Journal für
Mineralstoffwechsel
Zeitschrift für Knochen- und Gelenkserkrankungen
Orthopädie · Osteologie · Rheumatologie

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Journal für Mineralstoffwechsel

2003; 10 (3), 10-12

Indexed in SCOPUS/EMBASE/Excerpta Medica
www.kup.at/mineralstoffwechsel

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ÖGR
Österreichische Gesellschaft für Rheumatologie

Krause & Pachernegg GmbH · VERLAG für MEDIZIN und WIRTSCHAFT · A-3003 Gablitz

P.b.b. GZ02Z031108M, Verlagspostamt: 3002 Purkersdorf, Erscheinungsort: 3003 Gablitz

Osteoprotegerin Serum Levels in Rheumatoid Arthritis

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Osteoprotegerin (OPG) wird in der entzündeten Synovialis bei chronischer Polyarthritis von Makrophagen, dendritischen Zellen und B-Zellen produziert. OPG hemmt die Differenzierung von Osteoklasten und ihre Aktivierung durch die Bindung an RANKL (receptor activator of NFκBβ ligand). OPG könnte daher eine wichtige Rolle in der Verhinderung des Auftretens von Erosionen bei chronischer Polyarthritis spielen. In der vorliegenden Studie wurde untersucht, ob die Serumspiegel von Osteoprotegerin mit Entzündungsparametern oder der Gelenksdestruktion bei chronischer Polyarthritis korrelieren. Patienten und Methoden: In 85 Sera von 68 Patienten mit chronischer Polyarthritis wurden die Serumspiegel von OPG untersucht und mit klinischen Parametern, Laborbefunden und dem Larsen-Score verglichen. Zur OPG-Bestimmung wurde ein Sandwich-ELISA (enzyme-linked-immunosorbent-assay) mit zwei spezifischen OPG-Antikörpern verwendet. Mit dem bindenden Antikörper wurde eine Mikrotiterplatte beladen, der zweite Biotin-konjugierte Antikörper wurde durch Streptavidin-Peroxidase und TMB gemessen. Der Immunoassay mißt sowohl freies als auch an RANKL gebundenes OPG. Zur statistischen Auswertung wurde ein SAS-Programm verwendet, als Tests wurden der Mann-Whitney-Test, die Spearman-Korrelation und die Varianzanalyse (ANOVA) angewandt. Ergebnisse: Verglichen mit einer gesunden Kontrollgruppe (Alter 56–76 Jahre) lagen die Mittelwerte von Serum-OPG bei chronischer Polyarthritis höher. Wenn man den Mittelwert der Gesunden für die Einteilung in höhere oder niedrigere Serum-OPG-Werte bei Patienten mit chronischer Polyarthritis verwendet, zeigt sich im Mann-Whitney-Test eine signifikante Korrelation nicht nur mit BSG und Rheumafaktor, sondern auch mit dem Larsen-Score. Die OPG-Spiegel korrelierten mit dem Rheumafaktor, der BSG und dem Serum-CRP (C-reaktives Protein) signifikant positiv, der Larsen-Score zeigte nur einen statistischen Trend. Diskussion: OPG blockiert die Differenzierung von Osteoklastenvorläuferzellen und die Aktivierung reifer Osteoklasten. Es wird nicht nur durch Osteoblasten, sondern im entzündeten Synovium der chronischen Polyarthritis auch durch dendritische Zellen und B-Zellen produziert. Obwohl bei chronischer Polyarthritis im Synovialgewebe erhöhte Konzentrationen von RANKL und niedrige Konzentrationen von OPG gefunden wurden, zeigen unsere Untersuchungen, daß bei größerer Entzündungsaktivität auch das OPG im Serum höher ist als bei Gesunden. Die erhöhte OPG-Produktion ist aber offensichtlich nicht ausreichend, um die Effekte von RANKL zu blockieren.

Objective: Osteoprotegerin (OPG) is produced by macrophages, dendritic cells and B-cells in the inflamed synovium of patients with rheumatoid arthritis (RA). It inhibits osteoclast differentiation and activation by neutralizing the receptor activator of NF-κBβ ligand (RANKL). Thus OPG may play an important role in preventing erosions in RA. Methods: OPG serum levels were measured in 85 sera of 68 patients with RA and compared with clinical and laboratory parameters and the Larsen Score. A sandwich-type-ELISA based on two OPG specific antibodies was used. The catching antibody is coated to a microtiter plate, the second biotin-conjugated antibody is detected by streptavidin-peroxidase and TMB. This immunoassay measures both free and RANKL associated OPG. For the statistical evaluation a SAS-program, using Mann-Whitney-Test, Spearman correlation and analysis of variance was employed. Results: Mean OPG levels in the sera of patients with RA were elevated compared with a healthy control group (age 56–76 years). A highly significant correlation was found with rheumatoid factor, ESR and serum CRP. On the other hand, the Larsen Score showed only a borderline direct positive correlation with OPG serum levels. There was, however, a significant difference in ESR, rheumatoid factor and the Larsen Score between patients with low and high OPG serum levels. Discussion: OPG acts as a decoy receptor for RANKL thus blocking the differentiation of osteoclast precursor cells and the activation of mature osteoclasts. It is produced by osteoblastic cells and in the inflamed synovium of RA by dendritic cells and B-cells. Despite reports about increased expression of RANKL and low expression of OPG in the synovial tissue, our results show that the net effect in serum is an increase in OPG serum levels with more active disease. This can be explained by an increase in OPG production which is nevertheless insufficient to block the effects of RANKL. Thus the correlation between OPG in serum and the Larson score might be due to a RANKL/OPG disequilibrium in RA. ***J Miner Stoffwechs 2003; 10 (3): 10–12.***

Osteoprotegerin (OPG) is a soluble decoy receptor which blocks osteoclast differentiation and activation [1, 2] by neutralizing the receptor activator of NF-κBβ ligand (RANKL), also known as osteoclast differentiation factor, or OPG ligand. It has been shown, that OPG is produced by osteoblasts, dendritic cells and B-cells in the inflamed synovium of patients with rheumatoid arthritis (RA). Itonaga et al [3] reported, that RA macrophages could differentiate into osteoclasts, which is dependent on RANKL. In addition, activated RA synovial fibroblasts were shown to express RANKL [4]. Also OPG producing dendritic cells and B-cells, which attenuate osteoclast formation and activation, can be found in the inflammatory infiltrate in the RA synovium [3]. Reportedly, OPG levels in joint effusions of patients with RA are lower than in other joint diseases [5]. Thus OPG seems to play an important role in preventing erosions and osteoporosis in RA [6].

In a preliminary study OPG serum levels were measured in 85 sera from 68 patients with RA, and correlated to patient age, disease duration and markers of inflammation in order to determine whether OPG levels are dependent on clinical symptoms or findings in RA. In 17 patients OPG serum levels were compared with the development of erosions over the following years in order to estimate OPG's prognostic value.

Patients and Methods

68 patients (46 females, 22 males) with definite RA according to the ARA (American Rheumatism Association) criteria [7] were enrolled in the study. Clinical data are given in

Table 1. Clinical data of 68 patients, 22 males, 46 females, 51 pat. RF pos.

	Mean	Stand. Dev.	Min	Max
Age (y)	67	10.7	42	86
Disease duration (y)	9.3	10.6	1	56
Rheumatoid factor (IU/ml)	305.9	851.1	0	6634
ESR (mm n.W.)	36.5	20.1	3	83
CRP (mg/l)	31.3	26.7	0	120
Osteoprotegerin (pg/ml)	108.1	121.2	3.4	627.6
Ritchie Index	13.4	10.4	0	41
Disease Activity Score	3.8	1.3	0.4	7.1
Steinbrocker Stage	2.2	0.7	1.0	4.0
Larsen Score	42.3	43.1	0	178

From the [1] Rheuma-Sonderkrankenanstalt der SVAdGW, Baden, the [2]Ludwig Boltzmann-Institut für Experimentelle Endokrinologie, Vienna, and the [3]Institut für Rheumatologie, Baden
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table 1. Fifty-one patients (75 %) were seropositive. 10 Patients received low dose prednisolone for at least four weeks. 58 of 68 patients had experienced DMARDs (disease modifying antirheumatic drugs), however 47 did not receive DMARDs three months prior to blood sampling. 17 patients were seen twice. The first visit took place within the first two years after onset of the disease, the second up to seven years (x = 2.47a; s = 0.35) later. In all patients, a standardised physical examination [8] was performed and duration of morning stiffness, numbers of painful and numbers of swollen joints, overall pain on VAS (visual analogue scale) and general health were recorded. Laboratory parameters for inflammatory activity (ESR, C-reactive protein) were registered as well. From these data the Disease Activity Score [9] was calculated. In each patient plan X-rays of hands and feet were performed and the Larsen Score [10] was calculated.

Serum samples were collected in the morning from fasting patients and stored at –20 ° C. OPG serum levels were measured using a sandwich-type-ELISA based on two OPG specific antibodies (Biomedica GmbH, Vienna, Austria). The monoclonal catching antibody was coated to a microtiter plate and the second biotin-conjugated antibody detected by streptavidin-peroxidase and TMB. At this method, the mean value of a healthy control group consisting of 170 blood donors is 44 pg/ml (40 pg/ml for males, 47 pg/ml for females), the reference serum concentration 5–145 pg/ml (5^{th}–95^{th} percentile). OPG is somewhat age-dependent. The mean value for healthy persons from 56 to 76 years is 55 pg/ml [11]. The assay measures free and complexed OPG as well. The presence of rheumatoid factor does not interfere with the measurement. Clinical parameters, disease activity score and Larsen Score were compared to OPG serum levels by means of a SAS-program using the Mann-Whitney-Test, Spearman correlation and analysis of variance.

In patients who were seen twice, the OPG serum levels of the first visit were correlated with the Larsen Score from the last and also with the increase in the score per year (score difference between second and first visit divided by the interval between the two examinations in years).

Results

Mean OPG levels in the serum of patients with RA (Table 1) were higher than the mean values for healthy persons from 56 to 76 years as reported by Kudlacek et al. [11]. 17 patients (25.0 %) showed values higher than 145 pg/ml, 51 patients showed values within the reference range. When the different clinical parameters were correlated with OPG serum levels, the correlation with the rheumatoid factor was highly significant. ESR and CRP correlated significantly as well (Table 2). No correlation could be found within different joint scores, DMARD or corticosteroid therapy, gender, patient age or disease duration. The Larsen Score showed only a borderline positive correlation with OPG levels. However, as patients were divided into two groups with lower and higher OPG serum levels (below and above the mean value of normals), a significant difference could be found for ESR and rheumatoid factor by analysis of variance (Table 3). As with the correlation analysis, Larsen Scores showed only a statistical trend due to the high standard-deviation of OPG and Larsen Scores as well. When patients were divided in two groups with Larsen Scores below and above 20, the chi-square test was significant: Patients with lower Larsen Scores had more often OPG-values below 44 pg/ml ($p < 0.02$). When the OPG levels of 17 patients, who had been seen twice, were compared to the annual increase in the Larsen Score, no correlation could be found.

Table 2. Spearman correlation coefficient of serum OPG levels compared to clinical and laboratory parameters

	DAS	Larsen-Score	ESR	CRP	RF
Spearman coefficient	0.115	0.199	0.308	0.227	0.614

Discussion

OPG is a soluble member of the family of tumour necrosis factor receptors [12]. It is produced by osteoblastic cells and acts as receptor antagonist for RANKL inhibiting the differentiation of osteoclast precursor cells and the activation of mature osteoclasts [13–15]. High levels of OPG in mice are osteoprotective [12]. OPG-knockout mice show enhanced activity of osteoclasts and develop an impressive osteoporosis. In RA activated CD4-positive T-lymphocytes produce RANKL which leads to an activation of osteoclasts and thus to bone lesions. OPG is able to inhibit this process completely [16–17]. With the up-regulation of the production of several cytokines in RA, not only RANKL, but to a lesser degree OPG may be elevated as well. However, other cells as endothelial cells and fibroblasts are also capable of producing OPG [18, 19]. Patients with active inflammation may therefore show higher OPG-values due to an activation of several other cells.

This hypothesis correlates with our results: The mean OPG serum level in RA is significantly higher than in a healthy control group. Furthermore, patients with active RA defined by high ESR and high rheumatoid factor show much higher OPG levels calculated by analysis of variance. Similarly a smaller, however significant difference, could be found with the Larsen Score. In a large group of normal controls, OPG levels were shown to be significantly higher for females and to increase with age [11]. The increased levels found in older healthy persons were clearly below the mean value found in our study. We could not observe such age dependence in our small group of RA patients that had already significantly increased levels related to disease activity.

These results may be interpreted as mechanism of up-regulation of OPG production in response to an elevation of RANKL and/or sRANKL in patients with active RA. Expression of RANKL has been shown to be increased in tissues surrounding bone erosions, while OPG was remarkably absent from tissues of patients with active RA [20]. Thus, the measurement of OPG in serum is an indirect in-

Table 3. Results of analysis of variance (ANOVA) if patients with low and with higher serum OPG levels are compared

	Serum-OPG ≤ 44	Serum-OPG > 44	p <
ESR ($\bar{x}$, mm n.W.)	27.3	41.0	0.01
CRP ($\bar{x}$, mg/l)	21.8	36.0	0.04
RF ($\bar{x}$, IU/ml)	82.0	410.5	0.05
DAS ($\bar{x}$)	3.8	4.1	0.26
Larsen Score ($\bar{x}$)	29.6	48.8	0.08

dicator of active processes occurring in affected joints, irrespectively whether the elevation is due to complexed OPG – in this case complexed serum-RANKL would be elevated as well – or to free OPG. The local elevation of RANKL leading to activation of osteoclasts and bone destruction is obviously not fully balanced by local OPG. Thus our results correlate to the results of Itonaga et al. [3], where upon the inflammatory infiltrate in the RA synovium includes not only RANKL-expressing T-cells, but also OPG-producing dendritic cells and B-cells. In addition, an increase of OPG serum levels might be a combination of an increased local OPG production in the synovium and an additional systemic response to counteract sRANKL in the circulation.

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