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Osteoprotegerin serum levels in women: Correlation with age, bone mass, bone turnover and fracture status

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Serum-Osteoprotegerinspiegel bei Frauen: Korrelation zu Alter, Knochendichte, Knochenstoffwechsel und Frakturstatus

Zusammenfassung. In präklinischen Studien wurde gezeigt, dass Osteoprotegerin (OPG) einen hemmenden Einfluss auf die Osteoklasten ausübt und daher eine wesentliche Rolle im Knochenstoffwechsel spielt. Ziel dieser Studie war es den *klinischen* Stellenwert von OPG zu evaluieren. Ist auch bei Menschen ein hoher Serum-OPG Spiegel mit niedriger Knochenresorption und konsekutiv höherer Knochendichte verbunden?

Bei 177 gesunden Frauen (17–85 Jahre) und bei 48 unbehandelten Patientinnen (71 ± 5 Jahre) mit primärer postmenopausaler Osteoporose wurde OPG im Serum gemessen und in Relation zu Alter, Knochendichte, Knochenstoffwechselparametern und – im Fall der Patientinnen mit Osteoporose – zu prävalenten Wirbelkörperfrakturen gestellt.

Bei den gesunden Probanden korrelierte OPG positiv mit dem Alter (r = 0,25, p < 0,001), während kein Zusammenhang mit der Knochendichte oder den Knochenumbauparametern gefunden wurde. Bei den Osteoporose-Patientinnen hingegen zeigte sich eine klare Korrelation mit den Knochenmarkern: Serum-Crosslaps r = +0.82, p < 0.0001; Osteokalzin r = +0,69; p < 0,0001.

Nach Normalisierung der Serum-OPG Werte für die Knochendichte und Umbaumarker zeigten Patientinnen mit prävalenten Wirbelkörperfrakturen deutlich niedrigere Werte im Vergleich zu den Patientinnen ohne Frakturen (57 ± 8 vs. 97 ± 10 pg/ml; p < 0,01).

Der altersabhängige Anstieg von OPG als antiresorptiver Faktor könnte einen insuffizienten parakrinen Kompensationsversuch der Osteoblasten auf den Knochenverlust im Alter darstellen. Der klare Zusammenhang von OPG mit den Knochenumbaumarkern bei Patientinnen mit Osteoporose ist wahrscheinlich als Ausdruck einer erhöhten RANKL/OPG Expression zu werten.

Niedrige OPG-Spiegel scheinen mit einem erhöhten Frakturrisiko assoziiert zu sein. Der individuelle Serum OPG Wert könnte unter Berücksichtigung des Knochenstoffwechsels ein prädiktiver Faktor für das Frakturrisiko bei Frauen mit primärer postmenopausaler Osteoporose sein.

Schlüsselwörter: Osteoprotegerin, RANKL, Frakturrisiko, Knochenstoffwechsel, Knochendichte.

Summary. Pre-clinical data have shown that osteoprotegerin (OPG) inhibits osteoclast function and therefore plays an important role in bone remodelling. This study aimed to evaluate the *clinical* value of serum OPG. Do higher OPG serum levels reflect decreased bone resorption and perhaps higher bone mass in women?

Serum OPG levels were measured in 177 healthy women (aged 17–85 years) and in 48 untreated patients (mean age 71 ± 5) with established osteoporosis, and related to age, bone mass, markers of bone turnover and, in the case of patients with osteoporosis, to pre-existing vertebral fractures.

In healthy women OPG levels showed a positive correlation with age (r = 0.25, p < 0.001) but not to bone mass or markers of bone turnover. In women with osteoporosis, however, there was a strong relationship between serum OPG and markers of bone turnover (serum c-terminal crosslinked telopeptides of type I collagen (sCTX): r = +0.82, p < 0.0001; osteocalcin (OC): r = +0.69, p < 0.0001), with patients who had higher levels of bone-turnover markers showing higher serum levels of OPG. After adjustment for bone mass and bone markers, patients with pre-existing vertebral fractures had significantly lower serum OPG levels than patients without fractures (57 ± 8 vs. 97 ± 10 pg/ml, [mean ± SE], p < 0.01).

The age-dependent increase of OPG as an antiresorptive factor may reflect an insufficient paracrine mechanism of bone cells to compensate for bone loss in older age. In patients with osteoporosis, however, OPG correlated strongly with markers of bone turnover; this may point toward a higher level of RANKL/OPG expression in these patients.

Finally, low OPG serum levels seem to be associated with vertebral fractures. We hypothesise that low OPG levels in preset conditions of bone turnover may indicate a higher risk of fracture in patients with osteoporosis.

Key words: Osteoprotegerin, RANKL, fracture risk, bone turnover, bone mass.

Introduction

New aspects of bone remodelling mechanisms have been reported in recent years and, with the discovery of osteoprotegerin (OPG) [1–3] and its ligand RANKL (receptor activator of NF-κB) [3–5], a new paradigm of osteoclast differentiation, fusion, activation and survival regulation has emerged [1–9].

Animal studies indicate that OPG increases bone density and protects against bone loss [1, 10–12], whereas OPG-deficient mice develop low bone mass and a higher incidence of fractures [1, 10, 12].

This in turn suggests that, if circulating OPG levels reflect local OPG expression in bone, individuals with high OPG levels should experience less bone loss and have higher bone mass than those with low serum OPG levels. Recently published human data do not, however, support this completely [13–16]. In contrast, Yano et al. found an inverse relationship between OPG serum levels and bone mass in postmenopausal women [13], and recently published data showed similar findings in a cohort of healthy men [14]. Another study found that high OPG levels are associated with a higher incidence of hip fracture, though there was no correlation with bone mass at any measurement site [15]. On the other hand, a significant negative correlation of OPG with urinary excretion of deoxypyridinoline and parathyroid hormone (PTH) serum levels has been described in elderly men, with no association with bone density or fracture status [16], whereas Khosla et al. described a positive correlation with resorption markers in men older than 50 [14]. The conflicting results of these studies may be partly because different assays were used for OPG detection in serum and the cohorts investigated differed in gender, age, origin and health conditions.

OPG serum levels were significantly higher in a cohort of 26 patients on hemodialysis therapy than in healthy controls [17]. Furthermore OPG tended to be negatively correlated with osteoblast and osteoclast surface area and may be used as marker for trabecular bone mineralization.

The therapeutic potential of OPG in the treatment of osteoporosis was recently proposed in a short-term study in which postmenopausal women showed a significant decrease of bone-turnover markers when treated with a single subcutaneous dose of OPG [18].

We investigated OPG serum levels in a healthy female population in relationship to age, menopausal status, bone mass and markers of bone turnover, as well as to fracture status in untreated women with primary postmenopausal osteoporosis.

Participants and methods

Healthy volunteers

Between March and August 1999 we assessed bone density and analysed laboratory data in a total of 461 women with a median age of 46 years (range 17–85) who responded to our advertisement in a local newspaper. All patients were Caucasian, ambulatory and willing to answer a questionnaire on their medication, past medical history, lifestyle factors, fractures, and gynaecological data including menses, number of children, frequency and length of breast feeding, menopausal status, contraceptive use and hormone replacement therapy.

Women on hormone replacement (n = 67) or contraceptive medication (n = 30) or with irregular menstruation (n = 3) were excluded from further analysis, and those whose bone density measurements showed t-score values below 2.5 SD (standard deviation) at the spine or hip were classified according WHO criteria [19] as having osteoporosis (n = 82) and were also excluded. We further excluded women with a history of bone fractures (n = 17), steroid treatment (n = 7) or any medication affecting bone metabolism, such as bisphosphonates (n = 9), calcium and/or vitamin D supplements (n = 23), statins (n = 22) or diuretics (n = 5). Nineteen women had to be excluded when laboratory analysis revealed hepatic (n = 8) or kidney (n = 9) dysfunction, hyperthyroidism (n = 1) or hypocalcemia (n = 1).

The remaining 177 healthy women were divided according to their menopausal status into a premenopausal group with regular menses (n = 131) and a postmenopausal group (n = 46) without menstruation for at least two years.

Patients with osteoporosis

In the spring of 2001, female patients with newly diagnosed postmenopausal osteoporosis who attended our outpatient bone clinic were invited to participate in a detailed analysis of bone status, including a questionnaire and the collection of serum samples for storage and later analysis of OPG. Patients with a history of recent fractures (within the past year) were excluded from the study in order to eliminate any influence of increased bone remodelling, due to fracture healing, on bone-turnover markers or OPG serum levels.

Forty-eight women who were not receiving any medication known to influence bone metabolism and had no evidence of secondary causes of osteoporosis were suitable for further analyses.

These patients were divided into a fracture (n = 17) and a non-fracture (n = 31) group according to their fracture status (radiologically confirmed vertebral fractures yes/no).

In addition, patients with serum sCTX and/or OC levels above the normal range were assigned to a "high bone-turnover" group (n = 15) while the remaining patients formed the "normal bone-turnover" group (n = 33).

The study was approved by the Ethics Committee of the Karl-Franzens University and all patients gave written informed consent before undergoing the study protocols.

Laboratory analysis

Blood samples were taken the morning after an overnight fast and sera aliquoted. Routine serum parameters included total serum calcium (Ca, normal range 2.0–2.6 mM/l, corrected for total protein according to Husdan et al. [20], phosphate (P, normal range 3.4–4.6 mg/l), magnesium (Mg, 0.7–1.1 mmol/l), creatinine (Crea, 0.6–1.3 mg/dl), gamma glutamyl transpeptidase (GGT, normal range < 29 U/l) and alkaline phosphatase (ALP, normal range 55–170 U/l) and were measured the same day using an autoanalyzer (Hitachi 747). The remaining aliquots, including sera for OPG analysis, were stored at –80 °C for later analysis of thyroid stimulating hormone (TSH, normal range 0.1–4.0 mE, Behring, Marburg, Germany), 25-(OH)-vitamin D (vitamin D_3, normal range 9–45 ng/ml, Immunodiagnostic Systems, Boldon, UK), OC (CIS-bio-international, Gif-sur-Yvette, France, normal range 3–10 µg/l for the healthy volunteers and Nichols Diagnostics, San Juan Capistrano, California, normal range 1–35 ng/ml for the patients with osteoporosis). We also

analysed sCTX, normal range 1.4–4.5 nmol/l, Osteometer, Bio Tech, Denmark, for the healthy volunteers and used an ELISA (Nichols Diagnostics, San Juan Capistrano, California, normal range for postmenopausal women 1.0–2.84 nmol/l) for the patients with osteoporosis. Intact PTH (normal range 10–65 pg/ml, Nichols Diagnostics, San Juan Capistrano, California) was also analysed.

OPG was measured in all patients and healthy volunteers using a highly sensitive commercially available polyclonal antibody-based sandwich ELISA (Biomedica GmbH, Vienna, Austria) with an intra- and inter-assay variability of 9% and 10%, respectively. This assay uses two highly specific antibodies against human OPG. A monoclonal IgG antibody raised from a murine hybridoma cell line after immunizing a mouse with recombinant human OPG (rhOPG) is used as a capture antibody able to neutralize the biological activity of rhOPG. The detection antibody is a biotin-labeled polyclonal anti-human OPG antibody derived from a goat after immunization with rhOPG.

The assay detects both monomeric and dimeric forms of OPG, including OPG bound to its ligand. Undiluted samples were tested according to the manufacturer's instructions. The normal range established in the 177 healthy controls was 6–138 pg/ml.

The lowest detection level was 1.2 pg/ml. All samples were measured in duplicate and averaged. If the coefficient of variation (CV) was greater 20% the sample was re-assayed.

The same OPG assay was used by Szulc et al. [16].

X-rays and bone density measurements

All patients with osteoporosis had standardized X-rays of the spine and the films were analysed by an experienced radiologist. Using the method described by Genant et al., a pre-existing vertebral fracture was diagnosed when there was >20% reduction in any of the anterior-, middle- or posterior-height ratios of the vertebral body compared with undeformed adjacent vertebrae [21].

All participants underwent bone mineral density (BMD) measurements of the femoral neck and the lumbar spine (L1–L4) (Hologic QDR 4500 Acclaim). The in vivo CV for lumbar spine and femoral neck measurements using this equipment is 1.2% and 2%, respectively. Bone density measurements were expressed as t- and z-score values comparing individual results with the normative NHANES database. Osteoporosis was diagnosed according to WHO criteria [19], when DXA measurement revealed t-score values below 2.5 SD at one of the measured sites.

Statistical analysis

All data are presented as mean ± SE (standard error) unless otherwise indicated. Unpaired Student's t-tests (e.g. differences in serum OPG levels) or chi-square tests (e.g. pre-existing fractures yes/no) were used for between – group comparisons.

Correlation between various parameters was analysed using simple linear regression. The relationship between parameters was evaluated in a multiple regression analysis. A p-value <0.05 was considered significant. All data were checked for normal distribution using the Kolmogorow-Smirnov test.

The statistical software packages Stat View (Abacus Concepts, version 4.0, Inc., Berkeley, California) and Statistica (StatSoft, version 5.5, Inc., Tulsa, Oklahoma) were used for data analysis.

Results

Healthy volunteers

The characteristics of this group are given in Table 1. Twenty-six percent (n = 46) of the volunteers had been postmenopausal for at least two years, whereas the majority (n = 131) had regular menstruation.

Table 1. Baseline characteristics of healthy volunteers. Comparison between pre- and postmenopausal women. All parameters are given as mean ± standard error

	All women n = 177	Premenopausal women n = 131	Postmenopausal women n = 46	p-value
Age	44 ± 1	38 ± 0.9	63 ± 1.1	< 0.0001
BMI	24 ± 0.3	23 ± 0.3	28 ± 0.6	< 0.0001
Serum creatinine	0.98 ± 0.01	0.97 ± 0.02	1.0 ± 0.01	< 0.05
ALP	83 ± 2	79 ± 2	95 ± 3	< 0.01
Serum calcium	2.35 ± 0.01	2.35 ± 0.01	2.36 ± 0.02	N.S.
Serum magnesium	0.86 ± 0.01	0.86 ± 0.01	0.87 ± 0.01	N.S.
Serum phosphate	3.7 ± 0.04	3.7 ± 0.05	3.8 ± 0.08	N.S.
GGT	13.4 ± 1	12.6 ± 1.2	15.6 ± 1.7	N.S.
TSH	1.57 ± 0.09	1.6 ± 0.11	1.46 ± 0.15	N.S.
25 (OH) vitamin D	36 ± 1.1	38 ± 1.4	31 ± 1.9	< 0.01
sCTX	1.9 ± 0.07	1.8 ± 0.08	2.2 ± 0.15	< 0.01
Osteocalcin	5.7 ± 0.15	5.6 ± 0.16	6.1 ± 0.34	N.S.
OPG	77 ± 3.6	69 ± 3.7	101 ± 8.3	< 0.0001
Bone mineral density				
Femoral neck	–0.30 ± 0.08	–0.55 ± 0.08	+ 0.47 ± 0.16	< 0.0001
Lumbar spine	+ 0.04 ± 0.08	–0.14 ± 0.08	+ 0.58 ± 0.19	< 0.0001

Age (years), creatinine (0.6–1.3 mg/dl) to convert to SI units multiply by 88.1, alkaline phosphatase (55–170 U/l), serum calcium (2.0–2.6 mmol/l), serum magnesium (0.7–1.1 mmol/l), serum phosphate (3.4–4.6 mg/l), gamma glutamyl transpeptidase (< 29 U/l), thyroid stimulating hormone (0.1–4.0 mE/l), 25 (OH) vitamin D (9–45 ng/ml) to convert to nanomoles per liter multiply by 25, sCTX (1.4–4.5 nmol/l), osteocalcin (3–10 µg/l), OPG (6–138 pg/ml), bone mineral density (z-score ± SD); *N.S.* not significant.

Table 2. Baseline characteristics of the patients with osteoporosis. Comparisons between "high bone turnover" and "normal bone turnover" as well as "fracture" and "non-fracture" groups. As the majority of lumbar spine X-rays revealed osteoarthritis and/or vertebral fractures in most of the patients, results of lumbar DXA measurements are not given in the table. All parameters are given as mean ± standard error

	All patients n = 48	Bone turnover		p-value high/normal	Prevalent vertebral fracture		p-value yes/no
		high n = 15	normal n = 33		yes n = 17	no n = 31	
Age	71 ± 0.7	70 ± 0.62	71 ± 0.7	N.S.	71 ± 1.1	70 ± 1	N.S.
BMI	27.3 ± 0.05	7.7 ± 0.07	28 ± 0.09	N.S.	27.9 ± 0.09	27.7 ± 0.05	N.S.
Femoral neck Z-Score	–0.90 ± 0.07	–0.78 ± 0.13	–0.95 ± 0.08	N.S.	–0.85 ± 0.11	–0.92 ± 0.08	N.S.
Pre-existing vertebral fracture (%)	35	27	39	N.S.	N.A.	N.A.	N.A.
sCTX	2.6 ± 0.2	4.3 ± 0.17	1.8 ± 0.1	p < 0.0001	2.3 ± 0.02	2.7 ± 0.03	N.S.
Osteocalcin	25.9 ± 1.3	35 ± 2	22 ± 1	p < 0.0001	24 ± 2	27 ± 1.8	N.S.
intact PTH	40.4 ± 1.3	42.2 ± 2.3	40 ± 1.6	N. S.	40 ± 2.1	41 ± 1.7	N.S.
25 (OH) vit. D	21.6 ± 1.0	21.2 ± 1.7	21.8 ± 1.2	N.S.	23.1 ± 1.5	20.3 ± 1.2	N.S.
OPG	82.7 ± 7.6	146.8 ± 12	53.6 ± 3.3	p < 0.0001	56.7 ± 7.9	97 ± 10.2	p = 0.01

Age (years), z-score (± SD), pre-existing vertebral fractures (number of patients with fractures given in percent), sCTX (1.0–2.84 nmol/l), Osteocalcin (1–35 ng/ml), intact PTH (10–65 pg/ml) to convert to SI units multiply by 0.1, 25 (OH) vitamin D (9–45 ng/ml) to convert to SI units multiply by 2.5, OPG (6–138 pg/ml). *N.A.* Not applicable; *N.S.* not significant.

Postmenopausal volunteers showed significantly higher OPG, sCTX and ALP levels and lower vitamin D_3 levels than premenopausal individuals.

OPG correlated positively with age ($r = 0.25$, $p < 0.001$) (Fig. 1). This correlation was consistent in a multiple regression analysis adjusting for OC, sCTX, BMI, vitamin D_3 and bone mass at the femoral neck and the lumbar spine ($r = 0.32$, $p < 0.01$). There was no correlation between OPG and any other parameters.

Patients with osteoporosis

Characteristics of the postmenopausal patients with osteoporosis are given in Table 2, in which patients were stratified according to their bone turnover and fracture status. Approximately one-third of the women (n = 15) had elevated markers of bone turnover and were therefore assigned to the "high bone-turnover" group.

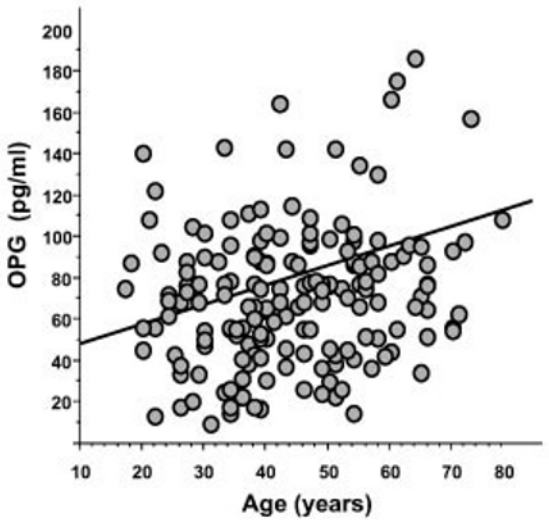

Fig. 1. Positive correlation of OPG serum concentrations and age in 177 healthy women aged 19 to 85 years ($r = 0.25$, $p < 0.001$)

OPG levels were found significantly higher in the patients with high bone turnover than in those with normal bone turnover (n = 33), although there was no difference in age, BMD, fracture status, vitamin D_3 and PTH.

A regression analysis showed a highly significant correlation of OPG with sCTX ($r = 0.82$, $p < 0.0001$) and OC ($r = 0.69$, $p < 0.0001$) in the group as a whole (Fig. 2). Further analysis found no correlation between OPG and sCTX ($r = 0.07$, $p = 0.68$) or OC ($r = 0.12$, $p = 0.5$) in those patients with normal bone turnover, but the correlation between OPG and both sCTX ($r = 0.73$, $p < 0.005$) and OC ($r = 0.73$; $p < 0.005$) was consistent in the group with high bone turnover.

When the groups with (n = 17) and without (n = 31) pre-existing vertebral fractures were compared, the only significant difference was in OPG serum levels. Patients in the fracture group had significantly lower serum OPG levels (57 ± 8 pg/ml) than patients without fractures (97 ± 10 pg/ml) (Fig. 3). This significant difference in OPG serum levels between the two groups persisted after adjustment for sCTX ($p < 0.02$). All other parameters were indistinguishable between the two groups (Table 2). There was no correlation of OPG with age, BMD, vitamin D_3 or PTH in the two groups.

Discussion

Osteoprotegerin (OPG) emerged as a bone "protector" from in vitro and pre-clinical work [1, 2, 6, 7, 9, 10, 11]. To our knowledge, there are as yet no clinical studies to support this. However, OPG substitution used as anti-resorptive therapy has shown the expected effects on resorption markers and confirmed the promising results seen in animal studies [18].

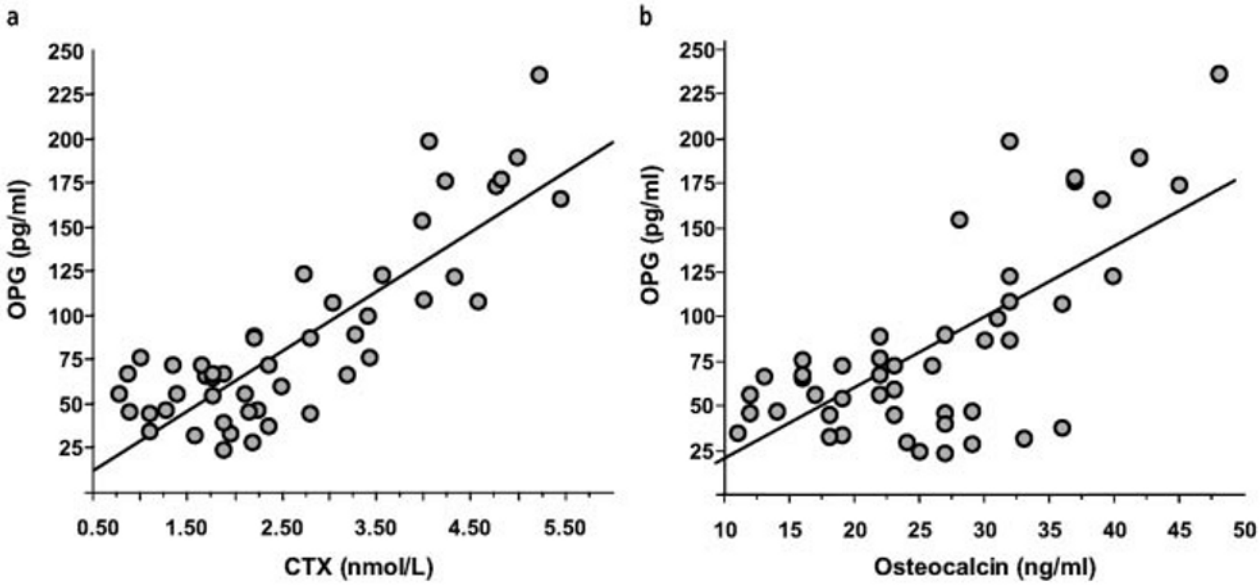

Fig. 2. Positive correlation of OPG and markers of bone turnover in 48 women with postmenopausal osteoporosis. **a** OPG to CTX ($r = +0.82$, $p < 0.0001$) and **b** OPG to OC ($r = +0.69$, $p < 0.0001$)

We were not able to show any relation between OPG and bone mass in our population of carefully selected pre- and postmenopausal healthy women; OPG correlated positively only with age in healthy women. This apparently contradicts the well known age-related decrease in bone mass. It also contradicts recently published in vitro data showing an age-related decline in OPG expression in human bone-marrow cells [22].

We can only speculate on the reason for the age-related increase of OPG in our sample. A possible explanation may be that all volunteers with osteoporosis ($n = 82$) were excluded from the study. Thus mainly elderly volunteers may have been dropped and only individuals with relatively high z-scores (see Table 1) included. Perhaps only patients with high OPG levels were able to maintain relatively high bone mass and were therefore classified as non-osteoporotic according to the WHO definition [21]. Another possible explanation is accelerated bone turnover in the postmenopausal individuals who were found to have higher sCTX levels than the premenopausal group. We also found a positive correlation of OPG with markers of bone turnover in a cohort of patients with accelerated bone turnover following solid organ transplantation [23, 24]. The increase in OPG serum levels with age and turnover could either be a paracrine response mechanism by bone cells in order to attenuate bone resorption or simply reflect accelerated bone remodelling.

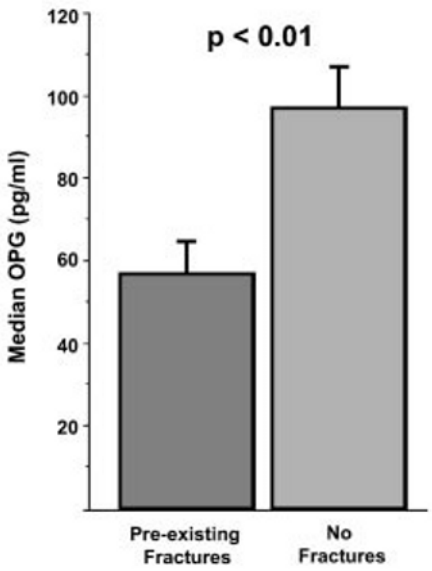

Fig. 3. Differences in OPG serum levels in postmenopausal patients with ($n = 17$) and without ($n = 31$) fractures expressed as mean ± SE (standard error). $p < 0.01$

The dependence of OPG serum levels on bone turnover was described earlier by Yano et al. [13], who also found a positive correlation between OPG and age in women. Browner et al. [15], who again found an age-related increase in OPG, evaluated patients with various comorbidities. As OPG is expressed not only by osteoblasts but also in high concentrations by a variety of tissues [1, 2, 7, 8, 25] and is a key regulator of the immune system [1, 2, 26, 27], interpretation of OPG serum levels in a cohort of patients, some of whom are in poor health, may be difficult. A direct comparison with our data may therefore be questionable.

A recently published study on 252 *healthy* men, however, also demonstrated an age-related increase in OPG, for which the authors postulated that the increase might reflect a mechanism protecting the skeleton against age-related bone loss [16]. Further, other authors have proposed that women with very severe osteoporosis and very high markers of bone turnover could have very high OPG levels to protect against bone loss [13]. Nevertheless, none of these studies reported an adjustment of OPG levels for bone-turnover markers in their analysis.

The interaction between bone turnover and OPG serum levels is emphasized by our findings in the patients with osteoporosis; those with accelerated bone remodelling had a clear turnover-dependent increase in OPG levels, whereas the patients with normal bone turnover showed no correlation of OPG with any marker of bone turnover.

OPG and RANKL are produced by cells of osteoblast lineage. The RANKL/OPG ratio is highest in mesenchymal bone-marrow stroma cells and decreases with osteoblastic cell differentiation [6–8], which is paralleled by a decreasing capacity to support osteoclastogenesis. Individuals with accelerated bone turnover probably have a larger pool of preosteoblasts and osteoblasts with consequently higher RANKL and OPG production, which would be a logical explanation for the increase in OPG as a function of bone turnover.

Of course the measurement of RANKL in serum will open new aspects and answers to some of these questions. It is nevertheless intriguing to speculate whether OPG rises *primarily* with age or in situations of high bone turnover in order to prevent bone loss or simply *secondarily* as a result of accelerated bone turnover with a large osteoblastic cell pool.

We found no correlation between OPG and bone mass in the patients with osteoporosis either, and no age-dependent relationship to OPG, perhaps because of the narrow age range in this group (65–79 years).

However, probably the most striking finding in our study was that patients with pre-existing vertebral fractures had significantly lower OPG levels than patients without fractures. Similar findings have been described in patients who had received cardiac transplants [24].

OPG is expressed by cells of osteoblast lineage, therefore a low level of OPG, or a level that is too low in relation to the degree of RANKL expression, may reflect quantitatively insufficient osteoblastogenesis with a consequent imbalance at the level of bone remodelling units, impaired healing of micro-damage and perhaps a higher risk of fractures. Recent studies have shown that OPG protects osteoblasts from TNFα-induced apoptosis [28] and have demonstrated a strong immunoreactivity for OPG, not only in osteoblasts but also in osteocytes and mineralized bone matrix [17, 28]. If low serum OPG levels indeed reflect decreased OPG deposition into the bone matrix by osteoblasts, then possible detrimental effects on osteoblast-/osteocyte survival rates or differences in bone mineralization may be new explanations, in addition to the antiresorptive effects of OPG, for the greater risk of fractures in these patients.

As a hypothesis, differences in OPG levels/OPG deposition in bone matrix might explain the occurrence of bone fractures in patients with normal bone mass, whereas others with low bone mass might not sustain fractures.

This study has several limitations: our population of healthy women was carefully selected, and the age-related increase in OPG may be biased because all women with osteoporosis and those with accelerated bone turnover were excluded.

The postmenopausal volunteers and the patients were not compared directly. The two groups differed in age, bone mass and, very likely, in turnover markers. Different assays were used for sCTX and osteocalcin in the two groups, making adjustment for bone turnover impossible.

Furthermore, we should keep in mind that circulating (measurable) OPG serum levels may only partly reflect the milieu of bone microenvironment and that our study is certainly limited by the lack of bone biopsy samples. Only histomorphometric and histological analysis could elucidate whether OPG serum levels correlate with the OPG content in bone matrix or OPG expression on eroded surfaces.

In summary, OPG may be only one part of a complex regulatory system of human bone remodelling. OPG in healthy individuals was normally distributed; furthermore we found no correlation of OPG with bone mass in our study. The importance of OPG may therefore be revealed only in the presence of pathological conditions or medication influencing bone metabolism [23, 24, 30, 31].

There was a clear correlation of OPG with bone turnover only in patients with accelerated bone remodelling; as a consequence, interpretation of OPG serum levels should always take bone-turnover status into account.

With respect to bone fractures, we suggest that OPG, independently of bone mass, and even after adjustment for bone turnover, may be an important indicator of prevalent vertebral fractures in patients with osteoporosis [24].

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