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Biologics Treatment Limits Disease Activity and Bone Metabolism in Patients with Rheumatoid Arthritis

Próchnicka RK[1,2], **Gorycka PA**[*3], **Mańczak M**[4], **Giemza FA**[2], **Wojciechowska M**[5], **Wojdasiewicz P**[1,6], **Maśliński S**[1], **Olesińska M**[2] **and Szukiewicz D**[1]

[1]*Department of General and Experimental Pathology, Warsaw Medical University, Poland*

[2]*Department and Polyclinic of Systemic Connective Tissue Diseases, National Institute of Geriatrics, Rheumatology and Rehabilitation, Poland*

[3]*Department of Biochemistry and Molecular Biology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Poland*

[4]*Department of Epidemiology and Health Promotion, National Institute of Geriatrics, Rheumatology and Rehabilitation, Poland*

[5]*Central Clinical Laboratory, National Institute of Geriatrics, Rheumatology and Rehabilitation, Poland*

[6]*Departament of Rheumoorthopedic Surgery, National Institute of Geriatrics, Rheumatology and Rehabilitation, Poland*

[*]**Corresponding author**: Gorycka PA, Department of Biochemistry and Molecular Biology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warszawa, Poland, Tel: +48 22 844-57-26; E-mail: paradowska_aga@interia.pl

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Abstract

Introduction: Generalized bone loss in rheumatoid arthritis (RA) is multi-factorial, and can be divided into erosions of the joint and osteoporosis. Biomarkers of bone remodeling provide a dynamic view of the remodeling process and they contribute to a better understanding of bone physiology and the pathogenesis of metabolic bone diseases. To examine whether treatment with biologics agents reduces of disease activity and changes the markers of bone metabolism, including osteocalcin (OC), N-terminal propeptide of type I procollagen (PINP), cross-linked C-terminal telopeptide of type I collagen (CTX), receptor activator of the NF-κB ligand (RANKL) and osteoprotegerin (OPG).

Materials and methods: 125 patients with active RA, who were treated with DMARDs (44 patients) and biologics agents including 49 patients on Etanercept, 16 patients on Adalibumab/Golibumab/Infliksimab and 16 patients on Rytuksymab/Tocilizumab were included in this study. Clinical and laboratory parameters of disease activity were measured at baseline and after 90 days of treatment and an index of disease activity (DAS-28) calculated. Changes in bone markers levels were measured by ELISA before start the treatment and after.

Results: As a result of treatment of the active RA with biologics agents, after 90 days, parameters such as DAS-28, VAS, number of swollen/tender joints, CRP and ERS decreased in the biologics group compared to the DMARDs group. The sRANKL/OPG ratio, and carboxylated (GLA)–OC and CTX levels were higher in RA patients compared to healthy subjects ($p<0.001$, $p=0.02$ and $p=0.002$, respectively). DMARDs therapy decreased the CTX level and sRANKL/OPG ratio in RA patients ($p=0.006$ and $p=0.06$, respectively). OPG was increased, whereas the sRANKL/OPG ratio was decreased in RA patients after 90 days biologics treatment.

Conclusion: The biologics therapy has protective effect against clinical disease activity as well as joint damage compared to conventional DMARDs in patients with RA.

Keywords DMARDs; Biologics agent; Rheumatoid arthritis; Bone remodeling markers; Disease activity; Bone loss; Joint erosion

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune connective tissue disease associated with a high prevalence of periarticular bone loss in the joints and chronic inflammation resulting in juxta-articular and generalized osteoporosis [1,2]. Osteoporosis, caused by a dysregulation of normal bone homeostasis, is one of the most important complications in patients with RA, which increased the risk of hip, vertebral fractures, reduced bone mass and microarchitectural deterioration of bone tissue [3,4]. The etiology of bone loss in RA patients is multifactorial and it may be mediated by a generalized pro-inflammatory state, high frequency of glucocorticoid use, relatively low levels of physical activity, classic risk factors for osteoporosis, as well as the disease itself, particular when uncontrolled [3,5,6]. Furthermore, the pathophysiological mechanisms of the bone remodeling are results of a disturbed balance between the processes of formation and resorption of the osseous tissue [3,4,7]. The intensity of these processes may be evaluated using biochemical markers of bone turnover/metabolism that are produced or released during bone remodeling.

Biomarkers of bone remodeling, which include bone formation markers such as osteocalcin (OC) and N-terminal propeptide of type I procollagen (PINP) and bone resorption markers such as cross-linked C-terminal telopeptide of type I collagen (CTX) provide a dynamic view of the remodeling process and they contribute to a better understanding of bone physiology and the pathogenesis of metabolic bone diseases [5,8]. Bone formation markers are direct or indirect products or enzymes of active osteoblasts, whereas bone resorption markers are resorption products of bone collagen [5]. Furthermore, they can be useful in predicting risk of fracture and bone loss,

monitoring the short-term effects of therapy, and indicating if an excessive slowing of the remodeling process is occurring [8].

Among mechanisms involved in bone remodeling, the complex system receptor activator of the NF-kB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG), which plays an important role in bone and immunity–significantly contributing to the emergence of the field of osteoimmunology–as well as organogenesis and disease conditions including RA, is the coupling factor between bone resorption and formation. In this system, OPG functions as a high-affinity soluble decoy receptor for RANKL and competes with RANK for RANKL binding. Therefore, OPG is an effective inhibitor of the effects of RANK–RANKL interactions [9,10]. Ultimately, the balance between RANKL and OPG determines the degree of activation and differentiation of the osteoclasts and regulate T cell/dendritic cell communications. Therefore, targeting the RANK/RANKL/OPG system may be effective in preventing bone damage in RA patients [1,5,11-13].

In the past decade, the most important advance has been the development of biological response modifiers or biologics, which are the newest class of drugs preventing partial or even total articular erosion in RA patients [4,5]. These drugs are directed against several proinflammatory cytokines involved in the pathogenesis of chronic inflammation, progression of joint structural damage and bone loss. However, the role of these drugs in preventing bone loss in clinical practice has not yet clearly assessed [14]. Therefore, the aim of this study was to determine the biomarkers of bone remodeling in the patients with RA in relation to disease activity and to investigate how expression of these molecules might change following biological treatment.

Materials and Methods

Study population

This study included a total of 125 patients with RA (115 (92%) women and 10 (8%) men) and of 42 healthy individuals. All patients fulfilled the American College of Rheumatology (ACR 2010) criteria for RA. Patients with RA were recruited at the Connective Tissue Diseases Department and Biological Therapy Center of the Institute of Rheumatology in Warsaw.

Formal consent was obtained from all patients. In terms of the treatment RA patients was divided into several subgroups. First group included 44 patients on disease-modifying anti-rheumatic drugs (DMARDs) standard therapy. Second group included 49 patients on combine therapy with Methotrexate and anti-TNF-α drug (Etanercept). Third group included 16 patients on combine therapy with Methotrexate and Adalibumab or Golibumab or Infliksimab. Fourth group included 16 patients on combine therapy with Methotrexate and CD20 blocker (Rytuksymab) or inhibitor IL-6 (Tocilizumab).

The control group consisted of 42 anonymous healthy volunteers (81% women *vs.* 19% men, range 18-65 years) who did not show any clinical or laboratory signs of autoimmune diseases. They were randomly selected from blood bank donors. Patients and control subjects had the same ethnicity, socioeconomic status and were from the same geographical area. They were Polish Caucasian.

The study was approved by Research Ethics Committee of the Institute of Rheumatology in Warsaw.

Demographics and disease activity data

A clinical study was conducted prior to initiation of therapy and during therapy about 90 days. All patients signed an informed consent, clinical and biochemical data were collected from patient's files and questionnaires and summarized in Table 1.

RA activity was assessed at 0 and 90 days of therapy by a disease activity score for 28 joints (DAS-28), which included the number of swollen and tender joints and erythrocyte sedimentation ratio (ESR); by a visual analogue scale (VAS): range 0–100 and patients global status and by the functional disability, which was calculated using the Health Assessment Questionnaires (HAQ): range 0–3. Levels of quantification C-reactive protein (CRP), platelets (PLT) and creatinine was also determined by standard methods. Furthermore, presence of rheumatoid factor (≥ 34 IU / ml) was determined by the nefelometric method, and presence of anti-CCP antibodies (≥ 17 U/ ml) was determined using commercial kits (Elecsys Anti-CCP assay; Roche Diagnostics GmbH, Basel, Switzerland) with measuring range 7–500 U/ml. The radiological progression was assessed by a Larsen method. These radiographs were evaluated by radiologists twice, once before treatment and second time after about one year.

Markers of bone metabolism

In a total of 125 patients serum samples were available for evaluation. Blood samples from all patients were taken in the morning after overnight fasting, separated immediately and stored at -70°C until analysis. Markers of bone turnover were measured at 0 and 90 days in 39 patients on DMARDs and after 90 days of therapy in the remaining RA patients (patients from 2 to 5 groups).

Bone formation was measured by OC and bone resorption was examined by CXC and PINP using ELISA kit (Takara Bio Inc. Japan) and an electrochemiluminescence immunoassay (Roche, Switzerland). Levels of osteoclast-regulating proteins, OPG and sRANKL, were determined using an ELISA kit from Biovendor Research and Diagnostics Products (Czech Republic). Intra- and inter-assay coefficients of variation were less than 7% and 10%, respectively.

Statistical analysis

Normality of the distribution of continuous variables was assessed using the Shapiro-Wilk test. Most of the variables were not normally distributed. Continuous variables were presented as the median and minimum and maximum values, categorical variables as number and percentage Differences between groups were assessed using: 1) Mann-Whitney test for two independent groups 2) Kruskal-Wallis test for more than two independent groups 3) Wilcoxon test for two related groups. Differences were considered statistically significant if p-value was <0.05.

Parameter	DMARDs group N=44	Infliksymab/Adalibumab/ Golibumabie Group N=16	Etanercept group N=49	Rytuksymab/Tocilizumab group N=16	P
	median (min-max)				
Age [years]	57 (22-77)	65.5 (41-79)	53 (22-76)	55 (34-67)	0.029
Disease duration [years]	10.5 (3-42)	16 (5-23)	8 (0-45)	11.5 (3-34)	0.035
Larsen	3 (1-5)	4 (2-5)	3 (1-5)	3.25 (1-5)	0.314
HAQ	1.94 (0.25-2.88)	1.94 (0.38-2.63)	1.13 (0-2.88)	0.63 (0.25-1.70)	0,002
Hemoglobin [g/dL]	12.7 (8.6-15.7)	12.46 (9.2-14)	12.2 (8.6-15.4)	12.35 (9.5-14.4)	0.663
PLT [$x10^3/mm^3$]	299 (140-626)	273 (173-948)	308 (174-704)	313 (156-641)	0.570
Creatinine	0.75 (0.5-1.14)	0.7 (0.5-1)	0.7 (0.5-2.6)	0.7 (0.5-1.1)	0.078
DAS-28-1	5.7 (4.0-7.1)	6.3 (3.7-8.0)	6.4 (2.6-8.2)	5.7 (4.5-7.6)	0.006
number of tender joints -1	10 (3-20)	17 (1-28)	16 (0-28)	11 (1-21)	0.008
number of swollen joints - 1	6 (1-18)	12 (1-20)	9 (0-20)	5 (3-20)	0.241
VAS [mm]- 1	50 (30-83)	77 (8-91)	66 (6-100)	55 (17-96)	0.030
ESR [mm/h] - 1	28.5 (2-109)	33 (7-53)	36 (7-106)	43.5 (3-86)	0.079
CRP [mg/L] - 1	13 (1-119)	17 (6-69)	17 (0.1-164)	26 (1-115)	0.891
DAS-28-2	4.1 (2.6-7.0)	3.3 (2.1-7.8)	4.0 (1.5-6.7)	2.9 (1.5-6.9)	0.005
number of tender joints -2	3 (1-16)	2 (0-22)	5 (0-23)	2 (016)	0.126
number of swollen joints - 2	3 (010)	1 (016)	1 (0-19)	0 (010)	0.0008
VAS [mm]-2	40 (10-89)	30 (7-80)	34 (6-90)	27 (9-82)	0.079
ESR [mm/h] - 2	22 (1-76)	26 (2-64)	19 93-96)	15 (1-78)	0.327
CRP [mg/L] - 2	8 (0.1-111)	9 (0.3-46)	7 (0.1-49)	6 (0-19)	0.460
	n (%)				
Women	31 (80%)	15 (94%)	48 (98%)	16 (100%)	0.008
Organ symptoms	33 (85%)	16 (100%)	39 (80%)	16 (100%)	0.124
anti-CCP presence	21 (70%)	13 (87%)	31 (76%)	11 (73%)	0.676
RF presence	31 (82%)	14 (87.5%)	43 (90%)	15 (94%)	0.583
Osteoporosis	12 (31%)	9 (60%)	20 (41%)	10 (62.5%)	0.168
Patients on glucocorticoid	31 (80%)	14 (87.5%)	44 (90%)	11 (69%)	0.208
Patients on methotrexate	13 (33%)	9 (56%)	38 (76%)	15 (94%)	<0.0001
Patients on sulfasalazin	11 (28%)	8 (53%)	23 (47%)	4 (25%)	0.119
1: values of clinical parameters before therapy; 2: value of clinical parameter after 90 days therapy					

Table 1: Demographic, clinical and biochemical characteristics of RA patients treated with different-drug regimens.

Results

RA patients clinical characteristic

To demonstrate the correlation between markers of osteoporosis and the effects of the biological therapy we divided RA patients for four groups. Changes in measures of disease activity of RA patients treated with different-drug regimens were presented in Table 1.

When the four groups were compared Larsen score, mean value of hemoglobin and PLT as well as number of swollen joints mean value of ESR and CRP measured at baseline were not different between study groups and making the groups of homogeneous.

The mean disease activity in RA patients measured before starting biologics therapy was higher in patients classified for biological treatment than in those who stayed on the standard therapy. DAS-28, number of tender joints and VAS were significantly higher in the biologics group compared to DMARDs group (p=0.007, p=0.008 and p=0.03, respectively). Moreover, number of swollen joints, CRP and ERS were also higher in the biologics group than in DMARDs group, although these differences were not statistically significant. As a result of treatment of the active RA with biologics agents, after 90 days, all these parameters decreased in the biologics group compared to the DMARDs group.

Comparative evaluation levels of biochemical markers of bone remodeling in RA patients and control group

The results of our study showed that the markers of bone remodeling were higher in RA patients compared to healthy subjects (Table 2). The sRANKL/OPG system levels were significantly higher in RA patients than in controls (p<0.001). A higher concentration of carboxylated (GLA)–OC and CTX in the serum of RA patients was found, compared to the values determined in healthy subjects (p=0.02 and p=0.002, respectively). Moreover, both undercarboxylated (GLU)-OC and PINP were also higher in RA patients compering with control group, however, these differences were not significant.

Parameter	RA patients	Controls	P
	Median (Min–Max)	Median (Min–Max)	
OPG [pmol/l]	5.59 (1.23–23.10)	4.47 (2.07–10.94)	0.001
sRANKL [pmol/l]	6.05 (0.17–73.79)	1.99 (0.55–12.84)	<0.0001
sRANKL/OPG [pmol/l]	1.42 (0.02–12.87)	0.44 (0.14–1.41)	<0.0001
GLA–OC [ng/ml]	7.04 (0.39–61.72)	5.78 (0.26–13.77)	0.025
GLU–OC [ng/ml]	2.55 (0.24–9.74)	2.10 (0.28–7.10)	0.110
CTX [ng/ml]	0.25 (0.03–0.80)	0.16 (0.08–0.35)	0.002
PINP [µg/L]	41.20 (6.61–135.70)	34.97 (10.06–105.20)	0.283

Table 2: Bone metabolic markers in RA patients and healthy subjects.

Evaluation of levels of bone remodeling markers in relation to DMARDs therapy

In our study we want to check if the different therapy may influence on the levels of the bone remodeling markers in patients with RA. That's way; we first compared the levels of these markers in RA patients on the DMARDs therapy at 0 and at 90 days (Table 3). The marker for bone resorption, CTX, was appreciable decreased at 90 days compared with baseline (p=0.007). Furthermore, the levels of osteoclast-regulating proteins, sRANKL/ OPG system, showed a tendency to decreased at 90 days compared with baseline (p=0.06). Analysis of the other markers of bone metabolism showed no statistically significant differences between both groups at baseline and after 90 days of DMARDs therapy). Moreover, we also observed that OPG as well as GLU-OC was increased at 90 days compering with baseline, although these differences were not significant.

Parameter	RA patients before therapy (at 0 days)	RA patients after DMARDs therapy (at 90 days)	P
	Median (Min–Max)	Median (Min–Max)	
OPG [pmol/l]	4.06 (1.90–9.27)	4.78 (1.23–10.80)	0.442
sRANKL [pmol/l]	10.48 (0.45–68.12)	7.90 (0.70–73.79)	0.129
sRANKL/OPG [pmol/l]	2.90 (0.07–22.74)	1.94 (0.16–12.87)	0.062
GLA–OC [ng/ml]	5.97 (0.65–55.64)	6.47 (0.39–17.46)	0.729
GLU–OC [ng/ml]	3.23 (0.47–12.59)	3.10 (0.34–9.74)	0.376
CTX [ng/ml]	0.25 (0.04–0.96)	0.19 (0.03–0.80)	0.006
PINP [µg/L]	40.45 (10.74–164.80)	35.62 (6.61–127.70)	0.372

Table 3: Changes in bone metabolic markers in RA patients on the DMARDs therapy at 0 and at 90 days (N=44 patients in both groups).

Effects of etanercept therapy on bone markers levels in RA patients

The effect of Etanercept therapy on the serum levels of bone formation/resorption markers was shown in Table 4. Serum level of OPG in RA patients after 90 days of treatment was significantly higher than at baseline (p<0.001). On the other hand, we observed that sRANKL/OPG ratio was reduced after 90 days compared to baseline (p=0.05). Other serum markers concentrations didn't changed after Etanercept therapy.

Parameter	RA patients on DMARDs therapy	RA patients on Etanercept therapy	P
	Median (Min–Max)	Median (Min–Max)	
OPG [pmol/l]	4.06 (1.36–9.27)	5.60 (2.14–23.10)	0.0006
sRANKL [pmol/l]	10.02 (0.45–68.12)	7.88 (0.17–66.65)	0.282
sRANKL/OPG [pmol/l]	2.96 (0.07–22.74)	1.59 (0.02–9.82)	0.051
GLA–OC [ng/ml]	5.81 (0.6 –55.64)	8.43 (0.91–61.72)	0.430
GLU–OC [ng/ml]	3.10 (0.47–12.59)	2.55 (0.26–7.00)	0.101
CTX [ng/ml]	0.26 (0.04–0.96)	0.26 (0.07–0.72)	0.830
PINP [µg/L]	41.30 (10.74–164.80)	40.80 (11.93–135.70)	0.433

Table 4: Changes in bone metabolic markers in RA patients on the DMARDs therapy (N=44) and in RA patients on Etanercept therapy (N=49) after 90 days.

Effects of Adalimumab/Golimumab/Infliksimab therapy on bone markers levels in RA patients

In this study we observed that the levels of bone metabolism markers in serum of patients with RA changed after 90 days of Adalibumab or Golibumab or Infliksimab (Table 5). The markers such as sRNAKL, sRANKL/OPG ratio, GLU-OC decreased, whereas the OPG, GLA-OC, PINP and CTX increased after 90 days compared to baseline. However, only the changes in the serum levels of the OPG and sRANKL/OPG ratio were statistically significant (p=0.002 and p=0.04, respectively).

Parameter	RA patients on DMARDs therapy	RA patients on Adalibumab/Golibumab/ Infliksimab therapy	P
	Median (Min–Max)	Median (Min–Max)	
OPG [pmol/l]	4.06 (1.36–9.27)	6.81 (3.85–10.82)	0.002
sRANKL [pmol/l]	10.02 (0.45–68.12)	5.26 (1.80–42.30)	0.063
sRANKL/OPG [pmol/l]	2.96 (0.07–22.74)	0.78 (0.57–3.91)	0.043
GLA–OC [ng/ml]	5.81 (0.65–55.64)	16.56 (3.75–37.63)	0.067
GLU–OC [ng/ml]	3.10 (0.47–12.59)	1.84 (0.31–5.20)	0.059
CTX [ng/ml]	0.26 (0.04–0.96)	0.29 (0.10–0.69)	0.887
PINP [µg/L]	41.30 (10.74–164.80)	50.06 (12.50–101.50)	0.284

Table 5: Changes in bone metabolic markers in RA patients on the DMARDs therapy (N=44) and in RA patients on Adalibumab/Golibumab/ Infliksimab therapy (N=16) after 90 days.

Effects of Rytuksymab/Tocilizumab therapy on bone markers levels in RA patients

When we compared RA patients at baseline and after 90 days on Rytuksymab or Tocilizumab therapy we found that only the levels of osteoclast-regulating proteins, sRANKL/OPG system showed statistically significant differences between examined groups (Table 6). OPG was increased (4.06 pmol/l *vs.* 6.39 pmol/l; p=0.003), whereas sRANKL (10.02 pmol/l *vs.* 2.97 pmol/l; p=0.003) and sRANKL/OPG ratio (2.96 poml/l *vs.* 0.48 pmol/l; p=0.001) were decreased in RA patients after 90 days therapy. Serum levels of other bone formation/ resorption markers didn't changed after 90 days drug/s application (Table 6).

Discussion

Our study showed that osteoporosis is a common finding in RA patients: 41.6% of RA patients had osteoporosis at one or more measured sites. Our results are contrast to some previous studies, which found a lower prevalence of osteoporosis in RA (from 22% to 36%) [15], whereas in accordance with other [7]. These differences might be explained by the multifactorial causes of osteoporosis, disparate ways of recruiting the study group, different reference populations and by the type of therapy. The factors, which have an influence on osteoporosis in RA patients, are: disease activity, seropositivity, aging, medication such as corticosteroids and methotrexate, low calcium intake or vitamin D deficiency [15].

The main finding of this study was that the serum levels of RANKL and RANK/OPG ratio were lower in RA patients treated with

biological agents than in DMARDs RA patients. Moreover, we also observed that in RA patients treated with biological agents the serum levels of OPG and GLA-OC was elevated. To the best our knowledge, this study is the first clinical research showing relationship between different biologic therapy and serum levels of bone metabolism markers in patients with RA. The individual groups of RA patients did not differ from each other in the following parameters: the radiological destructions of the disease, organ damage, presence of anti-CCP and RF, the average use of GKS, and co-occurrence with osteoporosis and osteopenia. This makes the treatment groups relatively homogeneous.

Based on a small but homogeneous group of RA patients, we found an increase of serum RANKL, OPG, RANKL/OPG ratio, GLA-OC and CTX in patients compared to controls. The results presented here are in line with a previous study showing higher levels of RANKL, GLA-OC and CTX in RA patients than in healthy subjects [16-23]. On the other hand, our results are inconsistent with the data showing the lower serum levels of OPG and the RANKL/OPG ratio in patients with RA comparing to controls [19-23]. We concluded that the increased local production of OPG, which is a naturally occurring decoy receptor for RANKL, may be a reflection of active inflammatory processes in patients with RA. Moreover, the increased levels of RANKL in inflamed joints lead to a high RANKL/OPG ratio, reflecting bone destruction, which is predictive of increased radiological progression. It has been also suggested that the RANKL/OPG ratio is a more important than single OPG and/or RANKL in regulating osteoclast formation and bone destruction. Additionally, a high RANKL/OPG ratio can help to estimate the severity of the disease and indicate the need for aggressive treatment. Studies from the last decade confirmed that the RANKL not only plays a part in physiological bone remodeling, but primarily occupies an important role in pathological processes [24]. Moreover, CTX, a degradation product derived from type I collagen, which is more specific to bone resorption than other measurements, have been shown to predict subsequent radiographic progression [25,26]. It is therefore fair to assume that RA patients with high disease activity will show elevated CTX-1 level. The discrepancies in the results associated with unregulated levels of bone formation markers (smaller, bigger, and unchanging) may be a result an imbalance of osteosis and osteogenesis processes in the course of RA, indicating a predominance of destructive processes over bone reabsorption. Moreover, Nakamura and Tanaka [27] studies suggested that pro-inflammatory cytokines such as TNF-α and IL-1 are primary pathological particles in stimulation of osteoclasts in rheumatoid arthritis, leading to bone destruction. The proinflammatory cytokines in the inflamed joints increase RANKL and OPG production by different cells including synoviocytes, osteoblasts, epithelial cells, T cells and B cells, thus promoting osteoclast development and subsequent local bone destruction [28,29]. The previous studies have shown that the markers CTX with RANKL/OPG complement each other and reflect different disease mechanisms. CTX reflecting generalized bone loss, whereas a RANKL/OPG ratio reflecting inflammation-driven, local bone loss around the joints [30].

At present, methotrexate (MTX) are one of the first-line drugs commonly used in RA therapy owing to its well-established efficacy and cheapness. MTX is indeed highly effective in inducing and maintaining disease remission, especially when used early in the course of disease, and when combined with biological DMARDs contributes to higher effectiveness, less drug immunogenicity and longer biological retention rates [31]. Our finding that chronic treatment with DMARDs reduces serum RANKL/OPG ratio and CTX levels is consistent with previous reports that these drugs affect bone remodeling and probably lead to reduction of inflammation [24,32,33]. It was found that MTX suppressed the expression of RANKL mRNA and protein, whereas increased the secretion of OPG, what is also agreement with our observation. Therefore, these authors suggested that MTX inhibits osteoclast formation in a dose-dependent manner, probably due to the modulation of the RANKL/OPG ratio since no direct osteoclast cytotoxicty was detected [34]. Our results also confirm observation that MTX through inhibiting the differentiation of early osteoblastic cells lead to suppression of disease activity, bone formation and reduction of joint damage (Table 1).

Biological therapy represents the most effective therapeutic modality available to patients with RA. Biologics usually work quickly to relieve the symptoms and swelling associated with RA. Joint destruction is also prevented, and good influences on bone remodeling are expected. Therefore, we set out to show that biological agents such as Etanercept, Infliximab, Adalibumab, Golibumab, Rytuksymab and Tocilizumab decreased bone remodeling markers and inhibits bone destruction highly effectively compering with traditional treatment. In the pilot study, Catrina et al. [35] demonstrated that TNF antagonists (such as Etanercept and Infliximab) in RA patients are able to modulate the RANKL/OPG ratio through upregulation of synovial OPG expression. In this study we observed that all used biological therapy reduce sRANKL/OPG ratio, but increased serum levels of OPG in RA patients after 90 days of treatment. Our results were similar to Isidoro Gonza'lez-Alvaro et al. study. They confirmed increase OPG serum level in 75 RA patients, receiving infliximab or adalimumab [36]. It should be also emphasized that the sRANKL levels in patients on a standard treatment was higher than those treated with biological agents.

Another very important finding in this study is that the largest decrease in the level of sRANKL and RANKL/OPG ratio as well as reduction in disease activity was achieved after application of Rituximab or Tocilizumab. Rituximab is a chimeric anti-CD20 B cell particle drug that demonstrates efficiency in inhibiting the progression of radiological [37,38]. However, the potential impact of rituximab on the bone metabolism is poorly documented. The results presented here are opposite to pilot study that assessed sRANKL and OPG level, before and after treatment with Rituximab. They concluded that a depletion of B cells by therapy with Rituximab negatively influences bone remodeling, because this therapy had no effect on the levels of bone remodeling markers [39]. Therefore, this is a pilot study showing correlation between RANKL/OPG system and Tocilizumab therapy. We have shown that Tocilizumab therapy reduced RANKL/OPG ratio and raise the OPG levels, which could also contribute to the radiographic benefit. In addition to the radiographic benefits, Tocilizumab and/or Rituximab improve signs and symptoms as well as functional evaluation with HAQ, whereas decreases of the disease activity. Nishimoto et al. [40] reported in the SAMURAI study, that Tocilizumab monotherapy was generally well tolerated and provided radiographic benefit in patients with RA. The results of this study confirmed that IL6 blockade can inhibit the osteoclast activation in RA. We concluded that a depletion of B cells and IL-6 pathway by therapy with Rituximab and/or Tocilizumab, respectively, positively influences bone remodeling, because this therapy had effect on the levels of bone remodeling markers. Moreover, both drugs have been proven efficient in reducing clinical signs and systemic inflammation in rheumatoid arthritis.

In conclusion, this study clearly demonstrates the superiority of biologics therapy in preventing clinical disease activity as well as joint

damage compared to conventional DMARDs in patients with RA. Thus, it is highly possible that these drugs may limit the risk of osteoporosis in RA patients. However, future investigation into the therapeutic effect on bone remodeling markers levels and disease parameters are also recommended in larger series.

Parameter	RA patients on DMARDs therapy	RA patients on Rytuksymab/Tocilizumab therapy	P
	Median (Min–Max)	Median (Min–Max)	
OPG [pmol/l]	4.06 (1.36–9.27)	6.39 (3.50–11.99)	0.003
sRANKL [pmol/l]	10.02 (0.45–68.12)	2.97 (0.40–52.91)	0.003
sRANKL/OPG [pmol/l]	2.96 (0.07–22.74)	0.48 (0.06–8.41)	0.002
GLA–OC [ng/ml]	5.81 (0.65–55.64)	22.13 (1.29–27.34)	0.371
GLU–OC [ng/ml]	3.10 (0.47–12.59)	2.73 (0.24–9.34)	0.701
CTX [ng/ml]	0.26 (0.04–0.96)	0.22 (0.09–0.59)	0.747
PINP [µg/L]	41.30 (10.74–164.80)	40.90 (21.90–118.00)	0.319

Table 6: Changes in bone metabolic markers in RA patients on the DMARDs therapy (N=44) and in RA patients on Rytuksymab/Tocilizumab therapy (N=16) after 90 days.

Competing Interests

All authors state that they have no conflict of interest related to this work.

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