

The Impact of Proinflammatory Cytokines of Rheumatoid Polyarthritis on the Generalized Loss of Bone Mass

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There is a bidirectional interaction between most immune cells and osteoblasts, osteoclasts and their precursor cells. The receptor activator of nuclear factor- κ B ligand (RANKL)/RANK/osteoprotegerin (OPG) system plays an essential role in the formation of osteoblasts, but it also has implications in osteoclast biology and implicitly on the diseases characterized by bone loss. Proinflammatory cytokines existing at synovial level function as direct or indirect stimulators of osteoclast differentiation, but also of its survival or activity, although some cytokines may also play an antiosteoclastogenic role. The fate of bone destruction is determined by the balance between osteoclastogenic and antiosteoclastogenic mediators. Our study has shown that the early initiation of the therapy with anti-TNF and anti-IL6 biological agents, in patients with rheumatoid arthritis, inhibits bone destruction, regardless of the anti-inflammatory activity in patients with rheumatoid arthritis.

Keywords: rheumatoid arthritis, systemic osteoporosis, proinflammatory cytokines, RANKL/RANK/OPG

Bone remodeling is disturbed by a series of conditions that are related to the skeleton, among which the most important are postmenopausal osteoporosis and rheumatoid arthritis. In these conditions, there is a local or systemic impairment of hormone levels or of the proinflammatory cytokines that are involved in stimulating or inhibiting bone resorption [1-3]. Chronic inflammation is a key mediator for the loss of local and systemic bone mass, especially in patients with rheumatoid arthritis. In these patients, cytokines are present in high concentrations in both, synovial arthritis and systemic circulation. Some proinflammatory cytokines function as direct or indirect stimulators of osteoclast differentiation, but also of its survival or activity. In addition to their proresorptive role, some of these cytokines may also play an antiosteoclastogenic role. The fate of bone destruction is determined by the balance between osteoclastogenic and antiosteoclastogenic mediators [3].

Proinflammatory cytokines, including TNF (tumor necrosis factor), IL1, IL6, IL17, disrupt the balance between osteoclast and osteoblast activity, usually resulting in obvious bone loss. These cytokines stimulate osteoclast differentiation, by increasing the mass of osteoclast precursors, upregulating the receptor activator of nuclear factor- κ B ligand (RANKL) expression in synovial osteoblasts and/or synovial fibroblasts, and synergizing with RANKL itself. Moreover, proinflammatory cytokines can disrupt certain signaling pathways, leading to aberrant osteoblast activity [4]. In joints affected by rheumatoid arthritis, proinflammatory cytokines, predominantly secreted by macrophages, fibroblasts and lymphocytes at the level of the inflamed synovium and the panus, mediate

the erosive process, increasing thus the differentiation and activity of the osteoclasts [5].

Proinflammatory cytokines cause not only bone resorption but also contribute to bone loss by directly inhibiting osteoblast differentiation. In vitro, TNF inhibits the differentiation of osteoblasts from pluripotent progenitor cells. TNF also induces osteoblast apoptosis, but it also interacts with other proinflammatory cytokines such as IL-1 β . It has been shown that continuous osteoblast exposure to IL-1 β in vitro leads to the inhibition of their differentiation. It has recently been proved that IL-1 β affects the migration of osteoblasts to chemotactic factors [6].

The RANKL/RANK/osteoprotegerin (OPG) system is a key system in the interaction between the immune system and the bone system [7]. Genetic studies that have shown that the members of the superfamily of tumoral necrosis factors have multiple functional roles in and outside the bone. The protection of the skeleton against excessive bone resorption is achieved by binding OPG to RANKL, preventing thus its binding to its ligand or RANK [8].

Factors that stimulate the formation and activation of osteoblasts are the same that stimulate RANKL expression at the level of osteoblast or stromal cells. In inflamed joints, RANKL is expressed by synovial cells and secreted by T cells. These sources are responsible for mediating joint destruction in patients with rheumatoid arthritis. Joint destruction in patients with rheumatoid arthritis is also mediated by TNF, through the systemic growth of osteoclast precursors and the promotion of their migration from bone marrow to peripheral blood and then into inflamed joints, where it supports the fusion of these cells with osteoclasts along RANKL and IL1 [8]. Both, RANKL and TNF, stimulate

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the release of the precursor cells of immature osteoclasts into circulation [9].

The stimulation of OPG expression is accomplished by most factors that determine the expression of RANKL at the level of osteoblasts. RANKL stimulation is associated with OPG reduction, or a decrease in OPG induction, so that the RANKL/OPG ratio changes in favor of osteoclastogenesis, a major determinant of bone mass. The role of OPG is osteoprotective [8].

The RANKL/RANK/OPG system plays an essential role in osteoblast formation, but it is also involved in osteoclast biology and thus in diseases characterized by bone loss [10, 11]. Bone destruction in arthritis occurs in the inflamed synovium, at the interface of the immune system and the bone. The IL-17 produced by Th17 cells superegulates the RANKL expression in synovial fibroblasts and induces the production of inflammatory cytokines, such as TNF- α , IL-6 and IL-1 from synovial macrophages. These cytokines continue to regulate RANKL expression in synovial fibroblasts and activate the precursor cells of osteoclasts [12-14].

The observation of the presence of systemic inflammation as a common pathophysiology mechanism for both, rheumatoid arthritis and osteoporosis, has motivated the onset of research in an attempt to gain a better understanding of the causes that lead to the acceleration of bone loss in this category of patients, ensuring thus a better patient management.

Experimental part

Materials and methods

The study was a prospective observational case-control study, conducted between 2017 and 2018, in order to determine the cumulative risk factors underlying the early onset and progression of osteoporosis in patients with rheumatoid arthritis. The study group consisted of 93 patients with rheumatoid arthritis who were re-evaluated 6 months after enrollment.

Patient selection was based on inclusion criteria (patients with ages ranging between 20 and 65, diagnosed with rheumatoid arthritis in stage I, II or III), as well as on exclusion criteria (patients under the age of 20 and over the age of 65, patients that associate endocrine-related comorbidities).

During the two evaluations, there were recorded the biological inflammation parameters (VSH, CRP), as well as certain biological parameters that determine possible complications of the basic disease or the side effects of the underlying treatment (haematological, hepatic, renal). We performed 25-OH vitamin D dosing, in order to determine possible correlations with bone loss and the disease activity. There were also determined the immunological markers of the disease (the rheumatoid factor by Latex method, Waaler Rose and Elisa tests, as well as the total anticitrulinic and antinuclear antibodies by Elisa tests). We performed the imaging of mineral-bone density in the hip and lumbar spine, in order to test the degree of bone loss in these patients. In 86 patients, we carried out (initially and after 6 months) dosages for inflammatory cytokines: IL1, TNF α and RANKL, by ELISA tests, in order to track their dynamics over time, correlated with the progression of the inflammatory disease and, at the same time, with the degree of bone loss. Internationally recognized disease scores were carried out: DAS 28, CDAI, SDAI, HAQ.

During this time, patients were treated with classical or biological dmARDs. Most of them received supplementary ones with vitamin D after the initial evaluation, because

this study group showed high or moderate vitamin D deficits. The data was processed by using statistical functions from SPSS 18.0 at the significance threshold of 95%.

Results and discussions

Out of the 82 patients with rheumatoid arthritis, re-evaluated after 6 months, 28 (34.1%) had systemic osteoporosis. The biochemical parameters monitored upon reevaluation after 6 months presented the following characteristics: upon reevaluation, the VSH varied from 4 to 115 mm/h, recording a series of homogeneous values ($p = 1.259$); the average level was 34.39 ± 22.25 mm/h, with no significant difference from the initial moment 34.18 ± 22.25 mm/h ($p=0.834$) ($p=0.834$). After treatment, the CRP varied very widely in the range of 0.40-102 mg/dL. The average level was 7.28 ± 8.63 mg/dL, slightly lower compared to the initial moment 7.55 ± 4.06 mg/dL ($p=0.710$).

The series of values for total seric calcium, after treatment, was homogeneous ($p=0.230$), with variations in the range of 8.10-10.20 mg/dL and an average level of 9.12 ± 0.40 mg/dL, significantly lower compared to the initial moment 9.20 ± 0.48 mg/dL ($p=0.002$).

The individual values of TGP and TGO varied widely and the skewness test ($p > 2$) suggested non-normal distributions (table 1). Upon reevaluation, TGO recorded values in the range of 14.20-89 mg/dL, with an average level of 25.47 ± 11.03 mg/dL, significantly higher compared to the initial moment 23.57 ± 14.76 mg/dL ($p=0.011$).

The Skewness Test with a value above 2 ($p = 3.28$) suggests that the IL1 α value series recorded a non-normal distribution, with variations from 0.13 to 3.07 and a median of 0.60 different from the average level of 0.64 ± 0.39 . Upon reevaluation, IL1 α recorded an average level of 0.64 ± 0.36 , significantly lower compared to the initial moment 0.68 ± 0.39 ($p = 0.001$) (fig. 1).

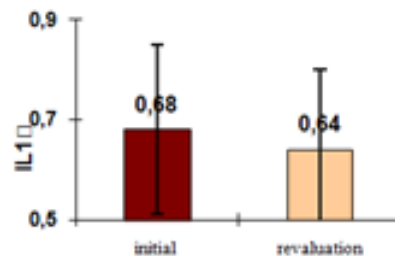


Fig. 1. Evolution of the average level of IL1 α

The Skewness Test with a value above 2 ($p = 4.26$) suggests that the TNF α value series recorded a non-normal distribution, with variations from 0 to 624.50 and a median of 0.01, much below the average level of 38.52 ± 109.41 . Upon reevaluation, the average level of TNF α remained at approximately the same level (38.70 vs 38.52; $p=0.842$) (fig. 2).

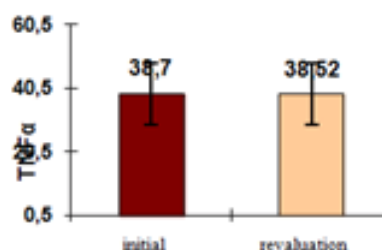


Fig. 2. Evolution of the average level of TNF α

The Skewness Test with a value above 2 ($p = 2.05$) suggests that the RANKL value series recorded a non-normal distribution, with variations from 11.87 to 130.57 and a median of 35.95 different from the average level of 39.15 ± 19.65 . uPON reevaluation, the average level of RANKL remained at approximately the same level (40.42 vs 39.15; $p = 0.055$) (fig. 3).

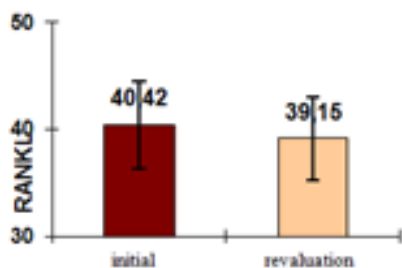


Fig.3. Evolution of the average level of RANKL

Upon reevaluation, lower levels of vitamin D and seric calcium were associated with higher HCV values (fig. 4). After 6 months, lower levels of vitamin D and seric calcium were associated with lower CRP (fig. 4).

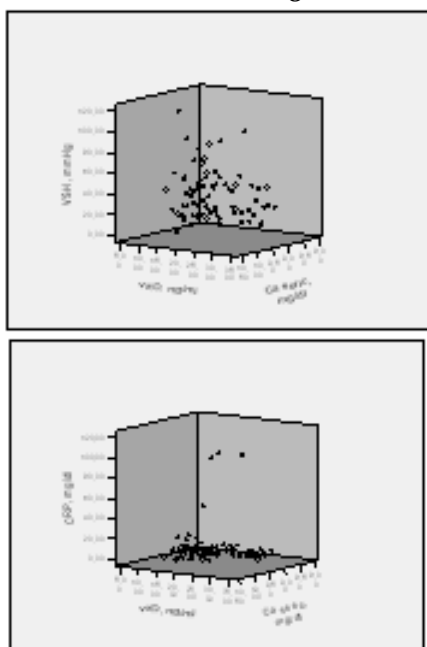


Fig. 4. Correlation of seric calcium and vitamin D with VSH vs Correlation of seric calcium and vitamin D with CRP

At the onset of the study, the correlation between seric calcium and RANKL was direct, reduced in intensity and statistically insignificant ($r = +0.132$, $p = 0.781$), an aspect which was maintained upon reevaluation ($r = +0.113$; $p = 0.708$). The correlation between vitamin D and RANKL was indirect, reduced in intensity and statistically insignificant ($r = -0.112$, $p = 0.331$), an aspect which was maintained upon reevaluation ($r = -0.129$; $p = 0.246$). Upon reevaluation, lower levels of vitamin D and seric calcium were associated with higher RANKL values.

At the onset of the study, the correlation between seric calcium and IL1 β was indirect, reduced in intensity and statistically insignificant ($r = -0.134$, $p = 0.244$), an aspect which was maintained upon reevaluation ($r = -0.104$; $p = 0.353$). The correlation between vitamin D and IL1 β was direct and reduced in intensity at the beginning of the study ($r = +0.107$, $p = 0.354$), but upon reevaluation these parameters seemed to be independent ($r = +0.027$; $p = 0.808$). Upon reevaluation, lower levels of vitamin D and seric calcium were associated with higher IL1 β values.

At the onset of the study, the correlation between seric calcium and TNF α was direct, reduced in intensity and statistically insignificant ($r = +0.145$; $p = 0.699$), but upon reevaluation these parameters seemed to be independent ($r = +0.045$, $p = 0.685$). Also, vitamin D and TNF α were apparently independent parameters at the onset of the study ($r = +0.088$; $p = 0.447$) and upon reevaluation ($r = +0.052$; $p = 0.685$). Upon reevaluation, lower levels of vitamin D and seric calcium were associated with higher TNF α values.

The RANKL - IL1 β correlation was indirect and reduced in intensity at the onset of the study ($r = -0.172$; $p = 0.134$) and upon reevaluation ($r = -0.180$; $p = 0.117$), but the results cannot be extrapolated to the general population (fig. 5.)

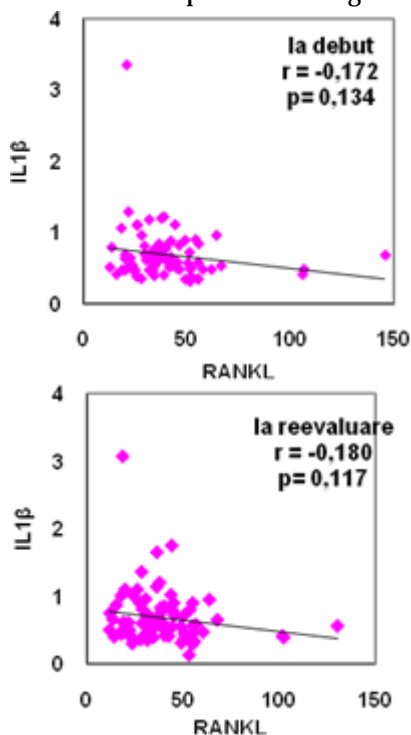


Fig. 5. RANKL - IL1 β correlation

RANKL and TNF α were apparently independent parameters at the onset of the study $r = 0.083$; $p = 0.471$) and upon reevaluation ($r = +0.091$, $p = 0.480$) (fig. 6).

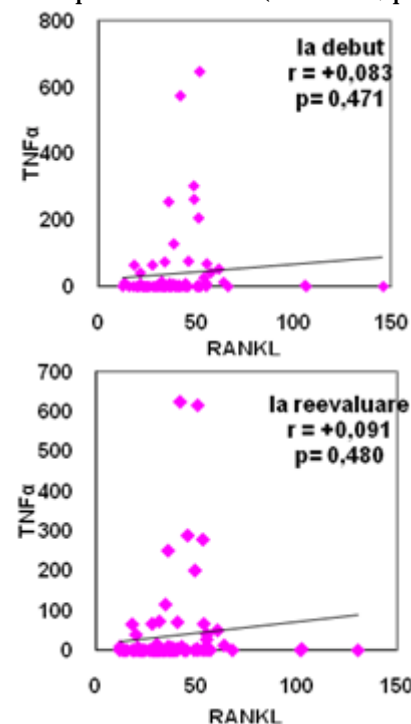


Fig. 6. RANKL - TNF α correlation

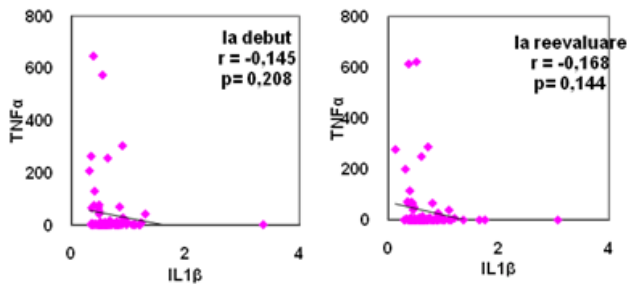


Fig. 7. IL1 β - TNF α correlation

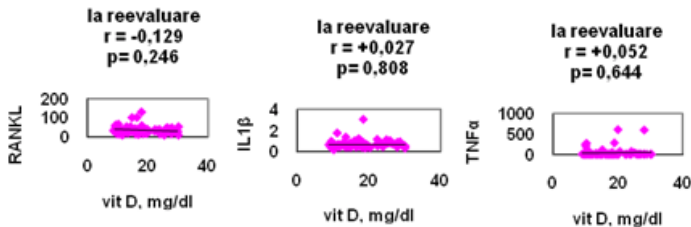


Fig. 8. Correlation of vitamin D with cytokines upon reevaluation

The IL1 β - TNF α correlation was indirect and reduced in intensity at the onset of the study ($r=-0.145$; $p=0.208$) and upon reevaluation ($r=-0.168$; $p=0.144$), but the results cannot be extrapolated to the general population (fig. 7). Upon reevaluation, vitamin D growth was accompanied by a RANKL decrease in 12.9% of patients and approximately the same level of IL1 β and TNF α . (fig. 8)

In the case of patients experiencing osteoporosis during the first evaluation, when reevaluated, the decrease in vitamin D was accompanied by a slight decrease in RANKL, IL1 β and TNF α , and a significant decrease in VSH and CRP. Upon reevaluation, the lowest average RANKL value was observed in patients treated with biological therapy, but the difference was not statistically significant ($p=0.709$).

The highest average value of IL1 β was found in patients treated with biological therapy associated with classic DMARDs and corticotherapy, but the difference was not statistically significant ($p=0.740$). The highest average TNF α value was found in patients treated with biological dMARDs associated with classic dMARDs and corticotherapy ($p=0.001$) (fig. 9).

The average cytokine level upon reevaluation was lower in patients with osteoporosis, but the only significant one from the statistical point of view was the TNF α level: (RANKL: 34.48 vs 40.51; $p=0.189$; IL1: 0.67 vs 0.68; $p=0.871$; TNF α : 21.75 vs 43.66; $p=0.04$). The series of values recorded for IL1a, TNF α and RANKL were non-homogeneous, but only the average level of IL1a significantly dropped upon reevaluation, with an improvement in the degree of inflammation.

A direct correlation between inflammatory cytokines and bone loss could not be established. In the case of patients experiencing osteoporosis during the first evaluation, when reevaluated, the decrease in vitamin D was accompanied by a slight decrease in RANKL, IL1 β

and TNF α , and a significant decrease in VSH and CRP. Upon reevaluation, the lowest average RANKL value was observed in patients treated with biological therapy, but the difference was not statistically significant.

It was established that, despite the studies showing an important deviation in the mineralous density in patients with rheumatoid arthritis, which is more frequent in older conditions, the rate of osteoporosis in the studied group was rather limited. Although we tried to analyze the involvement of other risk factors in the development of osteoporosis, which are independent of rheumatoid arthritis, such as: advanced age, menopause, immobilization or significant mobility limitation due to an inflammatory disease, the use of AINS and corticotherapy, we established that, in spite of the fact that we could not eliminate completely risk factors, the presence of systemic osteoporosis was lower than we would have expected. This result can be explained by the fact that the vast majority of patients benefited from a diagnosis in the relatively early stage of PR, as well as by the rapid onset of the immunosuppressant treatment, possibly leading to a limitation of the increase in the level of inflammatory cytokines and, implicitly, of osteoclast activation. The evaluation of inflammatory cytokines and RANKL was put in relation to other researched factors, as there is no standardization of their values.

Out of the total number of follow-up patients, we selected those, who had one or more treatment schemes that included biological agents, and it was established that this group of patients presented higher levels of bone-mineral density than those patients that were not subject to biological therapy. This fact supports those studies that show that blocking inflammatory cytokines leads to a decrease in osteoclastic activity and, implicitly, in bone resorption [15-17].

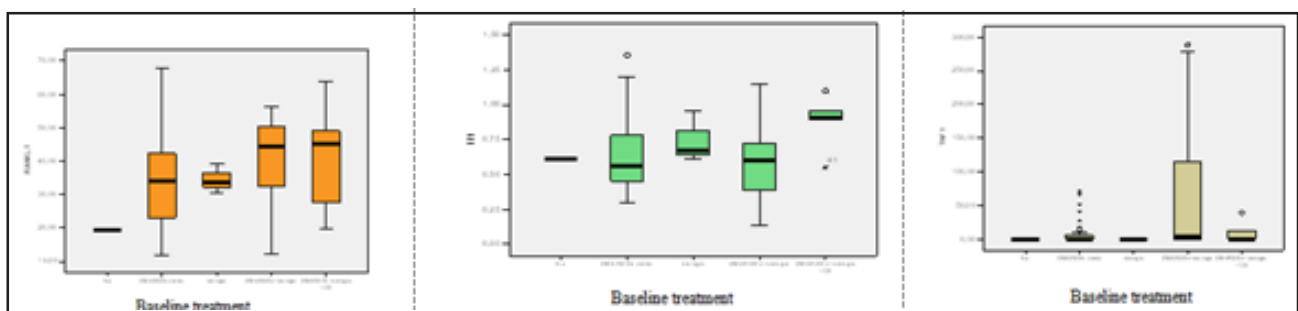


Fig. 9. Average values of RANKL, IL1 and TNF α , upon reevaluation, depending on the baseline treatment

Conclusions

Our study has shown that the early initiation of the therapy with anti-TNF inhibits bone destruction, regardless of the anti-inflammatory activity in patients with rheumatoid arthritis. In the evaluated cases, the treatment with TNF and IL6 blockers slowed down the progression of bone loss in patients with rheumatoid arthritis regardless of the duration of disease progression, the degree of activity of the disease, or the administered anti-inflammatory therapy.

In these patients, there was no systemic osteoporosis or only a slight decrease in the mineral density of bones, which is within the field of osteopenia.

Our increased interest in the pathophysiological mechanisms of bone loss in rheumatoid arthritis can lead to new therapeutic concepts on rheumatoid arthritis, including anti-erosive therapies. Despite the progress in osteo-immunology, many studies have shown that even today, a large proportion of patients with rheumatoid arthritis (up to 80%), especially those with associated risk factors, develop bone destruction during the progression of the disease. Thus, further research is necessary to completely elucidate the pathophysiology of bone loss directed by osteoclasts in patients with rheumatoid arthritis.

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