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Assessment of OPG, RANKL, bone turnover markers serum levels, and BMD after treatment with strontium ranelate and ibandronate in patients with postmenopausal osteoporosis

Ocena stężeń OPG, RANKL i markerów obrotu kostnego w surowicy oraz BMD u pacjentek leczonych z powodu osteoporozy pomenopauzalnej ranelinianem strontu i ibandronianem

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Abstract

Introduction: The aim of this study was to evaluate quantitative changes in OPG and RANKL proteins after treatment with strontium ranelate (SR) and ibandronate in patients with postmenopausal osteoporosis.

Material and methods: A total of 89 women with postmenopausal osteoporosis (PO), aged 51-85 years, patients of the Outpatient Clinic of Osteoporosis of the Military Teaching Hospital in Lodz, were enrolled in the study. The patients were randomly assigned to different therapies: ibandronate and (SR). Patients of the control group received only calcium and vitamin D_3 supplements. The patients' visits were repeated after three and six months. Measurements of β-CTX (C-terminal Telopeptide of type 1 collagen), osteocalcin, RANKL, osteoprotegerin (OPG), alkaline phosphatase concentrations in serum, as well as of total 24-hour calcium and phosphate levels in serum and urine, were carried out in material collected at baseline and after three and six months of therapy. Left hip and lumbar spine densitometry was done twice (at baseline visit and after six months).

Results: In all three groups there were no significant differences noted in the concentrations of OPG and RANKL serum protein levels during the study period. Both negative and positive correlations or tendencies of correlations were found between OPG serum concentrations and BMD changes in the SR group.

Conclusions: Both ibandronate and SR do not seem to cause any significant changes in OPG and RANKL protein serum levels during the first six months of treatment. OPG may play a role in osteoclast activity suppression in the course of treatment with ibandronate in patients with PO. OPG may play an important role in the mechanism of SR therapy and may be viewed as a potentially valuable parameter for monitoring and predicting the course of treatment with SR in PO. **(Endokrynol Pol 2016; 67 (2): 174–184)**

Key words: *osteoporosis, osteoprotegerin, RANKL, ibandronate, strontium ranelate*

Streszczenie

Wstęp: Celem badania była ocena ilościowych zmian stężeń białek OPG i RANKL po leczeniu ranelinianem strontu (RS) i ibandronianem u pacjentek z osteoporozą pomenopauzalną.

Materiał i metody: Do badania zakwalifikowano 89 kobiet z osteoporozą pomenopauzalną (OP), w wieku 51–85, będących pacjentkami Poradni Leczenia Osteoporozy Uniwersyteckiego Szpitala Klinicznego im. WAM w Łodzi. Pacjentki losowo przydzielono do dwóch grup terapeutycznych, otrzymujących RS i ibandronian. Pacjentki z grupy kontrolnej otrzymywały tylko wapń i witaminę D. Chore odbywały wizyty w ośrodku w chwili rozpoczęcia badania, po 3 i 6 miesiącach, w trakcie których oznaczano stężenia: β-CTX (C-terminalny telopeptyd kolagenu typu I), osteokalcyny, osteoprotegeryny, RANKL, fosfatazy alkalicznej w surowicy, a ponadto wapnia oraz fosforanów zarówno w surowicy, jak i w moczu całodobowym. Badanie densytometryczne biodra i odcinka lędźwiowego kręgosłupa przeprowadzano przed włączeniem terapii oraz po 6 miesiącach leczenia.

Wyniki: W żadnej z grup nie stwierdzono istotnych statystycznie zmian stężeń OPG i RANKL w surowicy. Jednakże w grupie leczonej SR, zaobserwowano liczne dodatnie i ujemne korelacje oraz trendy statystyczne, pomiędzy stężeniami OPG a zmianami BMD.

Wnioski: Zarówno ibandronian, jak i SR wydają się nie powodować istotnych zmian stężeń OPG i RANKL w surowicy, w trakcie pierwszych 6 miesięcy terapii. W hamowaniu aktywności osteoklastów OPG może odgrywać istotną rolę, w trakcie terapii ibandronianem u pacjentek z OP. Może też pełnić ważną funkcję w mechanizmie działania RS i w związku z tym, może być rozpatrywana jako wartościowy parameter w monitorowaniu i przewidywaniu efektów leczenia RS w OP. **(Endokrynol Pol 2016; 67 (2): 174–184)**

Słowa kluczowe: *osteoporoza; osteoprotegeryna; RANKL; ibandronian, ranelinian strontu*

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Introduction

In 1997, three independent research groups identified osteoprotegerin (OPG) [1–3]. OPG is a protein that belongs to the Tumour Necrosis Factor Receptor (TNFR) family and is produced by osteoblasts, as well as by other cell types, including peripheral blood lymphocytes [4–6]. OPG acts as a soluble decoy for the Receptor Activator for Nuclear Factor κ B Ligand (RANKL) [3]. It has also been proven that OPG inhibits apoptosis via binding to the ligand associated with tumour necrosis factor (TNF) (TRAIL, TNF-Related Apoptosis-Inducing Ligand). RANKL is produced by many cell types, including osteoblasts, endothelial cells, and active T lymphocytes [3, 7]. RANKL is present in cell-bound and soluble forms (soluble RANKL, sRANKL). The receptor for RANKL, called RANK (Receptor Activator for Nuclear Factor κ B), is present mainly on the surface of osteoclasts and dendritic cells. C-fms — the receptor for macrophage colony stimulating factor (M-CSF) — and RANK play an important role in osteogenesis [8]. Via binding to RANK in the presence of M-CSF, RANKL induces differentiation and activation processes in osteoclasts, prolongs their life, and strengthens their adhesion to bone surface [9, 10]. Many studies in recent years have confirmed the great importance of the OPG/RANK/RANKL system in the development of bone diseases [11]. Yano et al. [12] demonstrated that OPG serum concentration increases with age, but in women with postmenopausal osteoporosis it is higher in comparison with healthy controls. Furthermore, Kudlacek et al. [13] demonstrated that serum OPG concentrations significantly increase with age by an average of 2 pg/mL/year, both in women and men [13].

The relationship observed between Bone Turnover Markers (BTM) and the OPG/RANK/RANKL system has not yet been fully explored. In various studies, both positive and negative correlations were demonstrated between the concentrations of OPG protein and RANKL [14, 15] *vs.* Bone Mineral Density (BMD). However, some authors question the occurrence of these relationships at all [13, 16, 17]. In men, a reverse relationship was observed between deoxypyridinoline (DPD), a resorption marker, and parathormone (PTH) activities and OPG concentrations [16]. In turn, in women of post-menopausal age, a negative correlation was demonstrated between serum markers of bone resorption/formation and OPG concentrations, while no such correlation was found for either OPG or resorption markers in urine and PTH concentrations [18]. In a study on male and female populations (the inhabitants of Iceland), a positive correlation was obtained between osteocalcin (OC), a bone formation marker, and OPG, while a reverse correlation between tartrate-resistant acid phosphatase (TRAP) and OPG was demonstrated only in the subpopulation of women [19]. Similar conclusions may be drawn from a study carried out by the Fahrleitner-Pammer team [17], which provides firm lines of evidence for the correlation between high concentrations of OPG and high BT levels, but in postmenopausal women only [17]. The above-mentioned data are suggestive of a certain compensation mechanism: an increase in OPG concentration is a response to intensified activity of osteoclasts and enhanced bone resorption.

Ibandronic acid belongs to the group of aminobisphosphonates and represents the third and latest generation of bisphosphonates. The effectiveness and safety of ibandronate were proved in two important clinical trials: MOBILE and BONE [20, 21].

SR is used to treat postmenopausal osteoporosis in women and osteoporosis in men, exerting a double beneficial effect via several complex mechanisms on both osteoblasts and osteoclasts [22]. A number of clinical trials were performed to evaluate the efficacy of SR in the therapy of osteoporosis [23, 24].

The aim of the study was to determine whether OPG and RANKL are reliable indicators of effective postmenopausal osteoporosis treatment with SR and ibandronate.

Material and methods

Material

A total of 89 women, aged 51–85 years, and with diagnosed osteoporosis after menopause were enrolled into the study. All the participants were patients of the Outpatient Clinic of Osteoporosis of the Military Teaching Hospital in Lodz, Poland.

The patients were diagnosed with osteoporosis via hip and/or lumbar spine DXA (T-score ≤ –2.5 SD). All the enrolled women had had the last menstrual bleeding at least 12 months before the study onset. The exclusion criteria included:

— chronic administration of bone metabolism affecting agents: bisphosphonates, SR, sodium fluoride, denosumab, oestrogens or hormonal replacement therapy, selective oestrogen receptor modulators, glucocorticosteroids, anti-epileptic drugs, aromatase inhibitors, GnRH analogues, and chemotherapy;
— endocrine disorders significantly affecting bone resorption processes, such as: hyperparathyroidism, active hyperthyroidism, hyperprolactinaemia, and hypogonadism;
— chronic renal failure (glomerular filtration rate < 35 mL/min [25]);
— malabsorption;
— diagnosed cancer, malignant tumour, or process;

— chronic systemic disease that could have significantly affected bone metabolism;
— no written consent to participate in the study.

All the participants received supplementation of calcium (calcium carbonate, gluconate, or citrate) at a dose that ensured adequate intake of 1500 mg elemental calcium. In addition, all the patients received 400–800 IU of native vitamin D_3 per day in a formulation, either containing calcium salt or as a separate supplement. Following the obligatory recommendations, the dose was gradually increased throughout the study period up to 800–1000 IU per day. Calcium and vitamin D_3 supplements were purchased by the patients themselves. In order to determine calcium deficiency, the patients were questioned at the beginning of the study about their dietary habits. Twenty-nine patients, who completed the study, received oral ibandronate sodium in 150 mg (Bonviva, Roche) doses, every 30 days for six months. Twenty-four remaining study participants received orally 2.0 grams of SR (Protelos, Servier) once a day. Both study drugs were provided free of charge by their manufacturers. The women from the control group (15 patients) received only calcium and vitamin D3 supplementation. At study onset, the examined groups did not differ significantly in terms of age or BMD. The compliance and adherence to the therapy were assessed on the basis of patient declarations and returned empty drug packaging units. In cases of non-compliance to visit schedules, the patients were contacted by phone.

The patients, qualified to the study, paid visits every three months in order to replenish medication stocks, for evaluation of compliance to prescribed drug administration, and for planned examinations. Blood samples were collected from fasting patients at the inclusion visit and after three and six months to assay β-CTX, OC, sRANKL, OPG, total calcium, phosphates, and alkaline phosphatase.

In addition, all the patients provided a representative urine sample from 24-hour collection to determine 24-hour urine calcium and phosphate excretion rates. Hip and lumbar spine DXA were performed twice (at enrolment and after six months) in all the patients. All the enrolled patients remained under care of the Outpatient Clinic of Osteoporosis, Military Teaching Hospital in Lodz throughout the whole study period.

Methods

Densitometry

Hip (Neck, Trochanter, Total Hip regions) and lumbar spine (L2–L4 region) BMD was evaluated by dual-energy X-ray absorptiometry (DXA) on a Norland XR46 machine (Norland Corp., Fort Atkinson, WI, USA) at baseline and after six months of the therapy. The minimum acceptable precision for technologists in our facility does not exceed the values: 1.9% (LSC = 5.3%) for Lumbar Spine, 1.8% (LSC = 5.0%) for Total Hip, and 2.5% (LSC = 6.9%) for Femoral Neck.

Laboratory tests and OPG, RANKL, and bone marker concentration assessments

Biochemical assays, including serum concentrations of bone turnover markers, OPG, and sRANKL proteins, were carried out by means of the following methods and sets:

— the serum alkaline phosphatase concentrations as well as total serum calcium and phosphate levels and 24-hour urine excretion rates were assessed using a colorimetric method with an Olympus analyser (Olympus Life and Material Sciences, O'Callaghan's Mills, Ireland) and Beckman Coulter (Brea, California) kits, according to the manufacturer's instructions;
— the serum osteocalcin (OC) and β-CrossLaps (C-terminal telopeptide of type 1 collagen, β-CTX) concentrations were evaluated by the electrochemiluminescence method with an Elecsys 2010 analyzer (Roche Diagnostics, Penzberg, Germany) and Roche Diagnostics kits in compliance with the manufacturer's recommendations. Reference values for OC were 0.5 to 300 ng/mL and for β-CTX 0.01 to 6.0 ng/mL;
— OPG and sRANKL by the ELISA (Enzyme-Linked Immunosorbent Assay) method, using kits from Biomedica Gruppe (Vienna, Austria) and a Metertech ε960 spectrophotometer (Metertech Inc., Taiwan). The assays were carried out at wave length of 450 nm, following the manufacturer's instructions. Normal median sRANKL and OPG values for women were, respectively, 0.37 and 1.8 pomol/L.

The serum used to assay OPG, sRANKL, β-CTX, and OC concentrations was frozen immediately after centrifugation of collected blood and stored at –70°C until use. The other laboratory tests, i.e. calcium and phosphate levels in serum and 24-hour urine, as well as alkaline phosphatase concentrations, were measured immediately after sampling.

Statistical analysis

Statistical analysis covered the results of calcium-phosphate metabolism, serum RANKL and OPG concentrations, bone markers, and BMD

The study data are presented as means ± 0.95 confidence intervals. For all statistical analyses, the level of significance (p value) was determined at 0.05. The analysis of variance and the multivariate analysis of variance (ANOVA/MANOVA) were used, together with

Table I. *Baseline characteristics of studied patient groups*
Tabela I. *Podstawowa charakterystyka grup pacjentów uczestniczących w badaniu*

	Strontium renelete	Ibandronate	Control group
Age (years ± SD)	66.8 ± 7.4	68.1 ± 8.1	69.2 ± 8.1
BMI [kg/m^2]	24.8±3.8	26.8 ± 4.1	26.2 ± 2.9
Vertebral fractures (number/%]	3/12.5	8/28.6	1/6.7
Forearm fractures (number/%)	9/37.5	14/48.3	2/13.3
Hip fractures (number/%)	0/0	1/3.5	1/6.7
T-score ± SD/Neck BMD [g/cm^2 ± SD]	–3.10 ± 0.45/ /0.6246 ± 0.0523	–3.20 ± 0.55/ /0.6126 ± 0.0641	–3.21 ± 0.58/ /0.6109 ± 0.0677
T-score ± SD/Trochanter BMD [g/cm^2 ± SD]	–2.96 ± 0.66/ /0.4606 ± 0.0718	–2.88 ± 0.67/ /0.4714 ± 0.0767	–2.92 ± 0.34/ /0.4688 ± 0.0365
T-score ± SD/TH BMD [g/cm^2 ± SD]	–2.37 ± 0.59/ /0.6637 ± 0.072	–2.31 ± 0.63/ /0.6722 ± 0.0776	–2.35 ± 0.48/ /0.6671 ± 0.0589
T-score ± SD/L2–L4 BMD [g/cm^2 ± SD]	–2.35 ± 0.91/ /0.7230 ± 0.1457	–2.44 ± 0.59/ /0.7066 ± 0.0955	–2.37 ± 0.71/ /0.7175 ± 0.1145
Calcium concentration in serum [mmol/L]	2.53 ± 0.09	2.53 ± 0.08	2.54 ± 0.06
Phosphate concentration in serum [mmol/L]	1.2 ± 0.19	1.17 ± 0.17	1.16 ± 0.18
24-hour calcium urine excretion [mmol/24 h]	3.65 ± 2.35	4.73 ± 2.6	4.41 ± 3.13
24-hour phosphate urine excretion [mmol/24 h]	24.7 ± 13.05	20.54 ± 9.28	23.29 ± 9.82
Alkaline phosphatase concentration in serum [IU/L]	82.54 ± 20.79	88.72 ± 28.88	79.73 ± 14.94

repeated measurements to verify if BMD, OPG, RANK, and RANKL gene expression, alkaline phosphatase, calcium, and phosphate levels in serum and calcium, and phosphate urinary excretion rates changed after the applied treatment over the time period of the trial. Data distribution normality was checked in each group, using the Shapiro-Wilk's test. The homogeneity of variance was evaluated, using Hartley's F-max, Cochran's C, and Bartlett's chi-square tests. Mauchly's sphericity test was used to verify whether the criteria required for the use of variance analysis with repeated measurements were met. Multiple post-hoc comparisons were performed by Scheffe's test.

Correlation analysis
Spearman's rank correlation coefficient test was employed to evaluate the correlation between assessed parameters.

Results

Sixty-eight patients at the age of 51–85 years completed the study. The baseline characteristics of the study groups are presented in Table I. The patients on SR were those who most often discontinued the treatment (31.4%). A slightly lower percentage of women in the control group (25%) and only 14.7% of patients on ibandronate left the therapy before study completion; see Table II for withdrawal reasons.

Comparative analyses of parameters in and among study groups

Analysis of BMD
A non-significant (1.2%) increase of BMD in the femoral neck was observed after six months of therapy, both in the control group (1.2%) and in the SR group (1.6%). Regarding other regions, a slight BMD increase was

Table II. *Reasons for patient discontinuation*
Tabela II. *Przyczyny przerwania terapii przez pacjentki*

	The percent of patients, who withdrew per group	Withdrawal reasons		
		Side effects	Loss of interest in study and personal reasons	Loss of contact
Strontium ranelate	31.40%	100%	0%	0%
Ibandronate	14.70%	60%	0%	40%
Control group	25%	0%	80%	20%

also observed: 2.9% and 3.1% in the femur trochanter, 2.9% and 1.1% in Total Hip (TH), and 2.5% and 2.0% in the lumbar spine for the SR and ibandronate groups, respectively. The BMD differences were not statistically significant over time or between groups (data not shown).

Analysis of serum RANKL, OPG, OC and β-CTX concentrations

Serum sRANKL concentrations above detection limits were observed in a small number of the patients only: three in the control group, eleven on ibandronate and seven on SR.

Very low serum concentrations of sRANKL protein were seen throughout the entire study period in all the study groups (median value < 0.001 pmol/L *vs.* normal median value of 0.37 pmol/L, specified by the manufacturer). The results were insignificant among and within the groups (Fig. 1).

The differences in OPG concentrations did not reveal any significant changes under the applied treatment, nor were any significant differences observed among the groups (Fig. 2).

OC concentrations decreased by as much as 43% ($p < 0.001$) from baseline in the group on ibandronate (Fig. 3). Moreover, OC levels after six months on ibandronate therapy were significantly lower ($p = 0.049$) than in the control group; OC differences in the other groups did not attain significance.

β-CTX concentrations (Fig. 4) demonstrated significant falls of the marker levels in the group on ibandronate, namely by 55% and 73% after three and six months, respectively ($p < 0.001$). Moreover, β-CTX values after six months of ibandronate therapy were significantly lower than those in the control group ($p < 0.01$). In the course of the therapy with SR, the concentrations of the marker presented with a slight insignificant reduction by 28% after six months of therapy, but it was not statistically significant ($p = 0.41$).

Correlations among studied parameters (Table III)

Correlations between BMD and the other parameters (Table IV)

Correlations between femoral neck BMD and serum concentrations of bone markers, RANKL, and OPG proteins

We observed positive correlations between changes in femoral neck BMD values and OC ($R = 0.388$; $p < 0.05$) and β-CTX ($R = 0.407$; $p < 0.05$) concentrations in serum in the ibandronate-treated group.

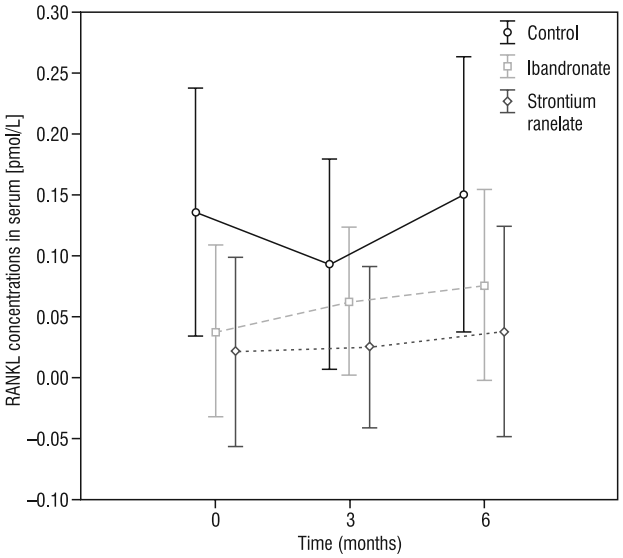

Figure 1. *Treatment-induced RANKL concentration changes in serum*

Rycina 1. *Zmiany stężeń RANKL w wyniku zastosowanej terapii*

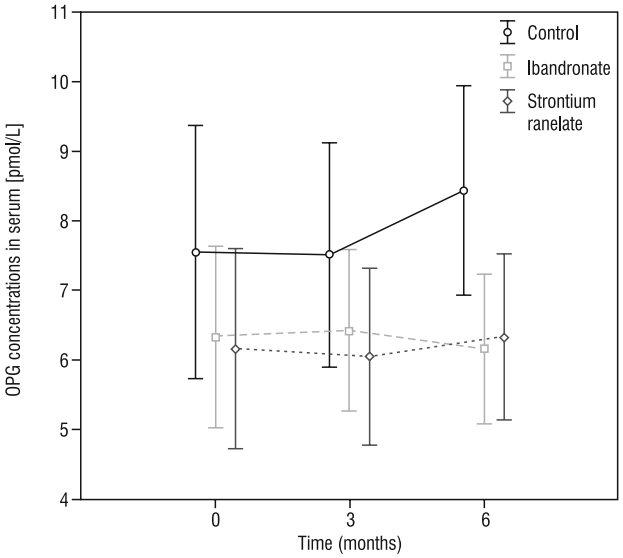

Figure 2. *Treatment-induced OPG concentration changes in serum*

Rycina 2. *Zmiany stężeń OPG w wyniku zastosowanej terapii*

In the SR group an insignificant positive correlation ($R = 0.375$; $p = 0.07$) between OPG concentration differences and changes in the femoral neck BMD was found.

In the other groups, no significant correlations were observed between changes of the above-mentioned parameters and femoral neck BMD or between femoral neck BMD increase/decrease and changes in the concentration of RANKL protein in serum.

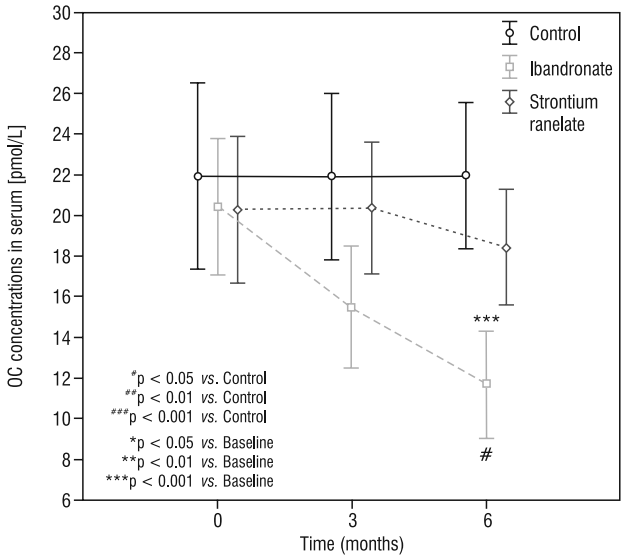

Figure 3. *Treatment-induced osteocalcin concentration changes in serum*

Rycina 3. *Zmiany stężeń osteokalcyny w wyniku zastosowanej terapii*

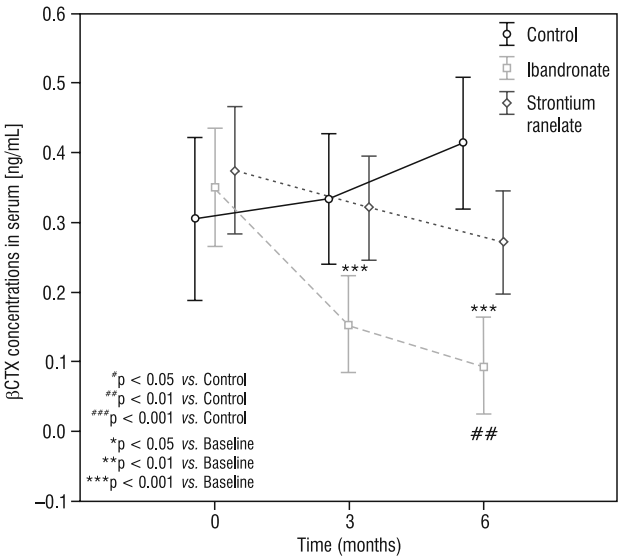

Figure 4. *Treatment-induced β-CTX concentration changes in serum*

Rycina 4. *Zmiany stężeń β-CTX w wyniku zastosowanej terapii*

Table III. *Correlations between changes of studied parameters*

Tabela III. *Korelacje pomiędzy zmianami badanych parametrów*

Correlation between	OPG			RANKL			β-CTX			OC		
	Group	R value	P value	Group	R value	P value	Group	R value	P value	Group	R value	P value
Neck BMD	**SR**	**0.375**	**0.07**	All	NS	NS	**IBA**	**0.407**	**< 0.05**	**IBA**	**0.388**	**< 0.05**
Trochanter BMD	All	NS	NS	All	NS	NS	All	NS	NS	**SR**	**–0.567**	**< 0.01**
Total Hip BMD	**SR**	**0.508**	**< 0.05**	All	NS	NS	All	NS	NS	All	NS	NS
L2–L4 BMD	**Control**	**0.648**	**< 0.01**	All	NS	NS	All	NS	NS	All	NS	NS
OC	All	NS	NS	All	NS	NS	**IBA**	**0.793**	**< 0.001**	N/A	N/A	N/A
OC	All	NS	NS	All	NS	NS	**SR**	**0.824**	**< 0.001**	N/A	N/A	N/A
OC	All	NS	NS	All	NS	NS	**Control**	**0.713**	**< 0.01**	N/A	N/A	N/A
β-CTX	All	NS	NS	All	NS	NS	N/A	N/A	N/A	**IBA**	**0.793**	**< 0.001**
β-CTX	All	NS	NS	All	NS	NS	N/A	N/A	N/A	**SR**	**0.824**	**< 0.001**
β-CTX	All	NS	NS	All	NS	NS	N/A	N/A	N/A	**Control**	**0.713**	**< 0.01**
OPG	N/A	N/A	N/A	All	NS	NS	All	NS	NS	NS	NS	NS
RANKL	All	NS	NS	N/A	N/A	N/A	All	NS	NS	NS	NS	NS

Correlations between femoral trochanter BMD changes and serum concentrations of bone markers, OPG, and RANKL

There was a significant, negative correlation (R = –0.567; p < 0.01) between femoral trochanter BMD changes and the differences in OC concentration in the group on SR. In the other groups, no significant correlations were found between increase/decrease of the above-mentioned parameter and femoral trochanter BMD differences or between femoral trochanter BMD changes and serum concentrations of OPG, RANKL, or β-CTX marker.

Correlations between TH BMD and serum concentrations of bone markers, OPG, and RANKL

A significant correlation was observed between increased OPG concentrations and BMD in the SR group (R = 0.508; p < 0.05). No significant relationship was found in the other groups between increase/decrease of the above-mentioned parameter and TH BMD differences or between TH BMD values and serum concentrations of RANKL protein, β-CTX, and OC markers.

Table IV. *Correlations between BMD changes and initial serum concentrations of OPG, RANKL, β-CTX, and OC*
Tabela IV. *Korelacje pomiędzy zmianami BMD a początkowymi stężeniami: OPG, RANKL, β-CTX i OC*

Correlation between	OPG			RANKL			β-CTX			OC		
	Group	R value	P value	Group	R value	P value	Group	R value	P value	Group	R value	P value
Neck BMD	**Control**	**0.723**	**< 0.01**	All	NS	NS	**IBA**	**–0.407**	**< 0.05**	**IBA**	**–0.442**	**< 0.05**
Trochanter BMD	All	NS	NS	All	NS	NS	All	NS	NS	All	NS	NS
Total Hip	**SR**	**–0.408**	**< 0.05**	All	NS	NS	All	NS	NS	**IBA**	**–0.391**	**< 0.05**
L2–L4 BMD	**SR**	**–0.534**	**< 0.01**	All	NS	NS	All	NS	NS	All	NS	NS

Correlations between lumbar spine BMD and serum concentrations of bone markers, OPG, and RANKL
A significant correlation was found ($R = 0.648$; $p < 0.01$) between increased lumbar spine BMD and serum OPG concentrations in the control group. Regarding other groups, no significant correlations were observed between increase/decrease of the above-mentioned parameter and lumbar spine BMD differences or between BMD values in that region and serum concentrations of RANKL protein and β-CTX and OC markers.

Correlations among the differences in serum RANKL, OPG, OC, and β-CTX concentrations
We observed positive correlations between OC and β-CTX concentration changes in all groups, i.e. in the ibandronate-treated group ($R = 0.793$; $p < 0.001$), the SR-treated group ($R = 0.824$; $p < 0.001$), and in the control group ($R = 0.713$; $p < 0.01$). No significant changes were observed between differences of the above-mentioned markers and RANKL and OPG, as well as between OPG and RANKL.

Correlations between BMD changes and the initial serum concentrations of OPG and RANKL proteins and OC and β-CTX bone markers

There were significant, negative correlations between the initial concentrations of OC ($R = –0.442$; $p < 0.05$) and β-CTX ($R = –0.407$; $p < 0.05$) in serum and femoral neck BMD changes in the ibandronate-treated group, as well as a significant correlation between OPG values and femoral neck BMD changes in the control group ($R = 0.723$; $p < 0.01$) (data not shown). Regarding other groups, no significant correlations were found between values of the above-mentioned parameters and femoral neck BMD differences or between BMD changes in the femoral neck and serum RANKL protein concentrations.

We observed a significant negative correlation ($R = –0.391$; $p < 0.05$) between TH BMD changes and OC levels in the ibandronate-treated group. Moreover, in the patients on SR, there was a negative correlation ($R = –0.408$; $p < 0.05$) between TH BMD differences and serum OPG concentrations. No significant correlation was found between the values of the above-mentioned parameters and TH BMD differences in the other groups nor between BMD changes in that region and β-CTX or RANKL concentrations.

A significant, negative correlation ($R = –0.534$; $p < 0.01$) was demonstrated in the group, treated with SR, between lumbar spine BMD changes and OPG concentrations in serum. No significant correlations were found in the other groups between the values of the above-mentioned parameter and lumbar spine BMD differences nor between BMD changes in that region and serum concentrations of RANKL protein, OC and β-CTX.

The observed relationships between femoral trochanter BMD differences and initial serum concentrations of bone markers and OPG or RANKL proteins were statistically insignificant.

Discussion

In the presented study, the observed differences in serum OPG and sRANKL concentrations were statistically insignificant. Moreover, the measurable values of sRANKL were obtained in a small number of the patients. Additionally, a significant decrease in serum OC concentrations was noted after six months, as well as of β-CTX and ALP after three and six months of therapy. After six months of therapy with ibandronate the values of the former two bone markers were significantly lower than in the control group.

Moreover, we found positive correlations between femoral neck BMD changes and OC and β-CTX concentrations in serum. The obtained results demonstrate favourable therapeutic effects of ibandronate.

The data, indicating a significant impact of bisphosphonates on RANKL and OPG secretion rates, have, in their majority, been obtained from *in vitro* studies [26, 27].

Reports that raise the issue of the effects of bisphosphonates, especially of ibandronate, on the OPG/RANK/RANKL system in *in vivo* studies with par-

ticipation of osteoporotic patients, are rather scarce in number, with equivocal conclusions.

Dobnig et al. [28] beside a significant improvement of BMD in the femoral neck and trochanter in patients with postmenopausal osteoporosis, submitted to a year long therapy with alendronate and risendronate, demonstrated a significant drop of CTX and OC concentrations — similar to our results — after just two months of therapy, which was maintained throughout the entire period of their study. Unlike in our results, OPG concentration significantly increased after six months of therapy, and that tendency remained for the subsequent six months, while the obtained values were significantly higher at all time points *vs.* the control group. Regarding sRANKL, as in our study, they did not demonstrate any significant differences, either in time or among the groups. Moreover, the occurrence of significantly positive correlations between OPG and CTX concentration changes after two months ($r = 0.49$; $p = 0.001$) was demonstrated, OPG and BMD of the femoral neck and trochanter after six and 12 months of therapy with bisphosphonates [28], which we did not see in our study.

Reyes-Garcia et al. [29], while having administered alendronate for 12 months to patients with osteoporosis after menopause, obtained a significant decrease of OPG concentration after 12 months of treatment, while the RANKL concentration increased at all the studied time points, i.e. after three, six, and 12 months from the therapy onset. Similarly as in our experiment, the concentrations of the bone fraction alkaline phosphatase and CTX demonstrated an expected fall under the therapy. However, the researchers also failed to demonstrate any correlations between the concentrations of OPG, sRANKL, and bone turnover markers, at the beginning and in the course of the study. They only found a correlation between CTX concentration changes ($r = –0.304$; $p < 0.05$) and BMD increase after 12 months [29].

Studying the effects of the risedronate on OPG concentration in serum, Karadag-Saygi et al. [30] obtained a significant fall in concentration of the above-mentioned compound in serum after one, three, and six months of therapy; however, the same effect was observed in the control group, which had been receiving the supplements of calcium and vitamin D_3 only. Moreover, analogously to our results, the authors of the study found a significant decrease in the concentrations of CTX and OC markers after three and six months of the therapy with risedronate, respectively [30].

Different results were obtained by the research group of Dundar et al. [31], who observed increased OPG and decreased RANKL concentrations in serum after three and six months of treatment with risedronate [31].

Anastasilakis et al. [32], despite a significant fall of RANKL concentration in serum of all studied groups, including the control, did not demonstrate any significant differences in RANKL or OPG concentrations during the period of six months among patients receiving risendronate in a dose of 35 mg/week, or proanabolic teriparatide (subcutaneously) [32].

In another study, after 15 months of therapy with alendronate a significant increase in BMD values was observed for the lumbar spine, with an insignificant increase of BMD for the femoral neck. After three months of mixed therapy, and in contrast to our results, the serum concentrations of OPG and RANKL demonstrated significant falls. The same was true with regards to CTX, but this time it was the same as in our study. All of the falls in the above-mentioned concentrations were maintained until the end of observation [33].

However, Choi et al. [34], having administered small (20 mg per week) doses of alendronate for 12 weeks to female patients with low bone mass, despite the obtained values of bone markers (OC, CTX), similar to our results, failed to obtain any changes in serum OPG concentration after the therapy [34].

Analysing the effects of antiresorptive medications, it is also worth mentioning the results of the study by Bashir et al. [35], who evaluated the influence of 12-month hormonal replacement therapy (HRT) with administration of raloxifene to female patients with postmenopausal osteoporosis. They demonstrated a significant drop of expression of all the three elements of the OPG/RANK/RANKL system, after just six months of using both therapy forms and a significant decrease of OPG concentration in serum after just one month of treatment, and that tendency was maintained until the end of the study.

However, similarly as in our results, the authors of the study obtained positive values of sRANKL only in a small group of patients; in all the other cases sRANKL values were below assayable level [35].

In our experiment, neither in any of the three groups nor among the groups were any significant differences observed in serum concentrations of OPG and RANKL protein. The changes of serum OC and β-CTX concentrations in the patients treated with SR were insignificant. A detailed statistical analysis demonstrated a significant, positive correlation and a positive dependence, although statistically insignificant ($p = 0.07$), between the differences in OPG concentrations and BMD changes, namely in TH and femoral neck in the group of patients on SR. Simultaneously, we demonstrated a significantly negative correlation between BMD changes in the femoral trochanter and changes of OC concentration in serum. Analysing the predictive values of OPG and RANKL among the patients treated

with SR, we found significantly negative correlations between the initial OPG concentration and the differences in TH and lumbar spine BMD values, which may indicate a need to evaluate OPG and RANKL when qualifications to optimal treatment are defined and/or considered.

The lack of expected, significant differences in the concentrations of bone markers, both of bone formation (OC) and bone resorption (β-CTX) markers in female patients receiving SR, may suggest a lack of therapeutic effectiveness of the above-mentioned drug in the evaluated group. Taking into account the reasonably well documented effects of SR on concentration changes of the following bone markers: bone fractions of ALP, CTX, N-terminal telopeptide of collagen type I, propeptides of collagen type I in randomised, double-blinded clinical studies [23, 24, 36], any other possible explanation for the observed lack of changes in BT marker values should in fact be sought in the poor compliance and persistence with prescribed therapy, despite declarations of the patients trying to present the opposite picture. However, the opinions are divided regarding the variability of serum OC changes under the effect of SR treatment [36].

The studies that undertook the issue of SR effects on the OPG/RANK/RANKL system are very scarcely represented by literature reports and were almost exclusively conducted in *in vitro* conditions [37–40], where cells of the osteoblastic line were predominantly the object of analysis, while *in vivo* studies on rodents were much less frequent [40, 41]. The lack of any studies in humans is a considerable impediment to any unequivocal conclusions from our study, based on available literature reports.

Peng et al. [41] evaluated the effects of an eight-week therapy with strontium in mice with inactivated genes for OPG and failed to demonstrate a favourable anabolic response, represented by an increased number of osseous trabecullae and increased bone volume. Nor did they find any suppression of osteoclastogenesis nor inhibition of bone resorption process. The above-mentioned effects were only observed in mice of wild-type strain [41].

Only in January 2012 a report was published [42], in which its authors indicate the role of osteoprotegerin in the therapeutic effects of strontium in a clinical study. Assays were performed on 2335 enrolled participants of the TROPOS trial, in whom serum OPG concentrations were determined at point zero and after 3, 6, 12, 24, and 36 months of therapy with SR. In contrast to our results, that research demonstrated that from the third month of drug administration, OPG concentrations were significantly higher than those in the control group and that the favourable effect was maintained until the end of the trial [42].

In our previous study [43] we showed that both ibandronate and SR seem not to exert any significant changes in gene expression levels of the OPG/RANK/RANKL system during the first six months of therapy. This study was the first attempt to evaluate the effects of ibandronate and one of the few to assess the influence of SR on serum OPG and RANKL concentration changes in female patients with postmenopausal osteoporosis. We are aware that our study has some limitations, i.e. only six-month observation period and rather small groups of patients. However, the available literature data, specifying the effects of either therapy on changes within the OPG/RANK/RANKL system, are also ambiguous and equivocal. It is suggestive of some additional, still concealed factors, which may significantly determine or control the activity of the above-mentioned system at transcription and/or translation levels [44–46].

There is currently no official position on the use of OPG and RANKL as bone metabolism markers in the diagnosis and treatment of osteoporosis [47–49]. The high diversity and heterogeneity of these markers' concentrations in the serum may result from their involvement in a number of other bone and joints diseases, as well as other systemic and metabolic disorders, including rheumatoid arthritis, ankylosing spondylitis, and hypertension [50–53].

Conclusions

1. According to our results, sRANKL and OPG do not seem to be reliable indicators of effective postmenopausal osteoporosis treatment with SR and ibandronate.
2. Neither ibandronate nor SR seem to induce any significant changes in the production of OPG and/or RANKL protein in serum during the first six months of therapy.
3. OPG may hold a significant function in the therapeutic mechanism of SR, and OPG concentration in serum may be considered as a potentially valuable parameter in monitoring and prognosing the further course of therapy with SR in postmenopausal osteoporosis. However, to state a clear conclusion further studies are needed on large groups of patients.
4. OPG may play a specific role in suppressing the activity of osteoclasts in the course of therapy with ibandronate administered to female patients with postmenopausal osteoporosis, but further studies are needed on large groups of patients.
5. Six-month therapy with SR and ibandronate administered in female patients with postmenopausal

osteoporosis is too short for a reliable evaluation of achieved improvement of BMD.

6. Bone turnover markers, especially βCTX, ALP, and OC, may be helpful in an evaluation of the therapeutic efficacy of ibandronate administered to patients with postmenopausal osteoporosis.

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