ORIGINAL ARTICLES

ASSESSING THE ROLE OF RECEPTOR ACTIVATOR OF NF-KB LIGAND IN DEVELOPING OSTEOPOROSIS IN PSORIATIC AND RHEUMATOID ARTHRITIS

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Abstract

Objectives: Evaluation of the bone remodeling by determining the serum level of nuclear receptor activator of factor Kappa B ligand (RANKL) involved in bone remodeling as a marker of reabsorption in psoriatic and rheumatoid arthritis; the comparison of the serum levels of RANKL with the values of bone mineral density, with the purpose of identifying some useful correlations in the early diagnostic of some complications and identifying the patients that will benefit from specific treatment.

Material and method: Our study has been realized on three groups of patients: group 1 (patients with psoriatic arthritis), group 2 (patients with rheumatoid arthritis) and group 3 (healthy patients).

Results: Our study demonstrated an elevation of RANKL serum levels in both types of arthritis, and comparing the serum levels of RANKL with the values of the bone mineral density has proven that there is a strong negative correlation between the RANKL serum levels and the lumbar T score both in the psoriatic arthritis group while in the healthy group the RANKL serum level is in a strong positive correlation.

Conclusion: The determination and the monitoring of the serum levels of RANKL could represent a parameter that would allow the appreciation and quantifying of the degree of activity of the disease, as well as a selection criterion of patients who will develop severe bone erosions and who can benefit in timely of specific therapy. Also the screening of systemic osteoporosis, which can lead to the occurrence of fractures, fractures that represent a feared complication due to the prolonged immobilization and the high costs it involves, represents an easily achievable objective these days with a special importance both for the patient and the sanitary system.

Keywords: psoriasis arthritis, rheumatoid arthritis, RANKL, osteoporosis

Introduction

In order to insure its functions, the bone tissue is continuously restored through a process of reshaping provided by two types of cells: osteoclasts cells, which are cells that reabsorb the bone matrix, and osteoblasts cells which are cells that synthesize a new matrix [1]. Under normal conditions, the reabsorbed bone is replaced by the same quantity of new bone, so that after each cycle of remodeling the total bone quantity remains constant. This remodeling allows the maintaining (preserving) of the bone mass throughout the course of

a normal adult life [2]. The recent progress registered in the study of the biology of the osteoclasts alongside with the known fact that the osteoclasts is an effectors cell which is important in the process of bony and articular destruction has contributed to the understanding of pathogenesis of bone destruction in psoriatic arthritis [3,4]. The bone complications in psoriatic arthritis appear due to transferred signals from cells that are immune to osteoclasts, cells specialized in bone reabsorption, by the pro-inflammatory cytokines produced in the areas of active inflammation. Therefore, osteoclasts represent key mediators in the process of bone loss which takes place in psoriatic arthritis [4,5]. Nuclear receptor activator of factor Kappa B ligand (RANKL) stimulates the formation and activation of osteoclasts, thus defending osteoporosis [5-8]. RANKL is an essential cytokine in the formation of osteoclasts. RANKL is a member of the tumoral necrosis family (TNF) and it exists in the form connected to the membrane of the osteoblasts ($RANKL_{MB}$) and a soluble form ($RANKLE_{SB}$ or $_{S}$RANKL). The $_{S}$RANKL is tied to a trans-membrane receptor (RANK), expressed on the surface of the osteoclasts and of the osteoclastic precursors [7,8]. The main action of RANKL consist in: differentiation and activation of the osteoclasts from the myeloid hematopoietic precursors alongside stimulation factor of the monocyte colonies (M-CSF); survival and activation of mature osteoclasts through inhibition of their apoptosis. The interaction device of the RANKL/RANK/OPG system in bone remodeling has as first stage the one where $_{S}$RANKL ties to the RANK receptor, forming the molecular RANK/$_{S}$RANK system, with a role in the growth of osteoclasto-genesis; therefore the bone reabsorption grows [9-12].

The purpose of our study consists in the evaluation of the bone reabsorption by determining the serum level of RANK, involved in bone remodeling as a marker of osteo-reabsorption in psoriatic arthritis; the comparison of the serum levels of RANKL with the values of bone mineral density, with the purpose of identifying some useful correlations in the precocious diagnostic of some complications and identifying the patients that will benefit from specific treatment.

Material and Method

Our study has been realized on three groups of patients: group 1 (patients with psoriatic arthritis who were diagnosed based on The Classification Criteria for Psoriatic Arthritis (CASPAR) criteria (is made up of 27 patients (n=27) out of which 15 male and 12 female with ages between 27 and 50 years, group 2 (patients with rheumatoid arthritis diagnosed based on the criteria of the American Rheumatology Association) is formed out of 21 patients (n=21) out of which 13 female and 8 male with ages between 25 and 52 years and group 3 (healthy patients with an average age of 30 years without rheumatoid, infectious, neoplastic or autoimmune conditions) which was formed of 20 patients (n=20) out of which 9 female and 11 male.

Determining the $_{S}$RANKL concentration. The serum level in the synovial RANKL (activating receptor of NF-kappa B ligand) has been determined through the immunoenzymatic technique of the ELISA sandwich type (Enzyme Linked Immuno Sorbant Assay).

Determining the mineral bone density. The mineral bone density has been measured at the level of the lumbar vertebral spine (L2-L4) through the DEXA method (dual energy X-ray absorptiometry). The osteopenia is defined based on a T score between -1 and -2 and osteoporosis with a T score ≤-2.5 according toms criteria and those of NIH Consensus Conference - National Institute of Health - 2006.

Statistical analysis:

The graphics and the statistical analysis in this study were done by use of Microsoft Office Excel 2007. We used: The elements of descriptive statistics: mean and standard deviation (mean±DS); the elements of interferential statistics: the t´ Student test and the Pearson test.

The t´ Student test was used for the comparison of data belonging to the psoriatic arthritis group comparative to the healthy group. The values of the p_1 were calculated (group 1 and healthy group). $p<0.05$ has statistically semnificative value.

The Pearson test was used for the comparison of data within the same group. The values of the r_1 were calculated. $r>0.5$ has good correlation (positive or negative)

Results

Serum levels of RANKL ($_{S}$RANKL)

In the group 1 (patients with psoriatic arthritis) the serum concentration of RANKL had values between 36.7 and 66.12 pg/ml (and average of 51.98 ±10.74 pg/ml; $p_1<0.003$- ES, significantly statistically intense in comparison with the witness set). In the group 2 (patients with rheumatoid arthritis) the serum concentration of RANKL had values between 58.45 and 69.96 pg/ml (an average of 63.71±2.06 pg/ml

Table I. Comparison between the RANKL serum levels with the analyzed groups

Parameter (media±DS)	Group 1 (n=27)	Group 3 (n=20)	Group 2 (n=21)
RANKL level (pg/ml)	51.98±10.74 p1<0.003	32.37±2.68	63.71±2.06 p2<0.002

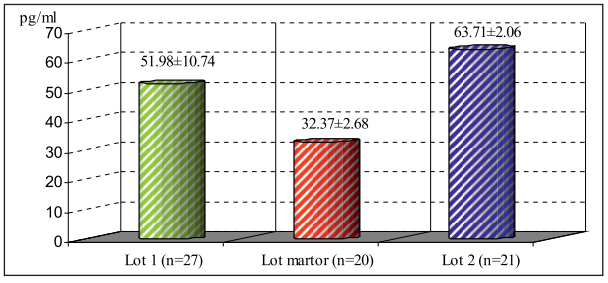

Figure 1. Comparison of RANKL serum levels in analyzed groups

p_2<0.002- ES, significantly statistically intense in comparison with the witness set). In the group 3 (healthy voluntaries) the serum concentration of RANKL had values between 29.65 and 37.14 pg/ml (an average of 32.27±2.68 pg/m) (Table I, figure 1).

By analyzing the results that were obtained from our study, we notice an elevation of RANKL serum levels: with group 1 an elevation of 160, 58% of the 51.98±10.74pg/ml average, as compared to the healthy group, and with group 2 an elevation of 196,81% of the 63.71±2.06 p/ml average as compared to the healthy group, as a consequence of the growth of bone reabsorption.

Comparison of RANKL serum levels with the values of bone mineral density

In the group1 (patients with psoriatic arthritis) the serum concentration of RANKL had values between 36.97 and 66.12 pg/ml (an average of de 51.98±10.74 pg/ml; p1<0.003- ES, significantly statistically intense compared to the healthy group), and the lumbar T score had values between 1.58 and -1.77 DS (an average of -1.68±0.06 DS; $p_{1'}$<0.01-S, statistically significant as compared to the witness set). In the group 2 (patients with rheumatoid arthritis (the serum concentration of RANKL had values between 58.45 and 69.96 pg/ml (an average of 63.71±2.06 pg/ml p_2<0.002-ES, significantly statistically intense compared to the witness set) and the lumbar T score had values between -2.51 and -3.41 DS (an average of -2.93±0.3 DS, p_2<0.001- ES, significantly statistically intense compared to the healthy group). In the group 3 (healthy voluntaries) the serums concentration of RANKL had values between 29.65 and 37.14 pg/ml (an average of 32.37±2.68 pg/ml), and the lumbar T score had values between 0.29 and 1.3 DS (an average of 0.82±0.32 DS) (Table II, Fig. 2, Fig. 3, Fig. 4).

Table II. Comparison between the serum levels of RANKL and the lumber T score in the analyzed sets

Parameter (means ± DS)	Group 1 (n=27)	Group 3 (n=20)	Group 2 (n=21)
Lumber T score (DS)	-1.68±0.06 p1'<0.01	0.82±0.32	-2.93±0.3 p2< 0.001
RANKL serum levels (pg/ml)	51.98±10,74 p1<0.003 r1=-0.80	32.37±2.68 r3=0,65	63.71±2.06 p2<0.002 r2=-0,56

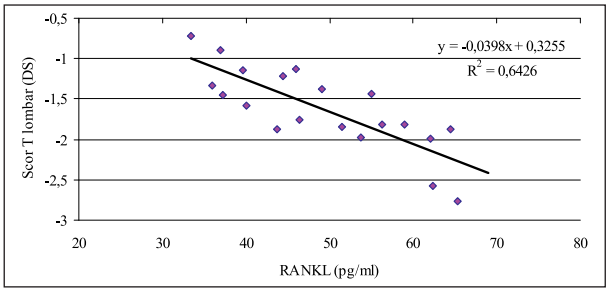

Figure 2. Correlation between serum levels of RANKL and the values of the lumber T score in patients with psoriatic arthritis

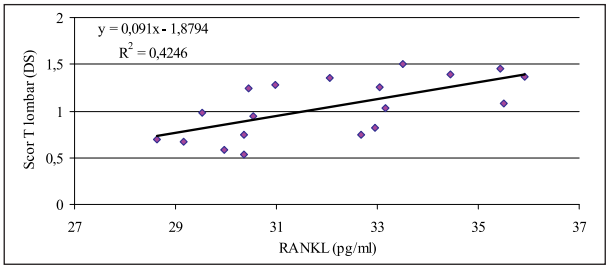

Figure 3. Correlation between the serum levels of RANKL and the lumber T scores in healthy voluntaries

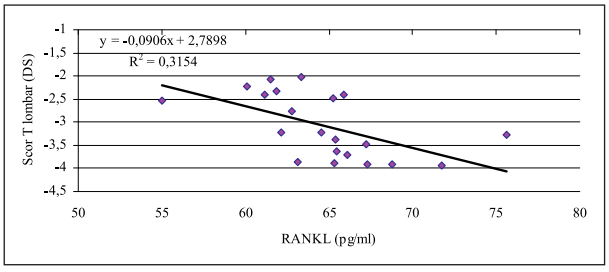

Figure 4. Correlation between serum levels of RANKL and the lumber T scores in patients with rheumatic arthritis

Discussions

In order to ensure its functions, the bone tissue is continuously remade through a process of remodeling ensured by two types of cells: osteoclasts, cells that reabsorb the bone matrix, and osteoblasts, cells that synthesize a new matrix [2-4]. The special importance that the RANKL has in bone remodeling is also proven by the results of the clinical trials about the role of RANKL in the initiation of the osteoclastogenesis, the RANKL levels in the peripheral blood and the synovial liquid in patients with psoriatic arthritis in comparison with healthy witnesses [5-7]. The results of these trials quoted in the professional literature have shown that in patients with psoriatic arthritis the RANKL was elevated both in the peripheral blood as well as in the synovial liquid, as compared to the healthy witnesses and that the osteoclasts represent the only type of cells that are capable of bone reabsorption and that these cells are not characteristic for psoriatic arthritis. Their role in this disease was documented by screening of an increased number of osteoclasts at the level of the affected joints. The mature osteoclasts have not been revealed at the level of the vascular lumen. The addition of RANKL at the cellular cultures coming from patients with psoriatic arthritis had caused the growth of the numbers and dimensions of the osteoclasts [9]. The results of our trial are according to the ones quoted by the professional literature and have demonstrated the rise of the serum concentration and the synovial liquid of RANKL in patients with psoriatic arthritis as compared to the healthy voluntaries (p1<0.003-ES, significantly statistically intensecompared to the witness set), but smaller thatn the patients with rheumatic arthritis. These results of our trial are in agreement with the clinical manifestations of rheumatoid arthritis in which the articular distructions are more accentuated than in psoriatic arthritis.These studies proove that RANKL is the key mediator of the articular distruction and of the loss of bone in psoriatic arthritis, and that he inhibationof the RANKL activity through OPG can prevent the distruction of cartilage - a critical and irreversible step in the pathogenesis of the psoriatic arthritis [10-13]. The observation that inhibating RANKL through OPG prevents bone loss and that it has a benefic effect over te cartilage in psoriatic and reumatoid arthitis, but does not affect the inlammation, as well as the knoledge of the fact that the pro-inflammatory cytokines TNF-α are mediators is critically dependant on the RANKL expression [12-14]. It's also demonstrated that the RANKL/RANK system plays an important role in the osteoclastogenesis both at the focale osteolitic lesions and the generalized ones [9]. This way, the bone distruction is mediated by RANKL, but the inflammatory answer can also be developed independently of the modifications of the bone metabolism of the bone destruction. Thus, it can be said that RANKL is the main mediator of bone destruction in the case of the psoriatic arthritis patients as well. These results prove once again the existence of a unique mechanism between the activation of the T cells, the production of cytokines and the activation of the osteoclasts and the articular destruction through the RANKL/RANK/OPG system, which explains the broad range of the skeletal destructions in psoriatic arthritis [13-15]. Our study has proved that there is a strong negative correlation between the elevation of RANKL levels in the blood and the decrease of bone mineral density, quantified by the values of the lumber T score in patients with psoriatic arthritis. This way the personal contribution of our trial regarding this subject consists in proving the apparition of the systemic osteoporosis in patients with psoriatic arthritis, a complication that is strongly dependent on the blood values of RANKL. As a consequence of these personal observations, it can be affirmed that monitoring he patients with psoriatic arthritis in order to identify precocious osteoporosis which can lead to fractures occurring, fractures which are feared complications due to the prolonged immobilization and the high costs they involve, represents an objective that is accessible these days and has a special importance both for the patient and the sanitary system. The personal contribution of our trial regarding this subject consists in proving the apparition of systemic osteoporosis in patients with psoriatic arthritis, a complication that is strongly dependent on the blood values of RANKL and which should be routinely monitored with these patients in order to prevent the occurrence of osteoporotic fractures, complication that involve both social and financial high costs and the determination and the monitoring of the serum levels of RANKL could represent a parameter that would allow the appreciation and quantifying of the degree of activity of the disease, as well as a selection criteria of patients who will develop severe bone erosions and who can benefit in timely of specific therapy. The precocious screening of systemic osteoporosis, which can lead to the occurrence of fractures, fractures that represent a feared complication due to the prolonged immobilization and the high costs it involves, represents an easily achievable objective these days with a special importance both for the patient and the sanitary system.

Conclusions

RANKL is the key mediator of the articular destruction and the loss of bone in psoriatic arthritis. The elevation of the serum RANKL levels represents a marker of the bone reabsorption which takes place in psoriatic arthritis. By

analyzing the results of our trial it can be concluded that there is an important raise of the RANKL blood concentration as well in both types of arthritis: psoriatic and rheumatoid, with the mention that, as expected, the rheumatoid arthritis shows a more accentuated elevation.

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