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Changes induced by DKK1 in rheumatoid arthritis patients who commence biologic therapy treatment

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Summary

Introduction: The aim of this study is to assess the relationship among inflammatory charge, cardiovascular risk and bone metabolism in patients with rheumatoid arthritis initiating biological therapy treatment.

Patients and methods: This is a prospective cohort study conducted in patients diagnosed with active rheumatoid arthritis (RA) assessed in the Rheumatology Unit and initiating biological therapy.
Patients will be selected consecutively, with preliminary data on 14 patients. We present preliminary data from 14 patients.

Results: Reduced Dickkopf-1 (DKK1) concentrations after commencing biological therapy were detected (baseline: 53.12±60.43 pg/ml vs 6 months 13.5±23.2 pg/ml, p=0.307) but without statistical significance. Changes were found in markers for bone remodeling with increased osteocalcin levels and CTX which were not statistically significant either.

Conclusions: We observed a nonsignificant decrease in DKK1 serum in patients with active RA treated with biologic therapy. Expanding the scope of study subjects and pending biochemical determinations will allow us, in the near future, to establish more precisely this link and the relationship of DKK1, bone remodeling, biological therapy and cardiovascular disease in RA patients.

Key words: *rheumatoid arthritis, DKK1, biological therapy.*

Introduction

Rheumatoid arthritis (RA) and other inflammatory rheumatic diseases such as ankylosing spondylitis and psoriatic arthritis have an increased cardiovascular mortality due to accelerated development of atherosclerosis[1]. Persistent chronic inflammation and genetic factors have been associated with the development of accelerated atherosclerosis and consequently cardiovascular events[2].

The Wnt pathway has been involved not only in altering the bone metabolism[3] but also in cardiovascular disorders[4,5], which may be the common link between these diseases. The implication of rheumatoid arthritis in this way explained by pro-inflammatory cytokines involved in its pathogenesis, such as tumor necrosis factor alpha (TNF-α), which plays an important role in the process of osteoclast differentiation by increasing the ligand receptor activator of nuclear factor kB ligand (RANKL) and Dickkopf-1 (DKK1) and sclerostin, both Wnt pathway inhibitors[6]. Thus, control of activity in patients with RA should entail not only an increase in BMD but also reduced cardiovascular risk.

The aim of the study is to assess the relationship of the inflammatory burden, cardiovascular risk and bone metabolism in patients with rheumatoid arthritis who start treatment with biological therapy. To do this, we have analyzed the relationship between inflammatory activity, serum concentrations of antagonists of the Wnt pathway (DKK1), the specific cardiovascular disease by the modified SCORE method for RA, the intima-media carotid and bone disease in patients with RA, at the start of treatment with biological therapy and at 6 and 12 months of treatment. In this paper, preliminary data from the study are presented.

Patients and methods

This is a prospective cohort study of patients diagnosed with active RA evaluated at the Rheumatology Unit, and commence biological therapy. For the diagnosis of RA 1987 ACR criteria were used. Inclusion criteria were the following RA diagnosis, over 18 years of age, presence of disease activity (DAS> 2.4) despite treatment with synthetic disease-modifying drugs and signed informed consent. We excluded patients with previous cardiovascular events, previous osteoporotic fractures, osteoporosis different metabolic bone disease, chronic renal disease, chronic liver disease, type 1 and 2 diabetes mellitus, neoplastic disease, pregnancy and lactation.

The study was approved by the Ethics Committee for Clinical Research of the University Hospital Rafael Mendez. All participants were informed of the type of study and its procedures, and provided informed consent before any study procedure. The study was designed and conducted in accordance with the ethical standards of the Helsinki Declaration.

The following variables were collected: DKK-1 serum levels, sociodemographic characteristics, blood pressure (BP), DAS-28 VSG, visual analogue scale (VAS) of the patient's disease on a 0 to 10 scale, duration of the disease determined in years, response to treatment assessed by EULAR, values for rheumatoid factor and anti-citrullinated peptide antibodies, blood count, general biochemistry with hepatorenal function, lipid profile (total cholesterol, HDL, LDL, triglycerides), C-reactive protein (CRP), and serum calcium, phosphorus parathyroid hormone (PTH), 25-hydroxyvitamin D3 (25-OH vitamin D3), bone turnover markers (bone alkaline phosphatase, osteocalcin, C-terminal telopeptide of type I collagen -u-CTX), thickness intima-media carotid (c-IMT), model SCORE (systematic coronary risk evaluation, systematic coronary risk Assessment) model modified for AR SCORE, and bone mineral density in the lumbar spine and hip measured by dual X-ray absorptiometry (DXA).

Biochemical determinations

The analysis of biochemical parameters was carried out by standard techniques.

Calciotropic hormone concentrations were determined by HPLC for 25-OH vitamin D3 and Elecsys systems for intact PTH. Remodeling biochemical markers were determined in an automated program (Roche Elecsys 2010).

DKK1 concentrations were evaluated by ELISA (Biomedica Medizinprodukte GmbH and Co. KG, Vienna, Austria) following manufacturer's instructions. For all other pending biochemical determinations, frozen samples at -80 ° C were used.

BMD Evaluation

Bone mineral density at the lumbar spine and femoral neck was assessed by dual X-ray densitometry (DXA) (Norland XR-800).

For postmenopausal women and men >50 years, the T-score was used to classify the central DXA in normal, osteopenia and osteoporosis. Z-score was used in the other cases; a Z-score <-2 was considered low bone mass.

Evaluation of c-IMT

The ultrasound evaluation of the carotid arteries was performed using Doppler ultrasound (Philips iU22) with a 9-3 MHz linear probe. The c-IMT was assessed and also the existence of plates. The c-IMT was measured in the distal third of both carotid arteries 1 cm above the bulb. The plate was defined as a greater focal thickening of 0.5mm within the arterial lumen or thickening >50% of the thickness of the adjacent intima or intimal thickness >1.5 mm.

Cardiovascular risk assessment

The patients' cardiovascular risk was determined by the SCORE model and modified to the AR SCORE. Those patients who had carotid ultrasound plates and/or c-IMT >0.9 were classified as patients at very high cardiovascular risk regardless of the SCORE obtained.

Table 1: Sociodemographic and clinical characteristics

N	14
Age, mean ± SD	47.14±14.06
Woman, n (%)	9 (64.3)
BMI, mean ± DE	29.89±7.41
HBP, n (%)	3 (21.4)
DLP, n (%)	3 (21.4)
Alcohol intake, n (%)	2 (14.3)
Smoking, n (%)	9 (64.3)

SD: standard deviation; BMI: body mass index; HBP: high blood pressure; DLP: dyslipidemia.

Statistical analysis
Data for continuous variables are expressed as mean ± standard deviation. The data for categorical variables are presented as percentages. Changes in quantitative variables before and after treatment were compared with Student's t test for paired samples. Categorical variables were compared by chi-square test.

Correlation analyzes were performed using Pearson's correlation (normal distribution) or Spearman (non-normal distribution). P values <0.05 were considered significant. For statistical analysis, the SPSS version 18.0 software (SPSS, Chicago, IL) was used.

Results

As of September 2015 14 naïve patients have been included for biological therapy. In this paper, we present the results at 6 months.

Demographic-clinical variables
The average age of the 14 patients was 47±14 years. 64.3% were women. 21.4% of the patients were hypertensive and 64.3% were smokers. The values of other variables are shown in Table 1.

Variables related disease
The average disease duration was 68±71 months (CI 10-240). The DAS 28-ESR mean baseline was 4.41±1, the average number of swollen joints 3±2, the average number of painful joints 4±3 and visual analogue scale (VAS) of the specific disease by 7±2 patient.

71.5% had positive rheumatoid factor and anti-citrullinated peptide antibodies. 64.3% of patients included in the study were taking disease modifying drugs (DMARDs) associated with biological therapy. The average prednisone dose was 3.7±2.5 mg. Only 44.4% of the patients had EULAR response to treatment at 6 months.

Analytical and variables related to bone metabolism
The values of these variables are represented in Table 2.

Correlation between bone remodeling, DKK1, disease activity and c-IMT
No significant correlation was found between disease activity measurement by DAS 28-ESR and levels of alkaline phosphatase, osteocalcin, CTX or DKK1. Nor did we observe any relationship between markers of remodeling or concentrations of DKK1 and intima-media thickness.

We found no association between DAS28-ESR-measured disease activity and cardiovascular risk assessed by SCORE and modified SCORE.

DKK1 changes after treatment and relationship with disease parameters
We found decreased levels of DKK1 after commencing biological therapy (baseline: 53.12±60.43 pg/ml vs 6 months 23.2±13.5 pg/ml, p=0.307) which was not statistically significant (Figure 1). No statistically significant association between decreased levels of DKK-1 and EULAR response to treatment was detected. As for bone remodeling markers, insignificant increased osteocalcin levels and CTX were detected.

Discussion

Epidemiological studies have shown an association between BMD loss and cardiovascular calcification, morbidity and mortality[7-9]. The Wnt pathway is involved in regulating vascular calcification and differentiation of smooth muscle cells to osteoblasts10. It has been shown to increase the DKK1 expression[11,12] in carotid atherosclerotic plaques and increased serum concentrations of sclerostin in patients with atherosclerotic disease and type 2 diabetes[13].

Furthermore, elevated levels of circulating DKK1 in patients with RA have been shown, linked to radiological damage[14-18], and sclerostin expression seems to correlate positively with levels of DKK1[19].

Our study showed a decrease in the levels of DKK-1 at 6 months of treatment, which is consistent with recently published by Briot et al.[20]. In this article, patients with active RA treated with tocilizumab experienced a decrease in DKK1 concentrations and a decrease in formation markers. However, in our study we found an increase in markers of formation and post-treatment resorption, but this did not reach statistical significance. Our limited sample size has certainly influenced our results.

In conclusion, we can say that in patients with active RA treated with biological therapy we have observed a nonsignificant decrease in serum concentrations of DKK1 and a significant increase in bone resorption. Expanding both the number of study subjects as well as more pending biochemical determinations would allow us in the near future to more precisely establish this association, and also the relationship between DKK1, bone remodeling, biological therapy and cardiovascular disease in patients with RA.

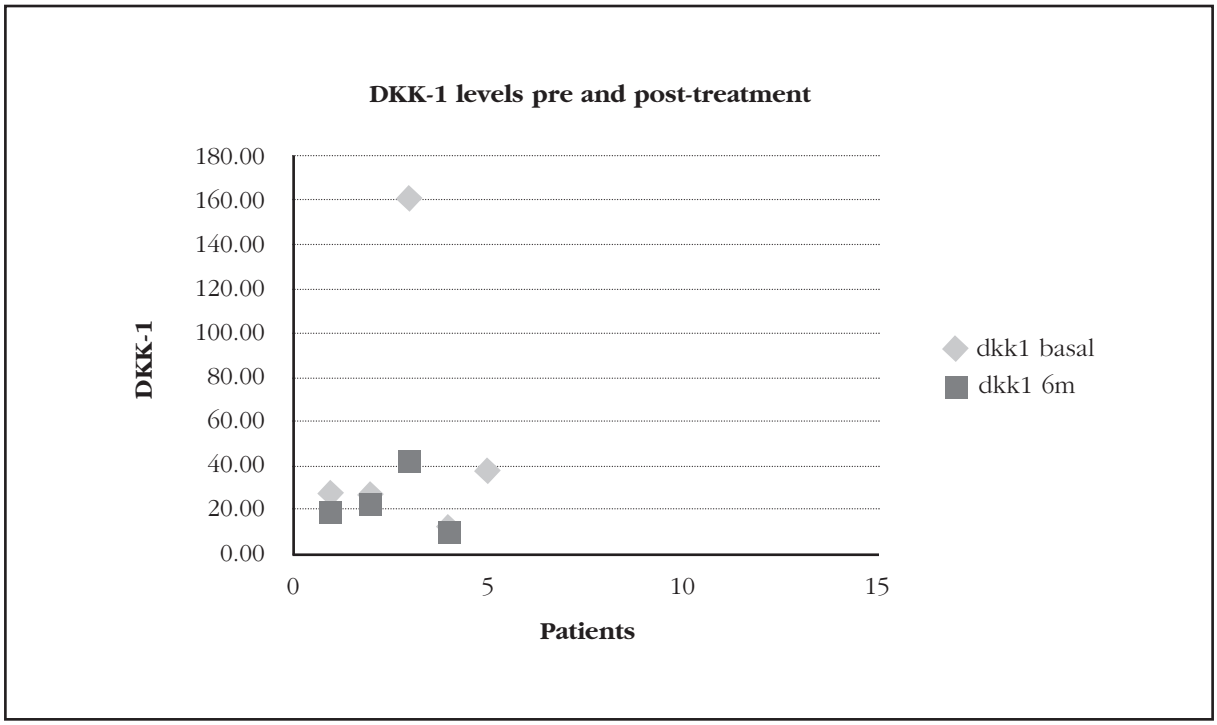
Competing interests: The authors declare no conflicts of interest regarding this article.

Table 2: Biochemical and related bone metabolism, disease activity and baseline cardiovascular risk and at 6 months

	Basal (N=14)	6 months (N= 9)
Total cholesterol (mg/dl), mean ± SD	198.85±37.09	195±55.91
HDL-cholesterol (mg/dl), mean ± SD	62.71±24.32	52±24.06
LDL-cholesterol (mg/dl), mean ± SD	129.57±34.24	138±59.07
Triglycerides (mg/dl), mean ± SD	117.85±43.95	154±76.80
CRP (mg/L), mean ± SD	8.62±10.50	6,66±9.34
IPTH (pg/ml), mean ± SD	37.85±12.76	52.01±14.88
25-OH vitamin D (ng/dl), mean ± SD	18.70±6.89	28.40±19.51
FAO (µg/dl), mean ± SD	11.82±4.20	11.50±4.13
Osteocalcin (ng/ml), mean ± SD	13.73±6.75	19.27± 11.54
CTX (ng/ml), mean ± SD	0.26±0.11	0.37±0.21
DXA central		
- Normal, n (%)	11 (78,6)	
- Osteopenia, n (%)	0 (0)	
- Osteoporosis, n (%)	1 (7,1)	
DAS 28-VSG, mean ± SD	4.41±0.99	3.38±1.51
EVA-disease, mean ± SD	7±2	3.66±2.82
SCORE		
- Low, n (%)	6 (42.9)	5 (55.6)
- Moderate, n (%)	8 (57.1)	3 (33.3)
- High, n (%)	0 (0)	1 (11.1)
- Very high, n (%)	0 (0)	0 (0)
SCORE modified		
- Low, n (%)	6 (42.9)	5 (55.6)
- Moderate, n (%)	6 (42.9)	2 (22.2)
- High, n (%)	2 (14.3)	1 (11.1)
- Very high, n (%)	0 (0)	1 (11.1)
SCORE-ultrasound		
- Low, n (%)	5 (35.7)	5 (55.6)
- Moderate, n (%)	1 (7.1)	2 (22.2)
- High, n (%)	1 (7.1)	1 (11.1)
- Very high, n (%)	7 (50)	1 (11.1)
c-IMT right (mm), mean ± SD	0.54±0.18	0.51±0.10
c-IMT left (mm), mean ± SD	0.64±0.20	0.49±0.08
Carotid plaques, n (%)	7 (50)	2 (25)

SD: standard deviation; CRP: C-reactive protein; FAO: bone alkaline phosphatase; CTX: C-terminal telopeptide of type I collagen; c-IMT: carotid intima-media thickness.

Figure 1. Changes in concentrations of DKK1 after biological treatment



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