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CLINICAL STUDY

Osteoprotegerin and Bone Mineral Density in Hemodiafiltration Patients

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A newly identified cytokine, osteoprotegerin (OPG) appears to be involved in the regulation of bone remodeling. In vitro studies suggest that OPG, a soluble member of the TNF receptor family of proteins, inhibits osteoclastogenesis by interrupting the intercellular signaling between osteoblastic stromal cells and osteoclast progenitors. As patients with chronic renal failure (CRF) often have renal osteodystrophy (ROD), we investigated the role of osteoprotegerin (OPG) in ROD, and investigated whether there was any relationship between serum OPG, intact parathyroid (PTH) (iPTH), vitamin D, and trabecular bone. Serum OPG combined with iPTH might be a useful tool in the noninvasive diagnosis of ROD, at least in cases in which the range of PTH values compromises reliable diagnosis. Thirty-six patients on maintenance hemodiafiltration (HDF) and a control group of 36 age and sex matched healthy subjects with no known metabolic bone disease were studied. The following assays were made on serum: iPTH, osteocalcin (BGP), bone alkaline phosphatase, 25(OH)-cholecalciferol, calcium, phosphate, OPG, IGF-1, estradiol, and free testosterone. Serum Ca^{++}, P, B-ALP, BGP, IGF-1, iPTH, and OPG levels were significantly higher in HDF patients than in controls, while DXA measurements and quantitative ultrasound (QUS) parameters were significantly lower. On grouping patients according to their mean OPG levels, we observed significantly lower serum IGF-1, vitamin D3 concentrations, and lumbar spine and hip bone mineral density in the high OPG groups. No correlation was found between OPG and bone turnover markers, whereas a negative correlation was found between serum OPG and IGF-1 levels ($r=-0.64$, $p=0.032$). Serum iPTH concentrations were positively correlated with bone alkaline phosphatase (B-ALP) ($r=0.69$, $p=0.038$) and BGP ($r=0.92$, $p<0.001$). The findings made suggest that an increase in OPG levels may be a compensatory response to elevated bone loss. The low bone mineral density (BMD) levels found in the high OPG group might have been due to the significant decrease in serum IGF-1 and vitamin D3 observed. In conclusion, the findings made in the present study demonstrate that increased OPG in hemodiafiltration patients is only partly due to decreased renal clearance. As it may partly reflect a compensatory response to increased bone loss, this parameter might be helpful in the

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identification of patients with a marked reduction in trabecular BMD.

Keywords osteoprotegerin, hemodiafiltration, renal osteodystrophy, bone mineral density

INTRODUCTION

Renal osteodystrophy is one of the most common complications affecting patients with chronic renal failure (CRF). Numerous factors are involved in the pathogenesis of the polymorphism of skeletal abnormalities, including phosphate retention, abnormalities in vitamin D and parathyroid hormone (PTH) (e.g., secondary hyperparathyroidism, hypoparathyroidism, PTH resistance of bone cells), hypogonadism, amyloidosis, immobility, poor quality dialysis, diabetes mellitus, or corticosteroid administration.[1,2] The term ''renal osteodystrophy'' includes a wide spectrum of skeletal alterations: from states of high turnover, such as osteitis fibrosa, characterized by increased osteoclast and osteoblast number and activity, to low bone turnover, as occurs in adynamic bone disease, characterized by a decrease in osteoclasts and osteoblasts and, therefore, by low bone resorption and formation. While it is known that hyperparathyroidism is involved in the development of osteitis fibrosa, the pathogenesis of adynamic bone lesions is not well understood. However, it has been suggested that this alteration depends on the oversuppression of bone turnover consequent to the increased use of calcitriol and calcium salts.[3]

A large body of new information on bone physiology has been reported in recent years. A new cytokine, osteoprotegerin (OPG), a soluble member of the TNF receptor family of proteins,[4] appears to be involved in the regulation of bone remodeling. In vitro studies suggest that OPG inhibits osteoclastogenesis by interrupting the intercellular signaling between osteoblastic stromal cells and osteoclast progenitors. Moreover, mice with targeted OPG ablation develop severe osteoporosis, due to a marked increase in the formation of osteoclasts, with subsequent bone resorption.[4–7] In vivo studies have shown that OPG is involved in the development of renal osteodystrophy. Hemodialysis patients have higher total OPG circulating levels than do normal subjects, but findings from studies investigating whether elevated serum OPG levels identify low or high bone turnover disease are contradictory. Haas et al. observed that circulating OPG levels, which are lower in patients with high-turnover bone disease, were higher than those in subjects with low to normal turnover bone disease and concluded that a combination of OPG and PTH may be used to in the correct diagnosis of renal osteodystrophy (ROD) types.[8] However, Coen et al. demonstrated that serum OPG values were significantly lower in patients with adynamic bone disease than in those with predominant hyperparathyroidism or mixed osteodystrophy.[9] A further study showed that OPG levels were 1.2-fold higher in hemodialysis patients with high PTH than in a low PTH group.[10]

However the assay of serum OPG in combination with PTH might be a useful tool for the noninvasive diagnosis of renal osteodystrophy, as in cases in which PTH values cast a doubt on the clinical diagnosis.[8–11]

The aim of the present study was, therefore, to investigate the role of OPG in uremic bone diseases, and to evaluate any relationships between serum OPG, PTH, vitamin D, other bone turnover markers, and lumbar spine and hip bone mineral density assessed using dual X-ray absorptiometry (DXA), and quantitative ultrasound (QUS) on the calcaneus and proximal phalanges of the hand.

METHODS

Subjects

Thirty-six patients (15 males and 21 females) on maintenance hemodiafiltration (HDF-Acetate Free Biofiltration, Integra Physio, Hospal, Bologna, Italy), performed for 3.5 hours, 3 times a week, with polyacrylonitrile filters (AN 69, Hospal, Bologna, Italy), for 27±9 months, were recruited after their fully informed consent had been obtained. All patients had undergone HDF for at least 12 months. The mean interdialytic increase in body weight was 3.2 kg (min, 2 kg; max, 4 kg). Blood and dialysate flows were set at a constant of 250–300 mL/min and 500 mL/min, respectively. All patients underwent three HDF sessions with a dialysis bath containing 1.75 mmol/L Ca^{++}, 2 mmol/L K^{+}, 0.5 mmol/L Mg^{++}, 139 mmol/L Na^{+}, and 5.56 mmol/L glucose. The ultrafiltration flow rate was constant throughout the session, and the total ultrafiltrated volume was <3.5 l for each subject.

Exclusion criteria were presence of clinical evidence of inflammatory syndrome, diabetes mellitus or other systemic diseases; change in calcium or calcitriol supplement dose during the 6 months prior to the study; and administration of corticosteroids, estrogens, androgens, anticoagulants, anticonvulsivants, or any drug influencing bone metabolism during the 6 months prior to the study. All patients were on calcitriol treatment (0.25 mcg/daily per os); 27 used calcium-containing phosphate binders, and nine were on sevelamer.

Table 1
Baseline subjects characteristics (mean ± SEM)

	Hemodiafiltration	Control
Age (years)	65 ± 2.3	61 ± 2
Males	15	15
Females	21	21
Duration of menopause (years)	19 ± 2.5	17 ± 2
Body mass index (kg/m^2)	24 ± 2.3	23 ± 0.5
Duration of HD (months)	40.76 ± 7.58	—

All dialyzed patients, except those with ADPKD, were treated with intravenous β-erythropoietin three times a week (mean dosage 23 ± 7 UI/kg body weight).

The control group, recruited from patients reporting to the Osteoporosis Center in the Department of Internal Medicine, consisted of 36 age and sex matched healthy subjects with no known metabolic bone disease.

Measurements

Predialysis blood samples were collected between 7:00 and 8:00 AM after an overnight fast. Serum samples were stored frozen at −80°C until assayed.

The following assays were performed on the serum samples: intact PTH, osteocalcin, bone alkaline phosphatase, 25(OH)-cholecalciferol, calcium, phosphate, OPG, IGF-1, estradiol, and free testosterone.

Bone alkaline phosphatase (B-ALP) was measured by a manual agarose assay with Automated Results (Sebia Inc., Norcross, GA). Analytical imprecision within runs was 0.5% coefficient of variation (CV) at a level of 458 U/I and between day imprecision was 2.2 CV% at a level of 357 U/I.

Ca^{++} was measured in a colorimetric assay with endpoint determination and sample blank on the Roche Modular systems (Roche Diagnostics Gmbh, Mannheim, Germany). Analytical imprecision within runs was 0.9% CV at a level of 8.48 mg/dL, and between day imprecision was 1.5 CV% at level of 8.38 mg/dL.

Phosphate was measured in a colorimetric assay with endpoint determination and sample blank on the Roche Modular systems (Roche Diagnostics Gmbh, Mannheim, Germany). Analytical imprecision within runs was 0.9% CV at level of 4.2 mg/dL and between day imprecision was 1.4 CV% at level of 5.2 mg/dL.

Serum intact PTH was measured using a commercial kit (Nichols Institute, San Juan Capistrano, CA, USA) immunoradiometric assay based on a double antibody technique; the normal range is 10–65 ng/L.

Plasmatic levels of estradiol were measured by electro-chemiluminescence assay (ECLIA) with an automatic analyzer, Elecsys 2010 (Roche Diagnostics SPA, Monza, Italy).

Serum osteocalcin (BGP) was measured by an immunometric assay that uses two monoclonal antibodies with specificity for intact human osteocalcin (1–49) (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The intra- and interassay coefficients of variation are 4.8% and 7.9% respectively.

Serum insulin-like growth factor (IGF-1) was measured by a chemiluminescence immunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The intra- and interassay coefficients of variation are typically below 10%.

Serum 25-hydroxicholecalcipherol was measured with a competitive protein-binding method, after purification on Sep-Pack C18 cartridges and extraction with acetonitrile. The normal range is 25–150 nmol/L.

Serum-free testosterone and 17β-estradiol were measured in men and women respectively by a radioimmunoassay (Biochem ImmunoSystem Italia SPA, Bologna, Italy). The intra- and interassay coefficients of variation are typically below 10%.

Osteoprotegerin was measured with a commercially available ELISA kit according to the protocol of the manufacturer (Immunodiagnostik, Bensheim, Germany). A monoclonal IgG antibody was used as capture antibody and biotin-labeled polyclonal antihuman OPG antibody was used as the detection antibody. This assay detects monomeric, dimeric, and ligand-bound forms of OPG [intra-assay coefficient of variation (CV) 8%; interassay CV 12%; lower detection limit: 0.14 pmol/L].

The bone mineral density (BMD) of the anteroposterior lumbar spine and hip (femoral neck, trochanteric, inter-trochanteric, and Ward triangle) was measured by DXA (HOLOGIC QDR 4500 W) immediately after hemodialysis. The instrument was calibrated on a daily basis according to the manufacturer's instructions. Reproducibility was calculated as a coefficient of variation (CV) obtained by weekly measurements of a standard phantom on the instrument, and by repeated measurements obtained in three patients of different ages. The CV of our instrument is 0.5% with the standard phantom; in vivo we calculated a CV of 1.1% for the lumbar spine, 1.5% for the neck, and 3.2% for Ward's triangle. The BMD data were expressed as grams per centimeter squared.

The QUS of the calcaneus was measured using an Achilles Express ultrasonometer (Lunar Corporation, Madison, WI) immediately after hemodialysis to avoid interference with peripheral soft tissue edema. To

Table 2
Biochemical and bone mineral density data of HDF patients and controls (mean ± SEM)

	Hemodiafiltration	Control	*p*
Ca (mmol/L)	2.75 ± 0.26	2.58 ± 0.02	<0.05
P (mmol/L)	1.62 ± 0.08	1.25 ± 0.025	<0.001
B-ALP (UI/L)	121 ± 15.7	76.8 ± 4.17	<0.01
BGP (mmol/L)	22 ± 4.05	4.9 ± 0.25	<0.001
IGF-1 (nmol/L)	20.17 ± 1.8	14.9 ± 0.61	<0.005
Vitamin D3 (nmol/L)	109.82 ± 7.48	117.312 ± 6.24	ns
iPTH (pmol/L)	238 ± 26.08	50 ± 2.83	<0.001
17β-estradiol (pmol/L)	77.09 ± 2.46	74.88 ± 066	ns
Free testosterone (pmol/L)	19.91 ± 2.49	26.02 ± 2.88	ns
OPG (pmol/L)	14 ± 0.88	5.7 ± 0.32	<0.001
Spine (g/cm^2)	1.06 ± 0.042	0.994 ± 0.018	ns
Femur neck (g/cm^2)	0.73 ± 0.023	0.81 ± 0.027	<0.05
Intertrochanteric (g/cm^2)	0.95 ± 0.02	1.043 ± 0.27	<0.005
Trochanteric (g/cm^2)	0.58 ± 0.022	0.68 ± 0.033	<0.05
Ward triangle (g/cm^2)	0.52 ± 0.023	0.61 ± 0.032	<0.05
BUA (dB/MHz)	102 ± 3.68	111 ± 1.06	<0.05
SOS (m/s)	1511 ± 4.96	1529 ± 4.5	<0.01
AD-SOS (m/s)	1801 ± 19	1895 ± 14.5	<0.001
UBPI	0.22 ± 0.025	0.3 ± 0.022	<0.01

minimize error sources, care was taken in positioning of the patient's leg and heel, and the heel skin was scrubbed with alcohol. Broadband ultrasound attenuation (BUA, dB/MHz) and the speed of sound (SOS, m/sec) were also measured.

The transmission of ultrasounds through the distal end of the first phalanx diaphysis in proximity of the condyles (ROI) of the last four fingers of the hand was also measured using a DBM Sonic Bone Profiler (IGEA, Carpi, Italy). The distal end of the diaphysis of the proximal phalanxes contains both cortical and trabecular bone, as well as a small medullary canal; the anatomic ROI is mostly cortical bone (approximately 60%).[12–14] The device calculates the amplitude-dependent speed of sound (AD-SOS) and the ultrasound bone profile index (UBPI). The UBPI, an optimum mathematical combination of three signal parameters developed to better discriminate fracture risk,[15] indicates the probability of a single subject belonging to the nonfractured group; its values are normalized, ranging from 0 to 1, the latter being attributed to the highest value obtained.[15] The final result is the average AD-SOS and UBPI over

Table 3
Biochemical, bone mineral density, and ultrasound data of HDF patients grouped according to PTH > or < 150 pg/mL (mean ± SEM)

	iPTH < 150 (21)	iPTH > 150 (15)	*p*
B-ALP (UI/L)	79 ± 11.29	173 ± 29.98	<0.005
BGP (ng/mL)	8.5 ± 0.91	40.96 ± 7.44	<0.001
IGF-1 (nmol/L)	19.38 ± 2.35	21.2 ± 3.1	ns
Vitamin D3 (nmol/L)	104.83 ± 14.91	117.31 ± 7.48	ns
Spine (g/cm^2)	1.048 ± 0.105	1.074 ± 0.140	ns
Femur neck (g/cm^2)	0.728 ± 0.037	0.727 ± 0.032	ns
Intertrochanteric (g/cm^2)	0.949 ± 0.027	0.955 ± 0.034	ns
Trochanteric (g/cm^2)	0.569 ± 0.026	0.602 ± 0.041	ns
Ward triangle (g/cm^2)	0.516 ± 0.034	0.526 ± 0.033	ns
BUA (dB/MHz)	103.8 ± 5.1	99.75 ± 6.09	ns
SOS (m/s)	1516 ± 4.6	1504 ± 10.56	ns
AD-SOS (m/s)	1784.4 ± 18.87	1821.25 ± 39.96	ns
UBPI	0.19 ± 0.025	0.26 ± 0.05	ns

Table 4
Biochemical, bone mineral density, and ultrasound data of HDF patients grouped according to OPG < and > 14 pmol/L (mean ± SEM)

	OPG < 14 pmol/L	OPG > 14 pmol/L	*p*
Number of patients	21 HD	15 HD	
Ca (mmol/L)	2.73 ± 0.18	2.78 ± 0.2	ns
B-ALP (UI/L)	101.4 ± 26.06	145.5 ± 14.65	ns
BGP (ng/mL)	23 ± 5.6	20 ± 6.5	ns
IGF-1 (nmol/L)	25.05 ± 2.46	14.45 ± 1.94	<0.005
Vitamin D3 (nmol/L)	132.13 ± 9.38	83.11 ± 9.63	<0.001
Spine (g/cm^2)	1.156 ± 0.06	0.911 ± 0.03	<0.005
Femur neck (g/cm^2)	0.759 ± 0.037	0.682 ± 0.025	ns
Intertrochanteric (g/cm^2)	0.967 ± 0.03	0.929 ± 0.02	ns
Trochanteric (g/cm^2)	0.627 ± 0.034	0.516 ± 0.054	<0.01
Ward triangle (g/cm^2)	0.582 ± 0.031	0.426 ± 0.015	<0.001
BUA (dB/MHz)	98.57 ± 4.42	114 ± 8.4	ns
SOS (m/s)	1509 ± 6.6	1517 ± 10	ns
AD-SOS (m/s)	1794 ± 28	1824 ± 11	ns
UBPI	0.21 ± 0.037	0.25 ± 0.016	ns

four fingers. At each measuring session, the reference speed of the subject's soft tissue is measured by applying the probes to the soft tissue area of the first interdigital space. The value is then automatically used by the device when measuring AD-SOS in the phalanx to take account of soft tissue interference.

In all subjects, AD-SOS was measured in the hand without a fistula by the same operator. All the osteosonogrammetry data were stored on a portable personal computer connected to the device.

Statistical Evaluation

Results were expressed as mean ± SEM. Data were analyzed by analysis of variance followed by a post hoc

Table 5
Biochemical, bone mineral density, and ultrasound data of HDF patients grouped according to combinations of OPG and PTH (mean ± SEM)

	Group A	Group B	Group C	Group D
Patient number	9	6	12	9
OPG	< 14 pmol/L	> 14 pmol/L	< 14 pmol/L	> 14 pmol/L
iPTH	> 150 pg/mL	> 150 pg/mL	< 150 pg/mL	< 150 pg/mL
B-ALP (IU/mL)	170 ± 47.33	177 ± 3.47	55.67 ± 6.49*,†	114 ± 24.98*,†
BGP (ng/mL)	39.43 ± 11.99	43.25 ± 10.94	11.32 ± 0.57*,†	4.73 ± 1.01*,†
IGF-1 (nmol/L)	28.03 ± 3.55	10.96 ± 0.02*	22.08 ± 3.85	16.8 ± 3.21
Vitamin D3 (nmol/L)	127.22 ± 11.10	112.44 ± 12.9	135.8 ± 15.57	63.57 ± 9.70*,†,‡
Spine (g/cm^2)	1.25 ± 0.11	0.89 ± 0.033*	1.107 ± 0.083	0.93 ± 0.06*
Femur neck (g/cm^2)	0.82 ± 0.03	0.63 ± 0.02	0.73 ± 0.06	0.73 ± 0.05
Trochanteric (g/cm^2)	0.69 ± 0.06	0.51 ± 0.016*	0.594 ± 0.042	0.52 ± 0.003*
Ward triangle (g/cm^2)	0.63 ± 0.02	0.42 ± 0.02*	0.558 ± 0.05	0.43 ± 0.03*
BUA (dB/MHz)	103 ± 9.3	91 ± 5.3	95 ± 4.7	93 ± 5.67
SOS (m/s)	1510 ± 16	1489 ± 9.8	1509 ± 4.5	1505 ± 12
AD-SOS (m/s)	1831 ± 13	1793 ± 23	1767 ± 26	1796 ± 28
UBPI	0.27 ± 0.023	0.21 ± 0.033	0.16 ± 0.032 +	0.22 ± 0.03

*p<0.05 vs. A.
†p<0.05 vs. B.
‡p<0.05 vs. C.

evaluation. Correlations between variables were assessed using linear regression analysis. The analyses were adjusted for age and bone mass index (BMI). A p value of <0.05 was considered statistically significant.

RESULTS

The clinical characteristics of all subjects participating in the study are shown in Table 1. There were no significant differences between groups for age, BMI, and years from menopause. The average time on dialysis was 41 ± 8 months.

The causes of renal failure were hypertensive nephroangiosclerosis,[16] glomerulonephritis,[9] tubulo-interstitial nephritis,[7] and ADPKD.[4]

The biochemical data obtained are summarized in Table 2. Serum Ca^{++}, P, B-ALP, BGP, IGF-1, iPTH, and OPG levels were significantly higher in hemodiafiltration patients than in controls ($p<0.05$). No statistically significant differences were found between the hemodialysis and the control groups for 25OHvitamin D3, estradiol, and free testosterone concentrations.

The DXA measurements at the hip and QUS parameters of the calcaneum and the phalanxes were significantly lower in patients than in control subjects, whereas the two groups had similar lumbar spine BMD values (Table 2).

The value of parathyroid (PTH) chosen in this study for subdividing the patients into two groups was 150 ng/L, since iPTH levels lower than 150 ng/L may well predict a low bone turnover disease.[16] On grouping all patients according to their intact PTH (iPTH) levels (below or above 150 ng/L), we found significantly higher B-ALP and BGP levels in the group with higher iPTH (Table 3).

On the other hand, on dividing the same patients into two groups according to their mean OPG value (14 pmol/L), we observed significantly lower serum IGF-1, vitamin D3 concentrations, and lumbar spine, trochanteric, and Ward's triangle bone mineral density in the high OPG groups (Table 4). No significant differences were observed in serum calcium levels between the two groups. On evaluating whether combinations of OPG and iPTH levels might segregate and, therefore, predict a particular group of patients, we obtained four subgroups (Table 5): high iPTH (>150 pg/mL)—high OPG (>14 pmol/L) (group B), high iPTH (>150 ng/L)—low OPG (<14 pmol/L) (group A), (shouldn't the order be A, B, C, and D) low iPTH (<150 ng/L)—low OPG (<14 pmol/L) (group C), low iPTH (<150 ng/L)—high OPG (>14 pmol/L) (group D). The B-ALP and BGP levels in groups C and D were significantly lower than were those in groups A and B. Group D patients had lower Vitamin D concentrations than did patients in Groups A, B, and C. The IGF-1 levels in Group B were significantly lower than those in Group A. Lumbar spine, trochanteric, and Ward's triangle bone mineral density were significantly higher in Group A than in Groups B and C. The UBPI was significantly lower in Group C than in Group A (Table 5). No significant differences were observed in serum calcium levels between these groups.

No correlation was observed between OPG and bone turnover markers, whereas a negative correlation was found between serum OPG and IGF-1 levels ($r=-0.64$, $p=0.032$). Serum iPTH concentrations were positively correlated with B-ALP ($r=0.69$, $p=0.038$) and BGP ($r=0.92$, $p<0.001$) levels.

DISCUSSION

The discovery of the OPG/RANLKL/RANK system was a major advance in our understanding of bone biology. Osteoprotegerin, a soluble decoy receptor in the TNF receptor super family, blocks OPG, or RANK, ligand interaction with its osteoclast receptor RANK, thus inhibiting osteoclast differentiation and activity.[4–6]

Contradictory results have been reported in the few studies[8–11,17] performed to evaluate the role of serum OPG levels in the pathogenesis of renal osteodystrophy and any relationship between its serum concentrations and other bone disease markers. In our study, serum total OPG was significantly higher in HDF patients than in controls, which is in agreement with other research.[8–11,17]

We found that patients with an iPTH value of >150 ng/L had a higher bone turnover than did those with an iPTH value of <150 ng/L. The finding of no differences between these groups for OPG levels or any correlations with bone turnover markers indicates that only iPTH concentrations are helpful in discriminating between high to normal, and a low ROD turnover.

Moreover, at both DXA and QUS assessment, no differences were found between bone mineral density in the two groups subdivided on the basis of PTH levels. However, in patients with high OPG values (or a high mean OPG value) the lumbar spine, the trochanteric, and Ward's triangle bone mineral density values were significantly lower than those in the group with low OPG levels. We, therefore, believe that an increase in OPG levels may be a compensatory response to high bone loss. Our data are in agreement with the finding made by other authors that serum OPG concentrations are significantly higher in women with postmenopausal osteoporosis than in age-matched healthy controls and that, within the osteoporotic group, OPG concentrations are higher in women with lower bone mineral density.[18]

Yet in another study, no difference was found between serum OPG levels in osteoporotic women and those in healthy postmenopausal women.[19]

The reduced bone mineral density we found in the high OPG group may have been due to the significant decrease in serum IGF-1 and vitamin D3 observed in this group with respect to the group with low OPG levels. The IGF-1 modulates bone turnover, and high IGF-1 levels stimulate bone formation, whereas low levels are associated with a bone turnover reduction.[20] The values for total and free IGF-1 were lower in hemodiafiltration patients with osteopenia than in those without osteopenia.[21] Moreover, we found a negative correlation between serum OPG and IGF-1 levels and this datum is in agreement with that reported an in vitro study showing that IGF-1 reduced OPG mRNA expression in bone cells.[21]

Our findings concerning any correlation between vitamin D3 and OPG were unexpected. Production of OPG may in fact be increased or decreased by vitamin D.[22–24] In our hemodiafiltration subjects, we found no correlation between these two parameters. We therefore assumed that the reliability of this parameter might enhance if a RANKL/OPG ratio was used to identify any relationship between vitamin D3 and this cytokine system. Moreover we ascertained whether combinations of OPG and PTH segregate particular groups of patients, enabling us to identify any differences in bone mineral density and QUS parameters across groups. The group with iPTH>150 ng/L and OPG>14 pmol/L had lower IGF-1 and trabecular bone mineral density than did the group with iPTH>150 ng/L and OPG<14 pmol/L. The OPG determination therefore may be used in patients with PTH>150 ng/L to identify higher trabecular bone loss.

The group with OPG<14 pmol/L and iPTH<150 ng/L had a lower B-ALP, BGP, and ultrasound bone profile index (UBPI) than did the OPG<14 pmol/L and iPTH>100 ng/L group. Consequently, in patients with OPG<14 pmol/L, PTH determination might be used to identify those with a poorer bone quality and bone turnover. It has, in fact, been shown that PTH stimulates not only bone reabsorption but also bone mineralization by increasing the number of osteoblasts, and that lower iPTH levels are associated with a lower mineralization rate;[25,26] it may be possible to diagnose the latter phenomenon, by means of UBPI.

However, it has been shown that AD-SOS and UPBS values are lower in hemodialysis patients with a high bone turnover than in those with a low bone turnover.[27] We, therefore, assumed that UBPI might be helpful in identifying hemodialysis patients with adynamic bone disease, with a diminished load-bearing bone capacity, therefore, at a major risk of fracture. In fact UBPI values are increased in ROD type I and II, but not in type III, patients.[28] On the other hand, it has been shown that ultrasound scanning of the phalanxes allows primary hyperparathyroidism and osteomalacia to be distinguished from osteoporosis.[29,30] However, BMD and heel ultrasound measurements are poor predictors of bone turnover and fracture risk in hemodialysis patients: one study on 104 patients in hemodialysis for at least 1 year showed that neither hip and lumbar spine DXA nor calcaneal ultrasound were predictive of fracture history.[31]

In the present study, OPG was measured in the peripheral circulation, and it is unclear as to how its extent reflects changes and activity in the bone microenvironment. Moreover, OPG is produced mainly by bone cells but also by a number of other tissue cells.[32] Therefore, serum changes may have been underestimated because nonskeletal sources may have increased background noise. Moreover, the methodology we used for measuring OPG in serum has not yet been fully validated.

However, it may be concluded that the increase in circulating OPG in patients on hemodiafiltration is due either to a decreased renal clearance, such as that demonstrated by Kazama et al., or to the absence of its removal through the hemodialysis membrane.[17] In either case, this occurrence may represent a compensatory response to increased bone loss and, therefore, might be helpful in identifying patients with a major reduction in trabecular bone density. However, currently a correct diagnosis of ROD can be achieved by performing bone biopsy. Further studies are required, therefore, in order to test the efficacy of a combined approach using the noninvasive bone renal disease evaluations now available, such as serological biochemical bone markers, DXA, and QUS parameters.

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