.29	13.46 ± 3.71	8.77 ± 3.77	NS
ObS-BS (%)	26.19 ± 13.00	7.72 ± 6.58	0.16 ± 0.29	NS
ES-BS (%)	12.74 ± 4.73	6.81 ± 3.65	2.15 ± 1.43	NS
OcS-BS (%)	4.67 ± 1.80	2.20 ± 1.18	0.51 ± 0.3	NS
MAR (mm/day)	1.1540 ± 0.4475	0.7593 ± 0.243	0.1286 ± 0.1604	NS
BFR/BS (μm^3/μm^2/day)	0.3467 ± 0.3114	0.0951 ± 0.0603	0.0042 ± 0.0097	$P < 0.01$
Aj.AR (μm/day)	0.8220 ± 0.4659	0.4943 ± 0.3421	0.0314 ± 0.0549	$P < 0.01$
Mlt (days)	23.39 ± 11.85	60.4 ± 77.93	657.84 ± 435.34	$P < 0.01$
OPG (pg/ml)	101.08 ± 68.76	81.88 ± 45.06	52.07 ± 33.85	NS

that decreased bone turnover in this pattern of osteodystrophy is not due to an excessive OPG production. Nevertheless, since data concerning local expression of OPGL are as yet not available, a low production of OPGL with a relatively high OPG/OPGL ratio cannot be excluded as a possible cause of the low bone turnover in ABD. However, the low osteoblastic surface with morphologically inactive osteoblasts that is typical of ABD, may be considered in accordance with a lower production of these cytokines. One case of ABD had a positive aluminum histochemistry and markedly elevated OPG levels, suggesting a different pathogenetic mechanism, which may advocate further research into the subject.

Serum OPG levels in the patients with HP and MO were on average higher than in the ABD group. Therefore, in histological patterns of renal osteodystrophy with high bone turnover and an active osteoblastic population, serum levels of OPG, like bone AP, were higher than in low turnover osteodystrophy. However, the inverse correlation of OPG with serum intact PTH, PTH 1–84 and total PTH levels, found in HP and MO patients with intact PTH levels $\leqslant$1000 pg/ml, is interesting. In addition, an inverse correlation was found between serum OPG and the histomorphometric parameters of bone resorption with special regard to ES/BS. While no correlation was initially found between serum OPG and the bone formation parameters, negative correlations were found between them after the exclusion of one patient with out-lying values. Including three cases with very elevated levels of PTH in the statistical evaluation, the negative correlations were again not observed.

PTH has been found to stimulate OPGL and reduce OPG secretion *in vitro* in osteoblastic-like cells [13–15]. Our findings in renal osteodystrophy patients suggest that PTH has a similar effect on osteoblastic cells *in vivo*. Excess PTH secretion could enhance bone resorption via the same mechanism already shown *in vitro*, which is through the modulation of OPG/OPGL cytokine system. However, the few cases with PTH levels above 1000 pg/ml had relatively elevated OPG levels, suggesting an increased production of OPG when the osteoblastic population is maximally stimulated by PTH.

As for the negative correlations between OPG and the formation parameters, one might hypothesize that the increase in PTH serum levels responsible for a reduction in OPG levels is also the cause of osteoblastic activation and bone sclerosis. A similar explanation can be given for the significant negative correlation between OPG and the parameter O.Th, and the borderline negative correlation of serum OPG with BV/TV.

In addition, serum OPG showed a significant positive correlation with calcitriol serum levels. The implication of this correlation is not yet clear. Calcitriol, a known factor of osteoblastic maturation, has been found to transiently stimulate OPGL expression in immature preosteoblastic cells, while this response is less evident in mature osteoblasts [13]. Increasing levels of calcitriol could be accompanied by a larger pool of mature osteoblasts, a relatively increased OPG production and reduced OPGL expression.

The present findings in renal osteodystrophy are the first observations of such a negative relationship between OPG, PTH and the parameters of bone

turnover in human metabolic bone disease, at least at PTH values $\leqslant$1000 pg/ml. Therefore, the OPG/OPGL cytokine system may be of importance in mediating the effects of PTH in renal bone disease. The effect of PTH on bone might also be controlled by several factors modulating this cytokine system in bone. An inverse correlation between PTH serum levels and OPG was also observed in normal subjects above 40 years of age [16].

An additional observation is the finding of a significant difference in OPG serum levels between patients with ABD and those with HP and MO at PTH levels of $\leqslant$300 pg/ml. It is known that PTH levels ranging from 100 to 300 pg/ml, define an area of uncertain bone turnover in chronic renal failure [2,23]. At PTH serum levels $\leqslant$300 pg/l, the average value of OPG, similarly to bone AP, was significantly lower in the patients with ABD than in HP and MO patients (Figure 3). This finding might suggest that serum OPG assays might be useful for distinguishing between low turnover bone disease and high turnover renal osteodystrophy, at least in the range of PTH values where a clinical diagnosis is in doubt.

In conclusion, the OPG/OPGL cytokine system appears to be directly involved in the development of low and high turnover bone disease in renal osteodystrophy. Serum OPG levels could be of help in the diagnosis of both disease entities.

Acknowledgements. This research has been supported by funds of the Second Medical Faculty of La Sapienza University, Rome. The skilled contribution of Ms Antonina Scuderi is acknowledged. The authors would like to thank Dr Thomas Cantor, Scantibodies Laboratories, San Diego, CA, for providing the kits for some of the PTH assays.

References

1. Quarles LD, Lobaugh B, Murphy G. Intact parathyroid hormone overestimates the presence and severity of parathyroid-mediated osseous abnormalities in uremia. *J Clin Endocrinol Metab* 1992; 75: 145–150
2. Coen G, Ballanti P, Bonucci E *et al*. Bone markers in the diagnosis of low turnover osteodystrophy in haemodialysis patients. *Nephrol Dial Transplant* 1998; 13: 2294–2302
3. Salusky IB, Kuizon BD, Belin TR *et al*. Intermittent calcitriol therapy in secondary hyperparathyroidism: comparison between oral and intraperitoneal administration. *Kidney Int* 1998; 54: 907–914
4. Monier-Faugere MC, Malluche HH. Role of cytokines in renal osteodystrophy. *Curr Opin Nephrol Hypertens* 1997; 6: 327–332
5. Gonzales EA. The role of cytokines in skeletal remodelling: possible consequences for renal osteodystrophy. *Nephrol Dial Transplant* 2000; 15: 945–950
6. Herbelin A, Ureña P, Nguyen AT, Zingraff J, Descamps-Latscha B. Elevated circulating levels of interleukin-6 in patients with chronic renal failure. *Kidney Int* 1991; 39: 954–960
7. Montalban C, Garcia-Unzueta MT, DeFrancisco ALM, Amado JA. Serum interleukin-6 in renal osteodystrophy: relationship with serum PTH and bone remodeling markers. *Horm Metab Res* 1999; 31: 14–17
8. Chambers TJ. Regulation of the differentiation and function of osteoclasts. *J Pathol* 2000; 192: 4–13
9. Hofbauer LC, Lacey DL, Dunstan CR, Spelberg TC, Riggs BL, Khosla S. Interleukin-1$\beta$and Tumor Necrosis Factor-α, but not Interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. *Bone* 1999; 25: 255–259
10. Simonet WS, Lacey DL, Dunstan CR *et al*. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; 89: 309–319
11. Mizuno A, Amizuka N, Irie K *et al*. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. *Biochem Biophys Res Commun* 1998; 247: 610–615
12. Hofbauer LC, Heufelder AE. Role of receptor activator of nuclear factor-κB ligand and osteoprotegerin in bone cell biology. *J Mol Med* 2001; 79: 243–253
13. Horwood NJ, Elliott J, Martin JM, Gillespie MT. Osteotropic agents regulate the expression of osteoclast differentiation factor and osteoprotegerin in osteoblastic stromal cells. *Endocrinology* 1998; 139: 4743–4746
14. Lee SK, Lorenzo JA. Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. *Endocrinology* 1999; 140: 3552–3561
15. Onya JE, Miles RR, Yang X, Halladay DL *et al*. *In vivo* demonstration that human parathyroid hormone 1-38 inhibits the expression of osteoprotegerin in bone with the kinetics of an immediate early gene. *J Bone Miner Res* 2000; 15: 863–871
16. Szulc P, Hofbauer LC, Heufelder AE, Roth S, Delmas PD. Osteoprotegerin serum levels in men: correlation with age, estrogen and testosterone status. *J Clin Endocrinol Metab* 2001; 86: 3162–3165
17. Coen G, Mazzaferro S, Ballanti P *et al*. Renal bone disease in 76 patients with varying degrees of predialysis chronic renal failure: a cross-sectional study. *Nephrol Dial Transplant* 1996; 11: 813–819
18. Maloney NAQ, Ott SM, Alfrey AC, Miller NL, Coburn JW, Sherrard DJ. Histological quantification of aluminium in iliac bone from patients with renal failure. *J Lab Clin Med* 1982; 99: 206–216
19. Parfitt AM, Drezner MK, Glorieux FH *et al*. Bone histomorphometry standardization of nomenclature, symbols and units. *Bone Miner* 1987; 2: 595–610
20. Ballanti P, Bonucci E, Della Rocca C, Milani S, LoCascio V, Imbimbo B. Bone histomorphometric reference values in 88 normal Italian subjects. *Bone Miner* 1990; 11: 187–197
21. Malluche HH, Faugere MC. Renal bone disease 1990: an unmet challenge for the nephrologist. *Kidney Int* 1990; 38: 193–211
22. Fournier A, Ghitu S, Bako G *et al*. Bone markers in the diagnosis of low turnover osteodystrophy in haemodialysis patients. *Nephrol Dial Transplant* 1999; 13: 2772–2774
23. Fournier A, Oprisiu R, Said S *et al*. Invasive versus non-invasive diagnosis of renal bone disease. *Curr Opinion Nephrol Hypertens* 199; 6: 333–348

Received for publication: 13.9.01
Accepted in revised form: 22.11.01