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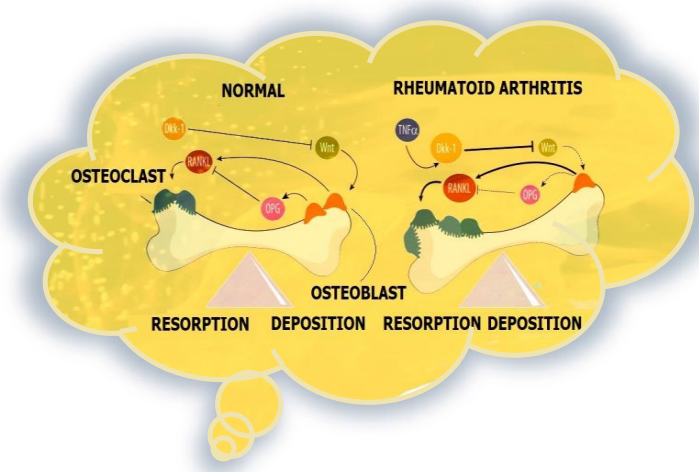

PHD THESIS - SUMMARY

Study of the Modulation Mechanisms Involved in the Early Onset of Systemic Osteoporosis due to Rheumatoid Arthritis

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PERSONAL CONTRIBUTIONS

MOTIVATION AND OBJECTIVES OF THE STUDY

Motivation of the study: the noticing of the presence of systemic inflammation as a common pathophysiological mechanism in both rheumatoid arthritis and systemic osteoporosis represents the motivation of our research, in an attempt to gain better understanding of the causes that lead to accelerated bone mass loss in this category of patients, as well as to achieve better patient management.

The fundamental ideas underlying this research were the very high incidence of the coexistence of the two conditions, namely rheumatoid arthritis and systemic osteoporosis, which is actually much higher than the current assessment and the poorer prognosis of patients in whom the two conditions coexist.

Objectives of the study

The study attempted to evaluate the early onset of osteoporosis in patients diagnosed with rheumatoid arthritis, the connection between the extent of the inflammatory process and the degree of bone loss, as well as the need to implement osteoporosis screening for the diagnosis of inflammatory rheumatic conditions. Many studies have shown a close connection between the inflammatory process and bone mass loss.

The research objectives of our doctoral thesis aimed to evaluate and elucidate the pathophysiological mechanisms of the interrelation between the extent of the inflammatory process and the degree of bone mass loss in patients diagnosed with rheumatoid arthritis.

Our study aimed to determine the connection between the degree of activity of the inflammatory disease and the extent of bone mass loss in patients with rheumatoid arthritis who are under active outpatient follow-up at the 1st Rheumatology Clinic of the Clinical Rehabilitation Hospital of Iaşi, by monitoring disease activity and determining the

functional impairment using internationally recognized score systems (DAS 28, CDAI, SDAI, HAQ).

MATERIAL AND METHOD

Study design: our prospective case-control study was conducted between January 2017 and June 2017 and attempted to identify the cumulative risk factors underlying the occurrence and progression of osteoporosis due to rheumatoid arthritis.

The data were uploaded and processed using the SPSS 18.0 statistical functions at the 95% significance threshold.

The primary processing, i.e. the systematization of the data by centralization and grouping, led to the obtaining of primary indicators, which are presented in the form of absolute values. Based on the primary indicators, the derived indicators were obtained through different statistical comparison, abstraction and generalization procedures. The derived indicators have the role of highlighting the qualitative aspects of an ensemble, aiming at the relations between different parts of a group of patients or different characteristics, and interdependence links between variables. The following derived indicators, described by the ANOVA test, were used:

- mean value indicators: simple arithmetical mean, median, module, minimum and maximum values;
- dispersion indicators: standard deviation, variation coefficient.

The Skewness test ($-2<p<2$) studies the normality of the sequence of values (continuous variable);

The χ^2 test - nonparametric qualitative test, which compares frequency distributions;

OR - RR share ratio - relative risk;

The Kruskall-Wallis correlation compares ordinal variables of 3 or more groups;

The t-Student test - parametric test comparing the mean values recorded in 2 groups with normal distributions;

The $F_{(ANOVA)}$ test used when 3 or more groups with normal distributions are compared;

The 'Pearson' correlation coefficient (r) is the correlation of 2 variables in the same group, the direct/indirect correlation being suggested by the coefficient sign.

Study population: our study included 93 patients diagnosed with rheumatoid arthritis who are under active outpatient follow-up at the 1st Rheumatology Clinic of the Clinical Rehabilitation Hospital of Iaşi, within the national program devoted to these conditions, and included the monitoring of their disease activity under therapy and the determining of the functional impairment using internationally recognized score systems.

Their initial assessment took 3 months and consisted of clinical, paraclinical and imaging examination. The subjects were divided into 2 groups, namely:

- **Group I** of 33 patients with osteoporosis
- **Group II** of 60 patients without osteoporosis

Only patients who met the appropriate diagnostic criteria for rheumatoid arthritis were included in the study (according to the EULAR 2010 guidelines).

The positive diagnosis criteria were:

- ✓ **clinical:** related to joint involvement (number of joints affected), symmetrical involvement of the proximal metacarpophalangeal and inter-phalangeal joints accompanied by joint pain and swelling);
- ✓ **paraclinical**:
  - biochemical inflammation parameters: ESR higher than 30 mm/hour and/or C-reactive protein (CRP) higher than 0.5 mg/dl,
  - immunological parameters: present/absent rheumatoid factor, present/absent antiCCP antibodies;
- ✓ **related to the persistence of the symptoms:** more than 6 weeks.

The inclusion and exclusion criteria were:

Inclusion criteria

- positive rheumatoid arthritis diagnosis;
- adults;
- patient's informed consent to be included in the study.

Exclusion criteria

- age under 18 years;
- presence of cancer;
- presence of other inflammatory diseases;
- endocrinological comorbidities (thyroid or adrenal conditions, diabetes mellitus);
- presence of other immunological conditions.

On reassessment, the study group consisted of 82 of the 93 patients with rheumatoid arthritis included in the study at the beginning of the research, the subjects being reassessed 6 months after the first examination (the time of enrollment in the study). Only 82 patients were present for the reassessment, while the remaining 11 could not be enrolled (1 death, 5 patients were hospitalized for other conditions, 2 patients were abroad and 3 patients changed their mind about their inclusion in the research). The patients with rheumatoid arthritis were tested to determine the internationally recognized disease activity scores: DAS 28, CDAI, SDAI, HAQ, in order to be able to monitor the activity of the disease in its dynamics and its functional impairment. In these 82 patients we measured the following inflammatory cytokines: IL1, TNF-α and RANKL.

During this time, the patients under active outpatient follow-up at the 1st Rheumatology Clinic of the Clinical Rehabilitation Hospital of Iaşi were given classical or biological disease modifying drugs (dMARDS). After their initial examination, most of them were also given vitamin D supplements, because a large or moderate vitamin D deficiency was detected in this study group.

Analyzed factors: the patients included in the study groups underwent dynamic assessments of some of their demographic parameters, life style, and clinical, biochemical, hematological, immunological and radiological parameters, and

disease assessment scales based on disease activity and functional capacity impairment were developed, as follows:

- ✓ socio-demographic factors: age; gender; background; education level; profession.
- ✓ lifestyle elements: smoking; alcohol consumption; sedentary lifestyle or physical activity level.
- ✓ clinical examination: number of painful joints (NPJ); number of swollen joints (NSJ); morning stiffness (MS).
- ✓ biochemical assessment:
  - o inflammation tests (erythrocyte sedimentation rate, C-reactive protein, fibrinogen);
  - o phosphorus-calcium tests (serum calcium, urinary calcium, urinary creatinine);
  - o hepatic and renal function (alaninaminotransferase, aspartataminotransferase, serum urea, serum creatinine);
  - o vitamin D_3 dosing 25 (OH);
  - o markers of bone formation and resorption (serum alkaline phosphatase);
  - o markers of inflammation (IL-1, TNF-α), RANKL dosage.
- ✓ hematological tests: complete blood count
- ✓ immunological tests: rheumatoid factor by Latex, Waaler Rose and Elisa method, anticitrullinated protein antibodies;
- ✓ imaging examination: X-ray of both hands; X-ray of both feet; musculoskeletal ultrasound scans of both hands and feet; osteodensitometry DXA test of lumbar spine and one hip;
- ✓ assessment of disease activity and functional impairment: using DAS28, CDAI, SDAI, VAS, HAQ scores.

RESULTS

Epidemiological characteristics

Classification on age groups at the beginning and on reassessment

Classification on age groups was homogeneous (Fig. 21). Most

patients were included in the 60-69 years (37.6%) and 50-59 years (33.3%) age groups. 12.9% of the patients were aged 70 and over. On reassessment, case distribution on age groups was also homogeneous (Fig. 23): age ranged between 22 and 82 years, with a median of 58.50 years and a Skewness test result p = -0.663.

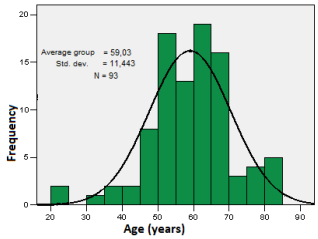


Fig. 21. *Initial patient distribution on age groups*

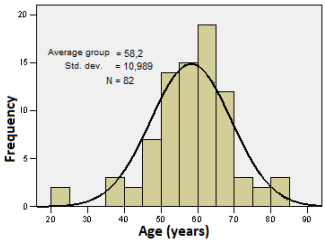


Fig. 23. *Patient distribution on age groups on reassessment*

Lifestyle characteristics

Smoking

We found that only 18.3% of the patients in this study group had been smokers, of whom 10.8% had been moderate smokers and only 2.2% heavy smokers (Fig. 31).

Alcohol consumption

Only 9.7% of the patients declared that they drank alcohol, of whom 6.5% mild alcohol intake and 3.2% moderate alcohol intake (Fig. 33).

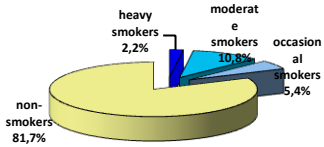


Fig. 31. *Case distribution according to smoking habits*

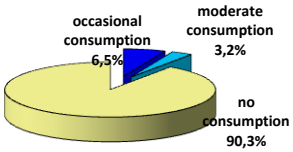


Fig. 33. *Case distribution according to alcohol consumption*

Physical exercise

Sedentary lifestyle and little physical activity were significantly correlated with the female gender (82.4% *vs.* 17.6%; p=0.046),

age group over 60 years (60.8% *vs.* 39.2%; p=0.029) and urban environment (70.6% *vs.* 29.4%; p=0.017) (Fig. 36).

Correlation of body weight assessed by BMI with osteoporosis

The mean BMI of osteoporosis patients was slightly lower (26.41 *vs.* 27.41 kg/m^2; p=0.347) (Tab. VIII).

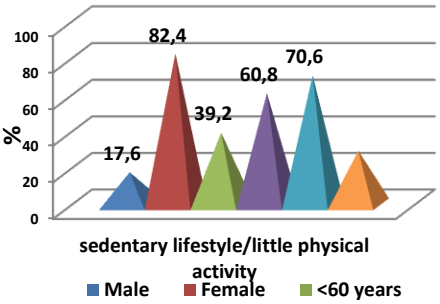


Fig. 36. *Structure of the patient group with sedentary lifestyle or little physical activity depending on their demographic characteristics*

Table VIII. Statistical BMI indicators (kg/m^2) depending on the presence of osteoporosis

Osteoporosis	N	Mean	Std. deviation	Std. error	Confidence interval		Min	Max	Chi2 testp
					-95%CI	+95%CI			
Yes	33	26.41	4.95	0.86	24.66	28.17	19.43	37.90	0.347
No	60	27.41	4.79	0.62	26.17	28.64	18.59	39.00	

Clinical parameters

Number of painful joints

NPJ is in a slight indirect correlation with the length of the disease, as a longer history of rheumatoid arthritis was associated with a lower number of painful joints in 12.8% of patients, but the result is not statistically significant (r = -0.128; p = 0.221) (Fig. 43). The mean NPJ did not differ significantly depending on the presence/absence of osteoporosis (7.97 *vs*. 9; p = 0.547) (Fig. 44).

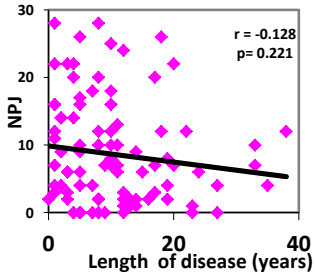


Fig. 43. *NPJ correlation with the length of rheumatoid arthritis*

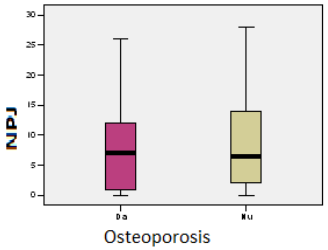


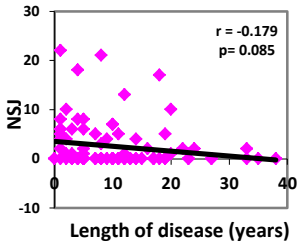
Fig. 44. *Mean NPJ depending on the presence/absence of osteoporosis*

Number of swollen joints

NSJ is in a slight indirect correlation with the length of the disease, as a longer history of rheumatoid arthritis was associated with a lower number of painful joints in 17.9% of patients, but the result is not statistically significant (r = -0.179; p = 0.085) (Fig. 46). The mean NSJ did not differ significantly depending on the presence/absence of osteoporosis (2.27 *vs*. 2.63; p = 0.722) (Fig. 47).

Visual analog scale (VAS) of pain

The VAS of pain ranged from 0 to 9. The value sequence mean was 4, which was close to the mean value of 4.7. The results of the Skewness test suggest that the VAS value sequence was homogeneous (p=0.172).



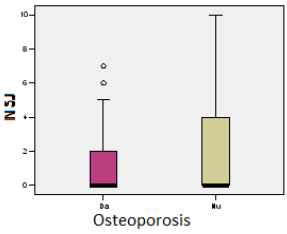
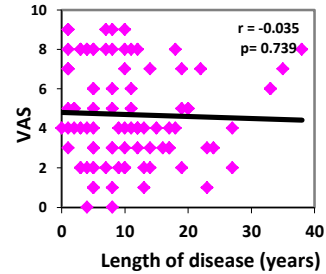


Fig. 46. *NSJ correlation with the length of rheumatoid arthritis*

Fig. 47. *Mean NSJ depending on the presence/absence of osteoporosis*

The mean VAS was 4.70 ± 2.45, with no significant differences between genders (4.67 vs. 4.71; p = 0.956), age groups (4.39 vs. 5.0; p = 0.233), backgrounds (4.47 vs. 5.12; p = 0.219), smoking (4.88 vs. 4.66; p = 0.735) or alcohol consumption (4.0 vs. 4.77; p = 0.540).

The VAS pain scale was not significantly correlated with the length of disease (r = -0.035; p = 0.739) (Fig. 49). The mean VAS level did not differ significantly depending on the presence/absence of osteoporosis (4.55 vs. 4.78; p = 0.656) (Fig. 50).



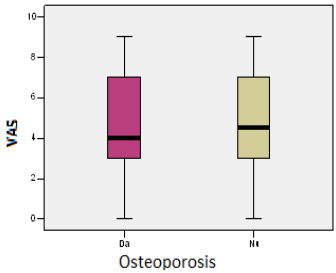


Fig. 49. *VAS correlation with the length of rheumatoid arthritis*

Fig. 50. *Mean VAS depending on the presence/absence of osteoporosis*

Disease Activity Score 28 - DAS$_{28}$

In the cases that we studied, the disease activity assessment score, DAS$_{28}$, showed a normal distribution (p = 0.450), with

variations from 1.10 to 8.22 and a median of the sequence of values of 3.79 (Table XIV). The mean DAS_{28} score was 3.97 ± 1.54, slightly higher in males (4.20 vs. 3.93; p = 0.541), over 60 years of age (3.88 vs. 4.07; p = 0.566), smokers (4.09 vs. 3.95; p = 0.726) and those who do not drink alcohol (3.61 vs. 4.01; p = 0.460).

In our study group DAS_{28} was not significantly correlated with the length of disease, although a longer length of disease was found to be associated with a lower DAS_{28} level in 15.9% of patients (r = -0.159; p = 0.129) (Fig. 52).

The mean DAS_{28} score did not differ significantly depending on the presence/absence of osteoporosis (3.81 vs. 4.07; p = 0.435) (Fig. 53).

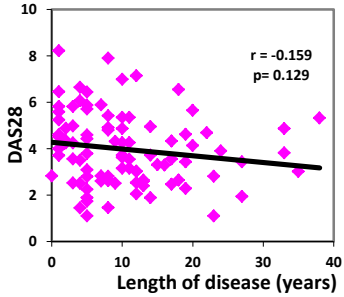


Fig. 52. *DAS_{28} score correlation with length of rheumatoid arthritis*

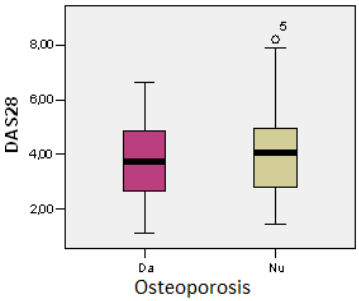
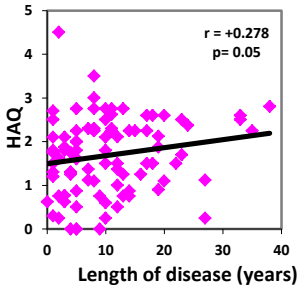


Fig. 53. *Mean DAS_{28} score depending on the presence/absence of osteoporosis*

HAQ (Health Assessment Questionnaire) functional parameter

In 27.8% of the patients in the studied group, the longer the disease, the higher the HAQ level (r = +0.278; p = 0.05); the correlation was direct, moderate in intensity, but statistically significant (Fig. 61).

The mean HAQ level did not differ significantly depending on the presence/absence of osteoporosis (1.71 vs. 1.67; p = 0.832) (Fig. 62).



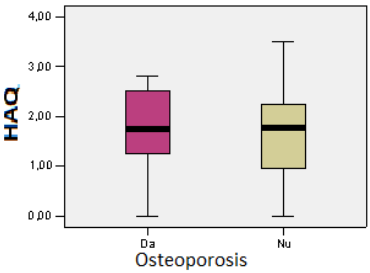


Fig. 61. *HAQ correlation with length of rheumatoid arthritis*

Fig. 62. *Mean HAQ level depending on the presence/absence of osteoporosis*

Biochemical parameters

The study of the **ESR** (erythrocyte sedimentation rate) inflammation marker showed that it ranged from 1 to 106 mm/h, recording a homogeneous sequence of values (p = 1.04), the mean level being 34.63 ± 25.12 mm/h. The **CRP** (C-reactive protein) inflammation marker study showed that it varied widely within the 0-149.3 mg/dl range, the mean level being 7.0 ± 22.63 mg/dl, i.e. much higher compared to the median value (2.80 mg / dl), and the Skewness continuity test (p = 5.18) showed a sequence of values with a non-homogeneous distribution.

The **serum calcium** value sequence was homogeneous (p= -0.03), ranging from 8 to 10.12 mg/dl and with a mean level of 9.22 ± 0.48 mg/dl.

Urinary creatinine was a variable with normal distribution (p=1.18), its values ranging between 0.42 and 2.0 g/24 h and mean level being 1.09 ± 0.26 g/24 h.

Serum creatinine also showed normal distribution (p=1.93), ranging from 0.35 to1.98 mg/dl, with a mean level of 1.09 ± 0.26 mg/dl.

Serum urea ranged from 10.60 to 95.30 mg/dl, exhibiting a homogeneous range of values (p = 1.47), the mean level being 35.69 ± 14.80 mg/dl.

Individual values of **AST** and **ALT** serum transaminases had wide variations, with the Skewness test (p> 2) suggesting non-normal distributions (Table XVIII).

Table XVIII. Statistical indicators of biochemical markers

		ESR	CRP	Serum Ca	Urinary creat.	Serum creat.	Serum urea	ALT	AST
N		93	93	93	93	93	93	93	93
Mean		34.63	7.00	9.22	1.09	0.85	35.69	26.53	23.10
Median		26.00	2.80	9.20	1.03	0.79	32.40	18.00	20.10
Standard deviation		25.12	22.63	0.48	0.26	0.28	14.80	30.63	14.05
Variance		630.9	512.2	0.24	0.07	0.08	219.0	937.9	197.5
Skewness Test		1.04	5.18	-0.03	1.18	1.93	1.47	4.37	3.65
Minimum		1.0	0.0	8.0	0.42	0.35	10.60	4.40	8.40
Maximum		106.0	149.3	10.12	2.00	1.98	95.30	236.0	111.0
Percentage	**25**	14.00	0.69	8.90	0.95	0.71	27.05	13.40	15.27
	50	26.00	2.80	9.20	1.03	0.79	32.40	18.00	20.10
	75	48.00	3.70	9.60	1.17	0.91	41.50	24.46	25.76

Immunological parameters

A positive titer was identified in 73.2% of the 82 reassessed patients, most commonly a high Elisa titer (39%), both at the beginning of the study and at the time of reassessment (Fig. 84).

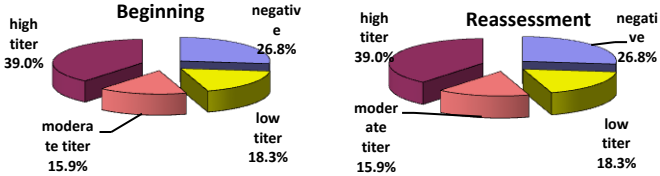


Fig.84. *Case distribution depending on the rheumatoid factor testing results by the Elisa method at the beginning of the study and at the time of reassessment*

Anticitrullinated protein antibodies

The positive titer for anticitrullinated protein antibodies was identified in 65.6% of patients, a high titer being the most common (52.7%).

Antinuclear antibodies

These antibodies were positive in 44.1% of patients, a low titer being the most common (31.2%)

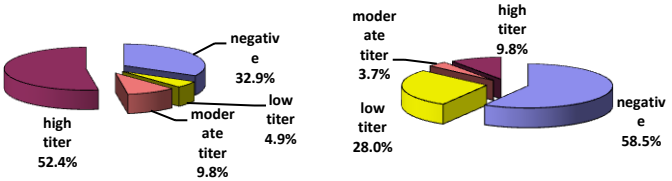


Fig. 87. *Case distribution depending on the anticitrullinated protein and antinuclear antibodies testing results*

Interleukin 1 alpha (IL-1α)

In the study group, IL-1α was not significantly correlated with the length of disease, however it should be noted that in 10.3% of patients, the longer the disease, the higher the IL-1α level (r = +0.103; p = 0.346) (Fig. 88). The IL-1α rank does not differ significantly depending on the presence/absence of osteoporosis (46.10 vs. 42.11; p = 0.480) (Fig. 89).

TNF-α tumor necrosis factor

In our group of patients, TNF-α and the length of disease seem to be independent parameters (r= -0.03; p=0.783) (Fig. 91).
The TNF-α rank does not differ significantly depending on the presence/absence of osteoporosis (43.28 *vs.* 43.62; p=0.946) (Fig. 92).

TNF-α tumor necrosis factor after 6 months

The mean TNF-α level virtually remained the same on reassessment (38.70 *vs.* 38.52; p=0.842).

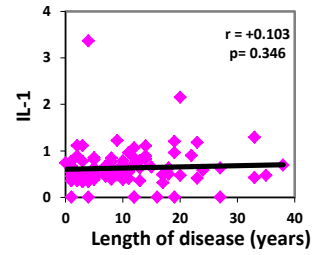


Fig. 88. *IL-1α correlation with the length of rheumatoid arthritis*

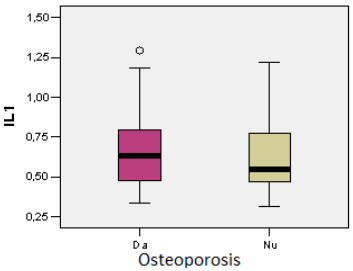


Fig. 89. *Mean IL-1α level depending on the presence/absence of osteoporosis*

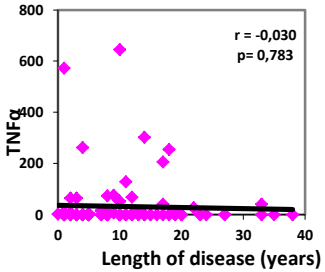


Fig. 91. *TNF-α correlation with the length of rheumatoid arthritis*

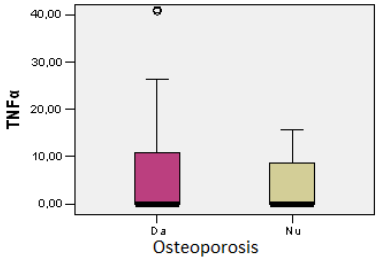


Fig. 92. *Mean TNF-α level depending on the presence/absence of osteoporosis*

NFkB receptor ligand (RANKL)

In our group of patients, RANKL and the length of disease seem to be independent parameters (r= +0.004; p=0.971) (Fig. 94).

The RANKL rank does not differ significantly depending on the presence/absence of osteoporosis (4.37 *vs.* 45.18; p=0.394) (Fig. 95).

Vit. D/Calcium/immunological parameters correlation

On reassessment, lower levels of vitamin D and serum calcium were associated with higher RANKL values (Fig. 112).

On reassessment, lower levels of vitamin D and serum calcium were associated with lower IL-1α levels (Fig. 115).

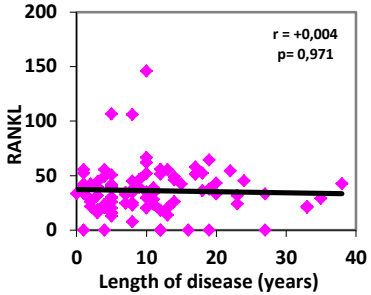


Fig. 94. *RANKL correlation with the length of rheumatoid arthritis*

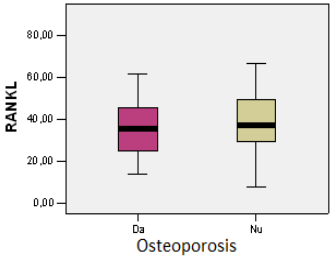


Fig. 95. *Mean RANKL level depending on the presence/absence of osteoporosis*

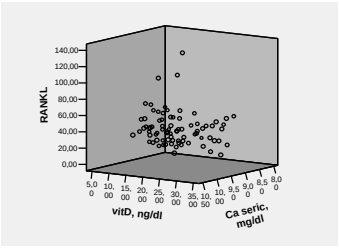


Fig. 112. *Correlation of serum calcium and vitamin D with RANKL*

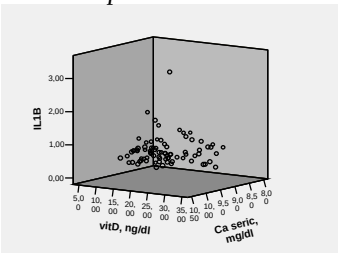


Fig. 115. *Correlation of serum calcium and vitamin D with IL-1α*

On reassessment, lower vitamin D and serum calcium levels were associated with lower TNF-α values (Fig. 118).

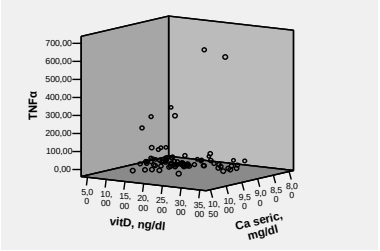


Fig. 118. *Serum calcium and vitamin D correlation with TNF-α*

DISCUSSIONS

X-ray examinations revealed three types of bone involvement in patients with rheumatoid arthritis. Joint borders are the place where the inflamed synovial is in direct contact with the bone, which leads to bone erosion specific to rheumatoid arthritis (169). Another bone area affected by this disease is the periartricular bone, which is caused by cytokines and factors expressed in the vicinity of the joints. The third type of injury is systemic osteopenia or osteoporosis, which involves the axial skeleton or the limb bones, and which is the consequence of the action of pro-inflammatory cytokines or other factors caused by the inflamed synovial tissue that enter the circulatory system and have various effects on these sites (170).

The patients included in the study had a wide variety of ages (ranging from 21 to 82 years), with a very high share of women (83.9% of the patients were female). The length of the disease was variable (10.8% of the patients had had the disease for about 1 year, while 6.5% of the total group had had it for more than 25 years; however, the mean length was 10.43 ± 8.38 years, significantly higher in women, with no significant differences by age group or background. Although literature data show that the average age of onset of the disease is between 25 and 40 years, 50.5% of the patients enrolled in the study were over 60 years old. In patients with systemic osteoporosis, the mean length of rheumatoid arthritis was significantly higher.

Most of the patients enrolled in the study had had, during the course of the disease, several treatment plans that alternated in different stages, with multiple associations between different DMARDs (disease-modifying anti-rheumatic drugs) conventional synthetic DMARDs (csDMARDs), biological DMARDs (boDMARDs - original biological agents), systemic corticotherapy (SC) with oral administration or intravenous pulse therapy, in various doses, depending on disease activity at a certain time, a situation that has led, over

the years, to the modification of the constellation of inflammatory cytokines.

35.5% of the patients included in the study suffered from systemic osteoporosis at the time of their examination, which was less than the osteoporosis mean reported worldwide in the rheumatoid arthritis population, which is over 38% in literature (178).

The mean NPJ was 8.63 ± 7.84 joints, and the mean NSJ was 2.51 ± 4.63 joints, with no significant differences between genders, age groups, backgrounds, smoking habits, alcohol consumption or the presence of systemic osteoporosis. The VAS pain scale was not significantly correlated with the length of the disease. Also, the mean VAS level was not statistically significantly altered by the presence/absence of systemic osteoporosis.

A longer history of disease was associated with lower DAS_{28} levels in 15.9% of the patients included in the research. This could be the consequence of effective treatment plans that manage to reduce disease activity, despite its long-term progression. In our study, the CDAI and SDAI disease activity scores were not significantly correlated with the length of disease or with the presence of systemic osteoporosis.

The results of our research showed a slight increase of the mean level of both ESR and CRP in patients with systemic osteoporosis. This finding is consistent with other studies in the field that failed to find a correlation between inflammatory activity quantified by measuring ESR or CRP and localized or generalized bone mass loss, the only certain conclusion being that the acceleration of hand bone mass loss, in the absence of radiological progression of the disease, may be directly related to inflammation in patients with good response to treatment after 6 and 12 months (3, 181). However, there are studies that have shown that the presence of a low degree of inflammation quantified by ESR and CRP values predicts bone mass loss and could be valuable in identifying patients at risk of osteoporosis. Some research has shown that a longer history of rheumatoid arthritis, a more intense disease activity and the occurrence of

the inflammatory syndrome, assessed by ESR and CRP follow-up, are potential risk factors for 10-year fracture probability (37, 179).

The biochemical and hematological parameters were not good predictors in the determinism of systemic osteoporosis.

As concerns hematological testing, the ROC curve did not reveal any of the hematological markers as a good predictor in the determinism of systemic osteoporosis. The connection between the disease activity level and the hematological parameters determined separately has been analyzed over the years in various studies. Most results have shown the existence of an inverse correlation between disease activity and hemoglobin level, as well as a direct correlation between thrombocytosis and intense disease activity. Evidence has shown that there is a close correlation between the mean platelet volume (MPV) and disease activity level, which is a marker of inflammation and disease activity. MPV increase may be determined by different cytokines and may lead, in the case of an active disease form, to higher platelet production levels within the inflammatory process. These young platelets are larger and carry a higher risk of atherosclerosis and cardiovascular disease in patients with active disease (182).

According to the current literature data, among the studied risk factors, smoking is involved in the occurrence of both rheumatoid arthritis and systemic osteoporosis (183); according to our study, 20% of the moderate smokers suffered from osteoporosis on their enrolment in the study group.

Rheumatoid factor positivity by Latex and Waller Rose agglutination tests was significant in smokers. The positive results of the rheumatoid factor determined by the Elisa method were identified significantly more frequently in patients over 60 years old and in patients with osteoporosis, which is consistent with the published studies in the field, where it was found that RF positivity increases with age (178).

Smoking and alcohol consumption were lower in patients with positive Elisa rheumatoid factor titer.

Systemic osteoporosis was present in 43.8% of those with moderate rheumatoid factor titer and only in 20% of those with elevated titer, significant percentage differences compared with the 37.5% of those with negative titer.

We have found in our research that sedentary lifestyle and little physical activity were significantly correlated with the female sex, our statistical analysis showing that 28.9% of patients with little physical activity had systemic osteoporosis.

There is a close connection between the severity of joint damage and the low mineral-bone density (184). This occurs because joint damage results in decreased daily physical activity, assessed by HAQ score, with implicit negative impact on muscle strength, resulting in low bone loading and thus reduced bone mineral density (BMD). However, we have noted no independent effect of HAQ on BMD, yet the decrease of physical leads to the onset of systemic osteoporosis only in combination with the other risk factors (74). In our study, the mean HAQ level does not differ significantly depending on the presence/absence of systemic osteoporosis. In 27.8% of the patients in the studied group, the longer the history of the disease, the higher the HAQ level; the correlation was direct, moderate in intensity, but statistically significant. Several studies revealed that a poor functional status, assessed by the initial HAQ score, is one of the best predictors of the subsequent progression of the disease. Since in most studies the HAQ score was monitored for at least 24 months, the assessment in our study at its beginning and after 6 months, which consisted of the completion of this disability questionnaire, cannot draw clinically relevant conclusions (181).

Among the assessed risk factors, we found that the female sex, age over 60 years and smoking increased the risk of developing systemic osteoporosis in patients with rheumatoid arthritis.

In the studied groups, the TNF-α rank does not differ significantly depending on the presence/absence of osteoporosis. We have also noted that TNF-α and length of

disease are apparently independent parameters. On reassessment, the mean TNF-α level remained virtually unchanged, which did not allow the establishment of a correlation between the TNF-α level and the progression of inflammation or osteoporosis. The mean TNF-α level was significantly lower in the patients who received vitamin D supplements during the 6 months of follow-up, which may correspond to the improvement of the underlying disease activity.

In the patients included in the study, the sequences of values recorded for IL-1α, TNF-α and RANKL were non-homogeneous, and the mean ranks did not differ between sexes, age groups, background, smoking habits, alcohol consumption or the presence of systemic osteoporosis.

In our study, IL-1α is not significantly correlated with the length of disease, however, it should be noted that, in 10.3% of patients, the longer the history of disease, the higher the IL-1α level. As with the TNF-α assessment, the IL-1α rank did not differ significantly depending on the presence/absence of systemic osteoporosis. During the 6 months of follow-up, we noticed an improvement in the disease parameters and lower mean IL-1α levels in patients who had received vitamin D supplements.

As for the other two parameters that we assessed in the patients in our study, the RANKL level showed no statistically significant differences depending on the presence/absence of systemic osteoporosis.

A direct correlation between inflammatory cytokines and bone loss could not be established. In patients who had systemic osteoporosis on their first examination, on reassessment, higher vitamin D levels were accompanied by a slight decrease in RANKL, a decrease in IL-1α and TNF-α, as well as a significant decrease in ESR and CRP.

On reassessment, the lowest mean RANKL value was detected in patients who had received biologic therapy, yet the difference was not statistically significant. Also, among patients with systemic osteoporosis, the highest mean RANKL level was

found in patients who had been administered vitamin D and biophosphonate, yet again the differences were not statistically significant.

Despite the results of a great number of studies, which show a significant impairment of the mineral bone density in patients with rheumatoid arthritis, which is all the more frequent as the disease has been progressing for a longer time, in our group, the frequency of osteoporosis is below the mean percentage reported by these studies. Although we have also tried to analyze the involvement of other risk factors than rheumatoid arthritis in the onset of osteoporosis, such as: old age, presence of menopause, immobilization or major limitation of mobilization through inflammatory disease, consumption of NSAIDs and corticosteroids, we have found that, although we could not completely rule out these risk factors, the presence of systemic osteoporosis was lower than we had expected. An explanation could be that the vast majority of patients were diagnosed in the early stages of their PR and immediately began an immunosuppressive treatment. Some patients underwent biological therapies, which most likely limited the increase of the inflammatory cytokine levels and, implicitly, osteoclast activation.

The assessment of inflammatory cytokines and RANKL was performed in relation to the other factors studied, as there is still no standardization of their values.

Among all the patients in our study, we have singled out those who had received one or more treatment plans that included biological agents. We noted that the patients in this group had better bone mineral density values than those who did not receive biological therapy. These data support studies that have shown that by blocking inflammatory cytokines a decrease in osteoclast activity and, consequently, bone resorption are achieved (195).

In the study that we have carried out, a great difficulty was represented by the selection of cases of rheumatoid arthritis according to the criteria that we have initially set, because this condition affects a low percentage of the general population,

and hence we could not select only the patients with the disease at its very beginning. We had very few patients with the disease at its very beginning or in whom the condition had been progressing for less than one year. All the patients undergoing continuous follow-up and treatment in this clinic (which is the clinic where over 90% of all the cases in the Moldova region are managed) were included in the study, with the observance of the inclusion and exclusion criteria. For this reason, many patients have received different treatment plans during the course of their disease progression, which were aimed at controlling disease activity. Some of them had taken a great diversity of drug associations between classical and biological dMARDS, sometimes with stages in which corticosteroids were also associated for certain time periods. Over the years, some patients had gone through all the treatment plans, even through several biological therapy plans. For this reason, it was difficult to establish which therapy had the greatest influence on bone resorption or whether they had had cumulative effects or enhanced one another.

In our research, the group of patients was not at all homogeneous from the view point of the length of disease; the patients did not undergo bone mineral density assessment either at the beginning of the study, or during its course. During the course of the progression of their polyarthritis, they also received various treatment plans, over various lengths of time, in different associations, so we cannot determine which therapy had the greatest influence on bone loss. However, we could note a low incidence of systemic osteoporosis in the patients included in our research, which was undeniably lower in the group of patients having received various biological therapies.

Another direction of analysis in our study was related to the implications of vitamin D in patients with rheumatoid arthritis. A significant vitamin D deficiency was detected in the patients included in the study group.

There is a growing interest in the role of vitamin D as a potential treatment for a number of inflammatory diseases. Vitamin D is a crucial secosteroid pro-hormone, with a wide

range of biological effects, ranging from the classical role of calcium and phosphorus metabolism mediator, which promotes bone mineralization, growth and healthy remodeling, to anti-microbial activity and cell differentiation modulation. Moreover, vitamin D exerts suppressive functions on the adaptive immune response cells, i.e. on the cells that are directly involved in PR development.

On reassessment, the analysis of our group of patients showed that the mean NPJ level was significantly lower, compared to the number of painful joints recorded in patients at the beginning of the study, which proves a positive evolution of the pain, correlated with the improvement of the serum level of 25 (OH) vitamin D. The same was true for the connection between 25 (OH) vitamin D and the mean number of swollen joints, unlike the published studies that analyzed the latter factors and did not detect their reciprocity (223).

There were negative correlations between the vitamin D level and the pain and disability scales. On reassessment, the mean VAS level was significantly lower compared to the VAS recorded before the treatment, also correlated with 25 (OH) vitamin D values. The VAS improved on reassessment, being correlated with improved NPJ and NSJ values, as well as with 25 (OH) vitamin D values.

The correlation between vitamin D and ESR was indirect, reduced in intensity, both initially and on reassessment. On the first assessment, lower levels of vitamin D and serum calcium were associated with higher ESR values. On reassessment, higher levels of vitamin D and serum calcium were associated with lower CRP values. These findings are in line with the published findings that identified that patients with more severe rheumatoid arthritis forms have lower 25 (OH) vitamin D serum levels, as well as higher ESR and antiCCP antibodies levels (223). The reassessment of our group revealed lower levels of vitamin D and serum calcium, which were associated with higher levels of anticitrullinic antibodies, yet the correlation was not significant.

There is also an inversely proportional ratio between vitamin D levels and increased disease activity (DAS28> 2.6), while the relation between vitamin D level and controlled disease (DAS28 <2.6) was not significant (224). On the 6-month reassessment conducted in our study, the mean DAS28 level calculated in patients was slightly lower compared to the one recorded on the first assessment, similar to the vitamin D values. These results confirm the correlation between the high disease activity and the low vitamin D levels (225).

As concerns the correlation between vitamin D and inflammatory cytokines, we found an indirect correlation between vitamin D and RANKL, which was reduced in intensity and statistically insignificant. The correlation between vitamin D and IL-1α was direct, reduced in intensity at the beginning of the study; on reassessment, these parameters were independent.

Our study failed to demonstrate the usefulness of inflammatory cytokines dosing in patients with rheumatoid arthritis in order to detect the risk of systemic osteoporosis. However, we noted that there were fewer patients under biologic therapy (TNF-α, IL-1 or IL-6 inhibitors) who had systemic osteoporosis than patients who had only received conventional DMARDs. Despite the high number of researches that have been concerned with this disease, it still requires further studies, especially regarding the protective effect of the new treatments used in rheumatoid arthritis on the generalized bone mass, as well as their role in stimulating bone formation.

The results show that vitamin D dosing at the time of rheumatoid arthritis diagnosis setting may be helpful in correcting the existing deficiency of this vitamin and hence in reducing disease activity and bone mass loss, but also in maximizing the response of the underlying disease to the its treatment.

CONCLUSIONS

1. The low frequency of polyarthritis in the general population has made it difficult for us to select only patients at the onset of the disease, so as to achieve a homogeneous study group.

2. There were many factors involved in rheumatoid arthritis progression that also influenced bone remodeling, making it difficult to distinguish between the roles played by each factor.

3. The patients were in different stages of disease progression, had had many treatment plans that alternated in different stages, with multiple associations, in various doses, depending on their disease activity at a certain time, a situation which led, in time, to the alteration of the constellation of inflammatory cytokines. Moreover, some patients underwent biological treatments with TNF-α blockers in many treatment plans that influenced the serum level of this cytokine as well as the level of mineral bone density.

4. Osteoporosis was confirmed in about one third of the patients with rheumatoid arthritis included in the study, which suggests an associative treatment.

5. A low percentage of patients had a positive correlation between the length of disease and a high IL-1α level.

6. The risk factors found to be associated with osteoporosis in patients with rheumatoid arthritis were mainly the female sex, age over 60 years and smoking, presence of inflammation, but also time of disease progression.

7. After 6 months, the mean NPJ and NSJ decreased significantly, in close correlation with VAS, CDAI and SDAI scores, which registered a significantly lower mean level on reassessment, while the disease activity score -DAS28- remained virtually at the same level.

8. The mean VAS pain score was statistically significantly higher in patients who had positive Latex or Waller Rose rheumatoid factor.

9. Patient reassessment 6 months after their enrolment in the study revealed an improvement of the inflammatory and

immunological syndrome, when they took their regular anti-inflammatory medication and also vitamin D supplements.

10. Significant inflammation revealed by high ESR and CRP values was correlated with low vitamin D levels and serum calcium values, both on the first assessment and after 6 months, which would suggest the appropriateness of concomitant vitamin D administration.

11. Since patient assessment consisted only of parameter measurements at the time of enrollment in the study and after 6 months, we cannot confirm at this point the direct connection between inflammatory cytokines and the extent of bone mass loss, as this would require a longer follow-up interval.

12. The results of the study suggest that, when setting the positive diagnosis of rheumatoid arthritis, it would be advisable to determine, as a basic standard for the assessment of these patients, the 25 (OH) vitamin D level and to perform a DXA scan of the lumbar spine to determine the risk of osteoporosis.

13. The correlation of the 25 (OH) vitamin D level with the T score in the lumbar spine and with the ESR and CRP inflammation markers could be an examination algorithm for determining the risk of osteoporosis in patients with rheumatoid arthritis, and at the same time it could be a prognostic factor for PR response to treatment and for disease remission.

14. The administration of vitamin D supplements, at the very beginning or in the early stages of the disease, in the case of patients with rheumatoid arthritis, could lead to an improvement of their status, both in terms of joint and muscle pain and improved mobility and stability, and in terms of inflammation reduction and bone mass loss slowing.

15. Treatment with TNF-α and IL-6 blockers slowed the progression of bone mass loss in patients with rheumatoid arthritis, regardless of the length of disease, disease activity or administered anti-inflammatory treatment. We detected no systemic osteoporosis or only a slight decrease in bone mineral density, within the osteopenia range, in these patients. Thus, our study has indirectly shown that early initiation of therapy with biologic anti-TNF agents

inhibits bone damage in patients with rheumatoid arthritis, irrespective of their anti-inflammatory activity.

16. Our great interest in the pathophysiological aspects of bone loss due to rheumatoid arthritis may lead to new therapeutic concepts in rheumatoid arthritis, including anti-erosive therapies. Despite all the breakthroughs in osteoimmunology, a high number of patients with rheumatoid arthritis suffer bone damage during the course of the disease. Thus, further research is needed to fully elucidate the pathophysiological mechanisms of osteoclast-directed bone loss in patients with rheumatoid arthritis, the effects of biological therapy on bone resorption mechanisms, and the biological markers that may be used to identify patients at risk for systemic osteoporosis.

List of 'in extenso' papers published by the author on topics tackled in the doctoral thesis

Articole cotate ISI

1. **Barzoi R**, Rezus E, Badescu C et al. The impact of proinflammatory cytokynes of rheumatoid polyarthritis on the generalized loss of Bone Mass. *Revista de Chimie*. 2018;69(9):2541-2545.

Articole BDI

1. Ciocoiu M, **Barzoi R**, Rezus E. Pathophysiological issues involved in the early onset of osteoporosis associated with rheumatoid arthritis. *Med Surg J - Rev Med Chir - Soc Med Nat*. 2017;121(2):296.
2. **Barzoi RO**, Rezus E, Petrariu FD, Badescu C, Ciocoiu M**,** Implications of vitamin d deficiency in inflammation due to rheumatoid arthritis. *Med Surg J - Rev Med Chir - Soc Med Nat.*2018;122(4):677-681.

Selective references

1. Jeremiah MP, Unwin BK, Greenawald MH et al. Diagnosis and Management of Osteoporosis. *Am Fam Physician* 2015;92(4):261-268.
2. Leistner DM, Seeger FH, Fischer A et al. Elevated Levels of the Mediator of Catabolic Bone Remodelling RANKL in the Bone Marrow Environment Link Chronic Heart Failure with Osteoporosis. *Circ Heart Fail* 2012;30:769-777.
3. Baum R, Gravallese EM. Impact of Inflammation on the Osteoblast in Rheumatic Diseases. *Curr Osteoporos Rep* 2014;12(1):9-16.
4. Jung SM, Kim KW, Yang CW et al. Cytokine-mediated bone destruction in rheumatoid arthritis. *J Immunol Res* 2014;2014:263625.
5. Weitzmann MN, Pacifici R. Osteoimmunology: Relation to Disease and Therapy. In Bronner F, Farach-Carson MC, Roach HI. *Bone-Metabolic Functions and Modulators*, 7, London: Springer; 2012, 237-250.
6. Jung SM, Kim KW, Yang CW et al. Cytokine-mediated bone destruction in rheumatoid arthritis. *J Immunol Res* 2014;2014:263625.
7. Ginaldi L, De Martinis M. Osteoimmunology and Beyond. *Curr Med Chem* 2016;23(33):3754-3774.
8. Sucur A, Jajic Z, Artukovic M, et al. Chemokine signals are crucial for enhanced homing and differentiation of circulating osteoclast progenitor cells. *Arthritis Res Ther* 2017;19(1):142.
9. Mirza F, Canalis E. Management of endocrine disease: Secondary osteoporosis: pathophysiology and management. *Eur J Endocrinol* 2015;173(3):R131-151.
10. Gravallese EM, Goldring SR, Schett G. The Role of the Immune System in the Local and Systemic Bone Loss of Inflammatory Arthritis. In: Lorenzo J, Horowitz M, Choi Y, et al. *Osteoimmunology*, 2, San Diego: Academic Press; 2016, 241-256.
11. Straub RH, Cutolo M, Pacifici R. Evolutionary medicine and bone loss in chronic inflammatory diseases—A theory of inflammation-related osteopenia. *Semin Arthritis Rheum* 2015;45(2):220-228.
12. Moon SJ, Ahn IE, Jung H et al. Temporal differential effects of proinflammatory cytokines on osteoclastogenesis. *Int J Mol Med* 2013;31(4):769-777.
13. Goldring SR, Purdue PE, Crotti TN et al. Bone remodelling in inflammatory arthritis. *Ann Rheum Dis* 2013;72(2):ii52-ii55.

14. Ralston SHM. Bone structure and metabolism. *Medicine* 2017;45(9):560-564.
15. Goldring SR. The osteocyte: key player in regulating bone turnover. *RMD Open*. 2015;1(Suppl 1).
16. Geusens P. The role of RANK ligand/osteoprotegerin in rheumatoid arthritis. *Ther Adv Musculoskelet Dis*. 2012;4(4):225-233.
17. Manson J. Rheumatoid Arthritis. In: Elselvier, editor. *Encyclopedia of Immunobiology*: Academic Press; 2016. p. 212-218.
18. Smolen JS, Redlich K. Rheumatoid Arthritis. In: Mackay I.R., Rose N.R., editor. *The Autoimmune Diseases*, Fifth Edition: Elsevier; 2014. p. 511–523.
19. Xue AL, Wu SY, Jiang L et al. Bone fracture risk in patients with rheumatoid arthritis: A meta-analysis. *Medicine*. 2017;96(36):e6983.
20. Hensvold AH, Joshua V, Li W et al. Serum RANKL levels associate with anti-citrullinated protein antibodies in early untreated rheumatoid arthritis and are modulated following methotrexate. Arthritis Res Ther. 2015;17:239.
21. Krishnamurthy A, Joshua V, Haj Hensvold A et al. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. *Ann Rheum Dis*. 2016;75(4):721-729.
22. Amarasekara DS, Yu J, Rho J. Bone Loss Triggered by the Cytokine Network in Inflammatory Autoimmune Diseases. *J Immunol Res*. 2015;2015:832127-.
23. Meednu N, Zhang H, Owen T et al. Production of RANKL by memory B cell: a link between B cells and bone erosion in rheumatoid arthritis. *Arthritis Rheumatol*. 2016;68:805-816.
24. Adamopoulos IE. Inflammation in bone physiology and pathology. *Curr Opin Rheumatol*. 2018;30(1):59-64.
25. Chang B, Quan Q, Li Y, Qiu H, Peng J, Gu Y. Treatment of Osteoporosis, with a Focus on 2 Monoclonal Antibodies. *Med Sci Monit*. 2018;24:8758-66.
26. Nagar M, Chera H, Daich J, Rosen Y. Vitamin D and Autoimmunity. In: Watson R, Preedy V, editors. *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases* (Second Edition): Academic Press; 2019. p. 203-220.