Egypt J Pediatr Allergy Immunol 2004; 2(1): 38-45.

Original article

Osteoprotegerin in juvenile rheumatoid arthritis: cross talk between the immune and the skeletal systems

Background: *Previous studies have linked the decreased local production of osteoprotegerin (OPG), an osteoclastogenesis blocking agent, in the inflamed joints of rheumatoid arthritis patients to the development of bone erosion.*

Objective: *We sought to assess OPG expression in juvenile rheumatoid arthritis (JRA) and to determine its relation to clinical and laboratory markers of disease activity, and radiologic evidence of bone resorption, as well as its relation to the type of onset, duration of illness and different therapeutic modalities.*

Methods: *The study included 40 children and adolescents with JRA, as well as, 20 clinically healthy age- and sex- matched subjects for comparison. The patients underwent clinical evaluation for disease activity by the summed joint index and investigations including assessment of ESR, CRP, antinuclear antibodies and rheumatoid factor. were Serum levels of osteoprotegerin were assayed by ELISA in the patient and control groups. Joints were evaluated radiologically using the modified Larsen index (LI).*

Results*: The serum levels of OPG in the patients [median (interquartile range): 0.474 (0.4) ng/ml] were comparable to those of the control group [0.495 (0.41) ng/ml] (p=0.29). However, patients with pauciarticular onset JRA had significantly lower OPG levels [0.3 (0.23) ng/ml] than the control group (p= 0.007). The OPG levels were below the 5th percentile of the control value in 60% of pauciarticular and 16.7% of polyarticular JRA cases. Patients with polyarticular JRA had significantly higher values of ESR, activity score and Larsen indices as well as serum OPG levels (p= 0.001, 0.001, 0.002 and 0.02, respectively). OPG levels did not correlate to the ESR or the activity score index values. On the other hand, the duration of illness showed a tendency to be negatively correlated to serum OPG (r= -0.309, p=0.05). LI correlated positively to the activity score index and to the ESR in the JRA patients, whether compiled in one group or classified into subgroups according to disease onset. However, OPG was not significantly correlated to the LI (r= 0.023). The different modalities of therapy did not seem to influence the serum levels of OPG ($\chi 2 = 4.21$).*

Conclusion: *Serum OPG expression was low in JRA, especially in the pauciarticular variety. OPG levels were higher in polyarticular JRA, but this does not necessarily have a protective effect since the proinflammatory process is known to promote also the expression of RANKL, an osteoclastogenesis enhancer. While clinical and biochemical parameters of activity, and LI did not correlate to OPG, the latter seemed to be adversely affected by increased disease duration.*

Key words: Osteoprotegerin, JRA, osteoclastogenesis, RANKL, bone resorption.

Hoda Y. Tomoum, Eman S. El-Hadidi*, Esraa M. F. El-Shahat*.

From the Departments of Pediatrics and Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.*

Correspondence:
Dr. Hoda Tomoum
Lecturer of Pediatrics, 10 El- Nagah Street, El Nozha, Cairo 11361, Egypt.
E-mail: hoda_tomoum @yahoo.com

INTRODUCTION

Rheumatoid arthritis (RA) is the prototype of autoimmune diseases that links the immune system with bone and cartilage metabolism [1]. The effects of rheumatoid arthritis on bone include structural joint damage (erosions) and osteoporosis, which are associated with increased morbidity and mortality. Osteoporosis in rheumatoid arthritis is characterized by a complexity of risk factors, including primary osteoporosis risk factors in addition to inflammation, immobilization, and the use of corticosteroids [2].

It has long been hypothesized that proinflammatory cytokines excessively produced in areas of active inflammation may transfer signals from immune cells to osteoclasts, however, the cellular and molecular context of this process remain elusive [1]. Recent evidence from experimental arthritis and progress in understanding the biology of osteoclasts has shed new light on the pathogenesis of skeletal manifestations [3].

Because osteoblast- stromal cell interactions with osteoclast precursors are required for subsequent osteoclast differentiation, an osteoclast differentiation factor expressed by these cells and recognized by osteoclast precursors was suspected. Such factor was identified as RANKL, receptor activator of nuclear factor -κB ligand. RANKL, a member of the tumor necrosis factor (TNF) ligand superfamily, is a transmembrane protein expressed in various cells and particularly on osteoblalst and activated T cells. RANKL can be cleaved and the soluble form is also active [4]. Both forms of RANKL act through binding to and activating receptor activator of nuclear factor (NF)-κB (RANK), a cell-bound receptor of the TNF receptor (TNFR) superfamily that is located on osteoclast precursor cells, mature osteoclasts, and dendritic cells. Following binding to its receptor, RANKL promotes osteoclast formation and activation, and inhibits osteoclast apoptosis [5].

The potent stimulatory effects of RANK by RANKL are counterbalanced by an endogenous antagonist, osteoprotegerin (OPG). Osteoprotegerin is a member of the TNF receptor family that lacks a transmembrane domain and represents a secreted receptor. Osteoprotegerin (Latin: os, bone; protegere, to protect) recognizes RANKL, and this decoy receptor blocks the interaction between RANK and RANKL. Osteoprotegerin has been observed to completely antagonize the effects induced by RANKL both in vitro and in vivo, leading to inhibition of osteoclast differentiation and activation [6]. Overexpression or exogenous administration of OPG have been shown to increase bone mass and prevent bone loss [7,8,9].

Several lines of evidence indicate that RANK/ RANKL/ OPG system is essential for bone metabolism, plays a critical role in the orderly development and function of the immune system, and may represent a molecular link between the immune system and bone metabolism [1]. The parallel roles of RANKL and OPG in regulating bone metabolism and the immune system may represent a potential therapeutic target in pathologic processes that are characterized by excessive bone resorption [6].

This study was aimed to assess OPG levels in sera of patients with juvenile rheumatoid arthritis (JRA) in comparison to healthy individuals and to determine its relation to markers of disease activity, whether clinical or laboratory, and to radiologic evidence of bone resorption, as well as its relation to the different types of disease onset, duration of illness and different therapeutic modalities.

METHODS

This study was carried out at the Pediatric Allergy and Immunology Unit of Children's Hospital of Ain Shams University in Cairo. The study was approved by the local ethical committee and consents were taken from the parents or care-givers before inclusion in the study.

The study was conducted on 40 children and adolescents with juvenile rheumatoid arthritis (JRA), all of them fulfilling the American College of Rheumatology criteria for diagnosis of JRA [10]. They were twenty-eight females (70%) and twelve males (30%), their ages ranged between 7 and 16 years with a median (interquartile range) of 13.0 (5.0) years.

Patients were subdivided into:

i- Polyarticular onset JRA: This group included 30 children (20 females and 10 males).

ii- Pauciarticular onset JRA: This group comprised 10 children (8 females and 2 males),

For comparison of results, a group of 20 age- and sex- matched clinically healthy children [median age (interquartile range): 12.56 (6.00) years] were included in the study.

The following was done for the enrolled patients:

I-Clinical evaluation: Stress was laid on the duration of illness, type of disease onset, number of affected joints, activity of arthritis, systemic manifestations and medications received.

Patients were assessed for clinical parameters of joint inflammation (activity score) using the summed joint index score [11,12]. For each of the clinical indices (the joint swelling, the pain on motion/joint tenderness and limitation of motion), the total articular activity for each patient was calculated, considering the affected joints only, as follows:

$$\textit{Activity score} = \frac{\textit{Sum of the clinical indices of the affected joints.}}{\textit{Number of affected joints.}}$$

II- Laboratory evaluation:

All participating individuals were subjected to the following investigations:

Sampling:

1. A sample on EDTA for measurement of ESR.
2. Clotted samples, the obtained serum was divided into portions, for the assay of CRP, Rheumatoid factor (RF) and antinuclear antibodies (ANA). A sample was stored at -20°C until assay of Osteoprotegerin level (OPG).

Analytical Methods:

- *Erythrocyte sedimentation rate assessment using the Westergren method.*
- Quantitative CRP assay by direct latex agglutination (QUIMICA CLINICA APLICADA, Spain).
- Rheumatoid factor (RF) assay by latex agglutination slide test (BIOTEC laboratories, UK)
- Antinuclear antibodies (ANA) using indirect immunofluorescence method (IMMCO diagnosis, NY, USA)
- Osteoprotegerin level using enzyme- linked immunosorbent assay (ELISA) method (Biovendor laboratories, Czech Republic).

Assay procedure: Diluted standards, quality controls and serum samples were added to microplate wells coated with capture monoclonal antiosteoprotegerin antibody. After incubation and washing steps, biotin- labeled polyclonal antihuman osteoprotegerin antibody solution was added for a further incubation step. Following a final washing step, hydrogen peroxide tetramethylbenzidine was incubated into the wells resulting in a coloured product. After the addition of oxidic stop solution, the colour was measured at 450 nm. The absorbance and colour intensity were considered proportional to the concentration of OPG in the sample. A standard curve was constructed and the concentration of OPG in the samples was determined [13].

III- Radiographic evaluation:

X- ray films were taken at the time of sampling. Evaluation was done according to modified Larsen scoring methods [14]. Joints examined radiologically were those of the hands, feet and knees. A mean value score of all joints was obtained for each patient (Larsen Index).

Statistical methods:

Statistical analysis was done using a software package (SPSS) version 10.0. Quantitative nonparametric variables were presented as median (interquartile range). Comparison between the different studied variables was done using Mann-Whitney U test. Correlation of different numerical variables was attempted using Spearman r correlation test. From statistical tables, the probability (p) value were calculated, p values less than 0.05 were considered significant. A cut-off value for OPG corresponding to the 5th percentile of the control group was calculated (equals 0.348 ng/ml) and values below it were considered as low OPG levels.

RESULTS

This study was conducted on 40 patients with JRA, their median age (interquartile range) was 13.0 (5.0) years. Thirty of the included patients had polyarticular onset JRA (median age 13.5 (4.63) years), while ten patients had pauciarticular onset JRA (median age of 12.0 (5.76) years. The duration of disease ranged between 1 and 11 years [median (interquartile range): 5.25 (4.0) years] (Details of the clinical characteristics of the included patients are summarized in **Table 1**).

The serum levels of OPG in our patients were lower than, though not significantly different from, that of the control group [median (interquartile range): 0.474 (0.4) and 0.495 (0.41) ng/ml, respectively] (z=1.05, p=0.29). However, when comparing subgroups of JRA patients (those with polyarticular and those with pauciarticular onset JRA) to the control group, we found that patients with pauciarticular onset JRA had significantly lower OPG levels [median (interquartile rang): 0.3 (0.23) ng/ml] than the control values (z= 2.65, p= 0.022) (Figure 1).

With a cut off value of 0.348 ng/ml, 60% of patients with pauciarticular JRA had OPG values below the 5th percentile of the control group, a result that is significantly different from that of patients with polyarticular JRA (16.7% only) (χ^2=7.06, p=0.014) (Figure 2).

Trying to evaluate the relation of OPG to disease activity, we correlated its levels with that of ESR and activity score index, but found no significant correlation (r= 0.071 and –0.03, respectively). Similarly, CRP positivity did not affect the serum levels of OPG (z= 0.03). The median Larsen index (LI) was 1.5 (1.9) (ranging between 0.33 and 4.33). Whether in the patients group or in either of the polyarticular or the pauciarticular patients subgroups, it was noted that LI had a significant positive correlation to the activity score index (r= 0.98, 0.98 and 0.87, respectively and p= 0.0001, 0.0001 and 0.001, respectively) and to the ESR (r= 0.96, 0.97 and 0.91, respectively and p= 0.0001). However, OPG did not show a significant correlation to LI (r= 0.023).

Comparing patients with polyarticular and those with pauciarticular onset- JRA, we found that

patients with polyarticular JRA had significantly higher ESR values, activity score and Larsen indices compared to pauciarticular patients. Furthermore, serum OPG levels were significantly higher in the polyarticular group than in pauciarticular- onset JRA (Table 2).

Comparing the OPG levels in different subgroups of polyarticular onset JRA, we found no significant difference when comparing patients with RF positive-, ANA positive- and seronegative-polyarticular JRA with each other [median (interquartile rang): 0.62 (0.47), 0.47 (0.29) and 0.47 (0.34) ng/ml, respectively].

The duration of illness showed a tendency to be negatively correlated to serum levels of OPG meaning that the longer the duration of illness was, the lower got the levels of OPG (r= -0.309, p=0.05). Comparing patients only on nonsteroidal anti-inflammatory drugs to those receiving immunosuppressive agents did not reveal significant difference in their OPG levels (z= 1.706, p=0.09). Similarly, patients on steroids and those receiving methotrexate had OPG levels comparable to those not receiving such drugs(z=1.25 and 0.015, p=0.22 and 0.99, respectively).

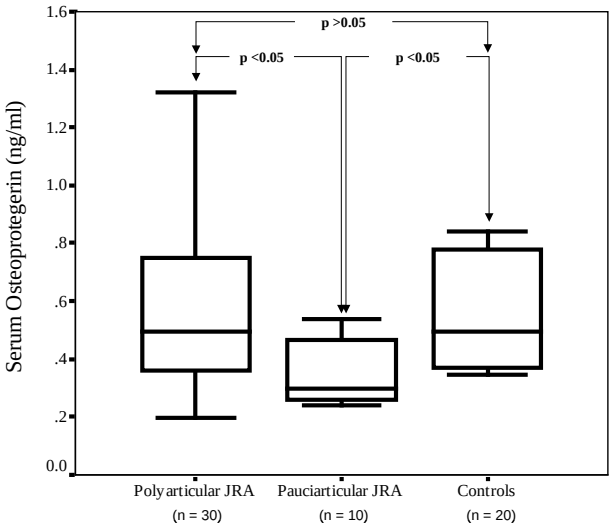
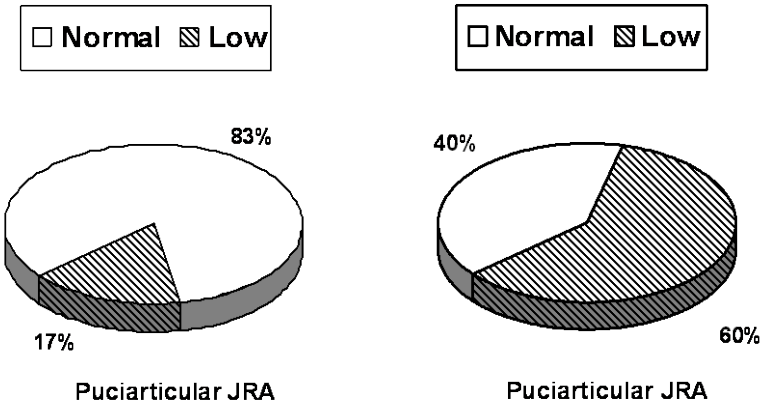


Figure (1): Comparison between osteoprotegerin levels in the studied sample.



χ^2=7.06, p=0.014

Figure (2): Variation of osteoprotegerin expression with the type of JRA onset

Table (1): Some clinical and laboratory data of the JRA patients

Age *in years*	
Median (interquartile range)	13.0 (5.0)
Subgroups of JRA *[number (%)]*	
Polyarticular JRA	30
RF- positive	6 (20%)
ANA- positive	10 (33.3%)
Seronegative	14 (46.7%)
Pauciarticular JRA	10
RF- negative	10 (100%)
ANA-positive	8 (80%)
Duration of disease *in years*	
Median (interquartile range)	5.25 (4.0)
Therapeutic modalities *[number (%)]*	
NSAIDs only	13 (32.5%)
Steroids Use	23 (57.5%)
Methotrexate	12 (30%)
Summed joint index score	
Range	2-11
Median (interquartile range)	4.0 (6.25)
Laboratory markers of disease activity	
ESR *in mm/h*	
Range	5-75
Median (interquartile range)	10 (30.0)
CRP positive cases *[number (%)]*	11 (27.5%)

ANA: antinuclear antibodies; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; JRA: juvenile rheumatoid arthritis; NSAIDs: nonsteroidal anti-inflammatory drugs; RF: rheumatoid factor.

Table (2): Variation of some studied parameters according to type of JRA onset

Parameters evaluated in median (interquartile range)	**Polyarticular JRA (n= 30)**	**Pauciarticular JRA (n=10)**	**Z value**
ESR *(mm/hr)*	20.0 (37.5)	5 .0 (5.0)	3.36**
Activity score	6.0 (6.38)	3 (0.8)	3.19**
LI	1.83 (1.83)	1.0 (4.17)	3.03**
Serum OPG*(ng/ml)*	0.5 (0.39)	0.3 (0.23)	2.28*

*p< 0.05, **p< 0.01
ESR: erythrocyte sedimentation rate; JRA: juvenile rheumatoid arthritis; LI: Larsen index; OPG: osteoprotegerin.

DISCUSSION

The pathogenesis of bone loss in rheumatoid arthritis is multifactorial; disease activity certainly is a major determinant of bone mass. Further pathogenic factors include effects of anti-inflammatory therapies (in particular glucocorticoids), reduced mobility, estrogen and/or androgen deficiency [15].

In our series, radiologic evidence of bone resorption was found to correlate to clinical and/or biochemical markers of disease activity of the JRA patients, whether compiled in one group or classified into polyarticular and pauciarticular subgroups. It has been previously reported that bone loss correlates well with measures of inflammation [16,6] and that proinflammatory cytokines as IL-1 and TNF-α are among factors triggering excessive osteoclastic activity, though the underlying mechanism was not completely understood. Recently it has been demonstrated that osteotropic factors and hormones such as PTH, 1,25(OH)2D3, IL-1, IL-1β, TNF-α or prostaglandin E2 act though a common pathway up regulating RANKL expression in osteoblast/stromal cells. By binding to its receptor (RANK) on osteoclasts precursors, RANKL promotes their differentiation and activation into mature osteoclasts [6].

The RANK/ RANKL system is completed by OPG that recognizes and blocks the interaction of RANKL. Measuring its levels in the enrolled patients, we found that values of OPG were lower than those of the control group, most evident in the pauciarticular JRA group. Furthermore, 60% of patients with pauciarticular JRA had serum OPG values below the 5th percentile of the control group.

To our knowledge, this is the first study assessing OPG levels in pediatric patients with JRA. Our results are supported by the findings of Takayanagi et al. [17] and Wong et al. [18] who reported that synovial fluid from adult patients with RA contained lower OPG levels compared to patients with other joint diseases, indicating a relative decrease of local production of OPG to compensate for the up-regulated RANKL expression seen in those patients. Similarly, Haynes et al. [19] reported that OPG expression on macrophage type synovial lining cells as well as on endothelial cells is deficient in RA patients with active synovitis, in contrast to that seen in patients with other joint diseases (spondyloarthropathy and osteoarthritis) or in healthy subjects. They suggested that this deficiency in OPG expression in the inflamed joints of RA patients may be important in the development of radiologically defined joint erosions. In support of these findings are the results of Wong et al. 18 who noted that IFN-α treatment of RA synovial fluid cells resulted in upregulation of OPG gene expression of those cells.

Comparing patients with polyarticular and those with pauciarticular JRA, we found a significantly higher clinical and biochemical markers of disease activity and higher OPG levels in patients with polyarticular compared to those with pauciarticular onset- JRA. The difference in the results of both groups can be explained by the underlying immunopathogenic mechanism of the polyarticular JRA which is characterized by proinflammatory response and manifested by increased proinflammatory cytokines [20] . In their study, Ziolkowska et al. [21] and Rifas et al. [22] stated that the proinflammatory cytokines enhance OPG and RANKL production in RA patients but the OPG:RANKL ratio is rather fixed or even decreased in view of the essentially lower OPG concentration in RA patients than controls. Recent studies have demonstrated that the control of osteoclastic differentiation, activity and survival and hence, bone resorption in the OPG/RANK/RANKL system resides, not in the absolute quantities of either OPG or RANKL, but rather in the proportion of the inhibitory OPG to the stimulatory RANKL [23,24] , which may explain why the higher levels of OPG in patients with polyarticular JRA did not have a protective effect against bone resorption. By comparison, the immunopathogenic mechanism underlying pauciarticular JRA is an anti-inflammatory response manifested by increased IL-4 and IL-10 mRNA [20] . Studies showed that IL-4 suppresses RANKL mRNA expression, even more, suppresses type I collagen breakdown, which may well explain the difference seen in our study between patients with polyarticular and those with pauciarticular onset JRA [25].

Correlating OPG levels to markers of disease activity, we found no significant

correlation to either laboratory or clinical markers of disease activity. This result is similar to that of Valleala et al. [26] and Feuerham et al. [13] who could not detect any relation between the disease activity and levels of OPG. Though most proinflammatory cytokines (IL-1, IL-11, IL-17 and TNF-α) are known to increase the levels of OPG and RANKL, others (IL-6 and IL-7) have no effect on OPG levels [23]. Furthermore, basic fibroblast growth factor (bFGF) inhibits OPG production by human fibroblast- like synovial cells by negating the direct stimulatory effect of the inflammatory cytokines in a dose- dependent manner [27].

Levels of OPG in our series did not significantly correlate to radiologic evidence of bone erosion (LI). Similar to our findings are those of Valleala et al. [26] who found no significant correlation between serum OPG levels and radiologic evidence of disease progression or even biochemical markers of bone metabolism. The complex interplay of cytokines in the regulation of the RANK/ RANKL/ OPG system may be a possible cause of this non significant relation. Also, none of the evidence so far excluded the possibility that other cells other than osteoclasts contribute to bone resorption. For example, the production of matrix metalloproteinases by the macrophages and synovial fibroblasts may directly increase bone erosion and play a role in preparation of bone surfaces for osteoclast attachment [28] .

The duration of illness showed a tendency to be negatively correlated to the level of OPG, it seems that longer exposure to proinflammatory cytokines, has a suppressant effect on OPG production. We could not trace similar data in the literature to compare our results.

It has been proposed that glucocorticoids [29,30] and immunosuppressants [31] support osteoblastic bone resorption by increasing the levels of RANKL and reducing OPG expression [23]; nevertheless, the different modalities of therapy did not seem to affect the level of OPG in our study. Valleala et al. [26] in their study on patients with RA stated that , at least, low dose corticosteroids- associated osteoporosis is probably not mediated through inhibition of OPG. Another plausible explanation is that the suppressant effect of the corticosteroids and immunosuppressant drugs on the inflammatory

process may play a role in antagonizing their direct action on the OPG/RANKL system.

In conclusion, serum OPG levels are lowered in patients with JRA, especially so in patients with pauciarticular JRA. While OPG levels were higher in patients with polyarticular than in those with pauciarticular JRA, this did not seem to have a protective effect on bone resorption since the proinflammatory process is known to equally promote higher levels of OPG and RANKL expression. While parameters of disease activity, whether clinical or biochemical, and degree of bone resorption did not show a significant correlation to OPG, its levels seem to be adversely affected by the increased duration of the disease. Steroids and immunosuppressive drugs did not seem to affect OPG levels and thus the known osteoporotic effect of these drugs may be mediated by other mechanisms.

Demonstration of the role of the newly discovered RANK/RANKL/OPG system provides hope that specific therapy can be provided for preventing bone loss and joint destruction. The primary consideration for the use of exogenous OPG to treat RA is that control of osteoclastic network depends on the ratio of OPG: RANKL. Thus, strategies that enhance local production of IL-4 or neutralize IL-1 and TNF-α together with OPG administration have been suggested. This may not only decrease bone loss but also inhibit the inflammatory process.

REFERENCES

1. **Hofbauer LC, Heufelder AE, Erben RG.** Osteoprotegerin, RANK, and RANK ligand: the good, the bad, and the ugly in rheumatoid arthritis. J Rheumatol 2001; 28(4): 685-7 .
2. **Haugeberg G, Orstavik RE, Kvien TK.** Effects of rheumatoid arthritis on bone. Curr Opin Rheumatol 2003; 15(4):469-75.
3. **Schett G, Redlich K, Smolen JS.** The role of osteoprotegerin in arthritis. Arthritis Res Ther 2003; 5(5):239-45.
4. **Saidenberg Kermanac'h N, Bessis N, Cohen-Solal M, De Vernejoul MC, Boissier MC.** Osteoprotegerin and inflammation. Eur Cytokine Netw 2002; 13(2):144-53 .
5. **Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, et al.** Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999; 402 (6759): 304-9.
6. **Roux S, Orcel P.** Bone loss. Factors that regulate osteoclast differentiation - an update. Arthritis Res 2000; 2 (6): 451–6.
7. **Itonaga I, Fujikawa Y, Sabokbar A, Murray DW, Athanasou NA.** Rheumatoid arthritis synovial macrophage-osteoclast differentiation is osteoprotegerin ligand-dependent. J Pathol 2000; 192(1):97-104 .
8. **Haynes DR, Crotti TN, Loric M, Bain GI, Atkins GJ, Findlay DM.** Osteoprotegerin and receptor activator of nuclear factor kappaB ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. Rheumatology (Oxford) 2001; 40(6):623-30.
9. **Ritchlin CT, Haas-Smith SA, Li P, Hicks DG, Schwarz EM.** Mechanisms of TNF-alpha- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. J Clin Invest 2003; 111(6):821-31.
10. **Cassidy JT.** Juvenile rheumatoid arthritis. In: Cassidy JT and Petty RE, editors. Textbook of pediatric rheumatology. 4th ed. Philadelphia: WB Saunders; 2001.p. 113-219.
11. **Giannini EH, Brewer EJ, Kuzmina N, Shaikov A, Maximov A, Vorantsov I, et al.** Methotrexate in resistant juvenile rheumatoid arthritis. Results of USA-USSR double blind, placebo controlled trial. The Pediatric Rheumatology Collaborative Study Group and The Cooperative Children's Study Group. N Engl J Med 1992; 326 (16):1043-9.
12. **Van Rossum MJ, Fiselier TJ, Franssen MJ, Zwinderman AH, ten Cate R, van Suijlekom-Smit LW, et al.** Sulfasalazine in treatment of juvenile chronic arthritis: a randomized, double-blinded, placebo-controlled, multicenter study. Dutch Juvenile Arthritis Study Group. Arthritis Rheum 1998; 41: 808-16.
13. **Feuerherm AJ, Borset M, Seidel C, Sundan A, Leistad L, Ostensen M, et al.** Elevated levels of osteoprotegerin (OPG) and hepatocyte growth factor (HGF) in rheumatoid arthritis. Scand J Rheumatol 2001; 30(4): 229-34 .
14. **Rau R, Herborn G.** A modified version of Larsen's scoring method to assess radiologic cases in rheumatoid arthritis. J Rheumatol 1995; 22(10): 1976-82.
15. **Brosch S, Redlich K, Pietschmann P.** Pathogenesis of osteoporosis in rheumatoid arthritis. Acta Med Austriaca 2003; 30(1): 1-5 [English abstract].
16. **Green MJ, Deodhar AA.** Bone changes in early rheumatoid arthritis. Best Pract Res Clin Rheumatol 2001; 15(1): 105-23 .

17. **Takayanagi H, Iizuka H, Juji T, Nakagawa T, Yamamoto A, Miyazaki T, et al.** Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum 2000; 43(2): 259-69 .

18. **Wong T, Majchrzak B, Bogoch E, Keystone EC, Fish EN**. Therapeutic implications for interferon-alpha in arthritis: a pilot study. J Rheumatol 2003; 30(): 934-40.

19. **Haynes DR, Barg E, Crotti TN, Holding C, Weedon H, Atkins GJ et al.** Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies and osteoarthritis and normal controls. Rheumatology (Oxford) 2003; 42(1):123-34 .

20. **Moore TL.** Immunopathogenesis of juvenile rheumatoid arthritis. Curr Opin Rheumatol 1999; 11(5); 377-83.

21. **Ziolkowska M, Kurowska M, Radzikowska A, Luszczykiewicz G, Wiland P, Dziewczopolski W, et al.** High levels of osteoprotegerin and soluble receptor activator of nuclear factor kappa B ligand in serum of rheumatoid arthritis patients and their normalization after anti-tumor necrosis factor alpha treatment. Arthritis Rheum 2002; 46(7):1744-53 .

22. **Rifas L, Arackal S, Weitzmann MN**. Inflammatory T cells rapidly induce differentiation of human bone marrow stromal cells into mature osteoblasts. J Cell Biochem 2003; 88(4):650-9 .

23. **Bolon B, Shalhoub V, Kostenuik PJ, Campagnuolo G, Morony S, Boyle WJ, et al.** Osteoprotegerin, an endogenous antiosteoclast factor for protecting bone in rheumatoid arthritis.. Arthritis Rheum 2002; 46(1):2 3121-35 .

24. **Ueland T, Bollerslev J, Mosekilde L.** Osteoclast function is regulated by neighbouring osteoblasts. Osteoprotegerin, RANK and RANK ligand constitute a unique regulatory system for bone resorption with important pathophysiological and therapeutic aspects. Ugeskr Laeger 2002; 164(27): 3526-3 [English Abstract].

25. **Lubberts E, Joosten LA, Chabaud M, van Den Bersselaar L, Oppers B, Coenen-De Roo CJ, et al.** IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion. J Clin Invest 2000; 105(12): 1697-710 .

26. **Valleala H, Mandelin J, Laasonen L, Koivula MK, Risteli J, Konttinen YT**. Effect of cyclical intermittent etidronate therapy on circulating osteoprotegerin levels in patients with rheumatoid arthritis. Eur J Endocrinol 2003; 148(5):527-30 .

27. **Yano K, Nakagawa N, Yasuda H, Tsuda E, Higashio K**. Synovial cells from a patient with rheumatoid arthritis produce osteoclastogenesis inhibitory factor/osteoprotegerin: reciprocal regulation of the production by inflammatory cytokines and basic fibroblast growth factor. J Bone Miner Metab 2001; 19(6): 365-72

28. **Romas E, Sims NA, Hards DK, Lindsay M, Quinn JW, Ryan PF, et al.** Osteoprotegerin reduces osteoclast numbers and prevents bone erosion in collagen-induced arthritis. Am J Pathol 2002; 161(4):1419-27 .

29. **Hofbauer LC, Gori F, Riggs BL, Lacey DL, Dunstan CR, Spelsberg TC, et al.** Stimulation of osteoprotegerin ligand and inhibition of osteoprotegerin production by glucocorticoids in human osteoblastic lineage cells: potential paracrine mechanisms of glucocorticoid-induced osteoporosis. Endocrinology 1999; 140(10): 4382-9.

30. **Vidal NO, Brandstrom H, Jonsson KB, Ohlssonn C.** Osteoprotegerin mRNA is expressed in primary human osteoclast- like cells: down-regulation by glucocorticoids. J Endocrinol 1998; 159(1): 191-5.

31. **Hofbauer LC, Shui C, Riggs BL, Dunstan CR, Spelsberg TC, O'Brien T, et al.** Effects of immunosuppressants on receptor activator of NF-kappa B ligand and osteoprotegerin production by human osteoblastic and coronary artery smooth muscle cells. Biochem Biophys Res Commun 2001; 280(1): 334-9.