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Effect of biological therapy on concentrations of DKK1 and sclerostin, cardiovascular risk and bone metabolism in patients with rheumatoid arthritis

DOI: http://dx.doi.org/10.4321/S1889-836X2018000100004

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Date of receipt: 24/09/2017
Date of acceptance: 12/12/2017

Work awarded with a 2015 FEIOMM Clinical Research Scholarship.

Summary

Introduction: Previous studies have linked the Wnt pathway in the alteration of bone metabolism and cardiovascular pathology. Also, the control of inflammation with biological therapy has a positive effect on bone mineral density (BMD) and cardiovascular risk. The aim of the study was to evaluate the effect of biological therapy in patients with rheumatoid arthritis, naïve to these therapy, on the inflammatory load and its relation with cardiovascular risk and bone metabolism.

Patients and methods: Prospective cohort study performed in patients diagnosed with active rheumatoid arthritis (RA) initiating biological therapy. Patients were selected consecutively not selected. The serum concentrations of Dickkopf-1 protein (DKK1) and sclerostin were collected, both by means of the ELISA method (Biomedica Medizinprodukte GmbH and Co. KG, Vienna, Austria); demographic and clinical variables, markers of bone remodeling, hip and lumbar spine BMDs were measured by dual energy X-ray absorptiometry (DXA), measurement of intima-media thickness (IMT), evaluation cardiovascular risk by Systematic Coronary Risk Evaluation (SCORE).

Results: 46.7% of patients presented EULAR response to treatment at 12 months. Only in this subgroup of patients, we found in the subgroup of patients an increase in the concentrations of DKK1 following the initiation of biological therapy (baseline 20.55±8.13 pg/ml vs 12 months 31.20±4.88 pg/ml, p=0.03). Regarding markers of bone remodeling, an increase in osteocalcin levels (baseline: 11.25±3.28 ng/ml vs 12 months 15.78±4.11 ng/ml, p=0.01). There was no change in IMT or SCORE at 12 months of treatment.

Conclusions: In patients with RA treated with biological therapy who presented EULAR response we observed a significant increase in serum concentrations of DKK1 at 12 months of treatment not associated with changes in bone metabolism and cardiovascular risk.

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

Introduction

Rheumatoid arthritis (RA) and other inflammatory rheumatic diseases have a higher cardiovascular mortality due to the development of accelerated atherosclerosis[1]. Persistent chronic inflammation, as well as genetic factors, have been implicated in the development of accelerated atherosclerosis and, consequently, in cardiovascular events[2]. Likewise, an increased risk of osteopenia and osteoporosis has been demonstrated in these patients[3,4].

The Wnt pathway has been involved not only in bone metabolism alteration[5], but also in cardiovascular disease[6,7], so it could be one of the common links between these diseases.

Previous studies have found higher serum levels of Dickkopf-1 protein (DKK1) in patients with RA than in the control group, which has been correlated with erosions and inflammation[8]. DKK1 has also been implicated in bone loss brought on by inflammation[9] and vascular calcification processes, with data showing a relationship between DKK1 levels and atherosclerosis in humans[10,11].

Rheumatoid arthritis involvement in this pathway has been explained through the proinflammatory cytokines involved in its pathogenesis, such as tumor necrosis factor alpha (TNFα), or interleukins 1 and 6, which have a significant role in the osteoclastic differentiation process, increasing the receptor activator of nuclear factor kB ligand (RANKL) as well as DKK1 and sclerostin, both Wnt pathway inhibitors[12,13]. Therefore, the control of activity in patients with RA should induce an increase in bone mineral density (BMD), while reducing cardiovascular risk.

Our study attempted to assess the effect of biological therapy in patients with RA who had not previously received biological therapy, on the inflammatory load was analyzed along with its relationship with cardiovascular risk and bone metabolism. To this end, the inflammatory activity, serum concentrations of Wnt pathway antagonists (DKK1 and sclerostin), the presence of cardiovascular risk determined by the modified SCORE method for RA, carotid intima-media thickness and bone disease in patients with RA, at the beginning of treatment with biological therapy and at 6 and 12 months of treatment.

Patients and methods

A prospective cohort study was carried out on patients diagnosed with active RA evaluated in the Rheumatology Unit who initiated biological therapy. The patients were selected consecutively and not selected. To diagnose RA, the 1987 American College of Rheumatology criteria (ACR) were applied. The inclusion criteria were the following: diagnosis of RA; older than 18 years; presence of disease activity defined by the Disease Activity Score 28 (DAS-28) with ESR >2.4 despite treatment with synthetic disease modifying drugs (DMARDs). Signed informed consent was also required. Patients with previous cardiovascular events, previous osteoporotic fractures, metabolic bone disease other than osteoporosis, chronic kidney disease, chronic liver disease, diabetes mellitus type 1 and 2, neoplastic disease, pregnancy and lactation were excluded.

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

The following variables were collected: serum levels of DKK1 and sclerostin; sociodemographic characteristics; blood pressure (TA); DAS-28 with VSG; Visual analog scale (VAS) of the disease by the patient measured from 0 to 10; duration of the disease determined in months; response of the disease to treatment assessed by EULAR response; rheumatoid factor values and anti-citrullinated peptide antibodies; hemogram; general biochemistry with hepatorenal function; lipid profile (total cholesterol, HDL, LDL, triglycerides); C reactive protein (CRP); calcium and serum phosphorus; parathormone (PTH); 25-hydroxyvitamin D3 (25-OH vitamin D3); markers of bone remodeling (bone alkaline phosphatase, osteocalcin, C-terminal telopeptide of collagen type I -CTX-); thickness of the carotid intima-media (CIMT); SCORE model (Systematic Coronary Risk Evaluation); modified SCORE model for RA; and BMD in the lumbar and hip spine measured by dual X-ray absorptiometry (DXA).

Biochemical determinations

Biochemical parameters were analyzed using standardized techniques.

Calciotropic hormone concentrations were determined by HPLC for 25-OH vitamin D3 and ELECSYS for intact PTH. The biochemical markers of remodeling were determined automatically (Roche Elecsys 2010).

The concentrations of DKK1 were evaluated by ELISA (Biomedica Medizinprodukte GmbH & Co. KG, Vienna, Austria) according to the manufacturer's instructions. The determinations of DKK1 were expressed in pg/ml. Sclerostin concentrations were assessed by ELISA (Biomedica, Vienna, Austria) following the manufacturer's instructions. Sclerostin determinations were expressed in pmol/l, and the lower limit of detection was 10 pmol/l.

Evaluation of disease activity

Disease activity was assessed according to DAS-28 with VSG.

Changes in disease activity were expressed by relative changes of DAS-28 with ESR at 6 and 12 months with respect to baseline, and by the EULAR response to treatment at 12 months.

Bone mineral density assessment

Lumbar spine and femoral neck BMD was evaluated through dual X-ray densitometry (DXA) (Norland XR-800). BMD was defined as the bone mineral content divided by explored area expres-

sed in g/cm^2. For postmenopausal and male patients >50 years, the T-score was used to classify central DXA into normal, osteopenia and osteoporosis. In the rest of the cases, the Z-score was used, considering low bone mass a Z-score <-2.

Changes in BMD were expressed as changes relative per year compared to the baseline.

C-IMT evaluation

Ultrasonographic evaluation of the carotid arteries was carrying out through echo-Doppler (Philips iU22) with a 9-3 MHz linear probe. C-IMT and the existence of plaques were assessed. C-IMT was measured in the distal third of both primitive carotid arteries, 1 cm before the bulb. The plaque was defined as a focal thickening greater than 0.5 mm within the arterial lumen or a thickening >50% of the thickness of the adjacent intima or an intimal thickness >1.5 mm.

BMI changes and the existence of plaques were expressed as relative changes at 6 and 12 months regarding the baseline.

Cardiovascular risk assessment

The patients' cardiovascular risk was determined using the modified SCORE and SCORE model for RA[14]. Those patients who presented plaques and/or c-IMT >0.9 mm in the carotid ultrasound were classified as very high cardiovascular risk patients regardless of the SCORE obtained.

Statistical analysis

The data of quantitative variables are expressed as mean ± standard deviation (normal distribution) or median (non-normal distribution). Qualitative variables data are presented as percentages. The changes in the quantitative variables before and after the treatment were compared with the Student t test for paired samples. The categorical variables were compared through the χ^2 test.

The correlation analyzes between quantitative variables have been carried out using the Pearson (normal distribution) or Spearman correlation (non-normal distribution). The analysis of the association between dichotomous quantitative and qualitative variables was carried out using the Student t test for independent samples (normal distribution) and the Mann-Whitney U test (non-normal distribution). ANOVA was used for polychromatic variables (normal distribution) and K independent samples (non-normal distribution). Values of $p<0.05$ were considered significant. The SPSS program, version 18.0 (SPSS, Chicago, Illinois, USA) was used for the statistical analysis.

Results

Twenty patients who had not previously received biological therapy were included in the study, of whom 18 completed the study at 6 months and 15 patients at 12 months.

Demographic-clinical variables

The average age of the patients was 45.22±14.47 years, with 72.2% being women. 11.1% of the patients included were hypertensive and 44.4% were smokers. The values of the rest of the variables are shown in table 1.

Variables related to the disease

The average duration of the disease was 79.16±69.75 months, median 55.50. The mean baseline DAS-28-VSG was 4.51±1; the number of swollen joints average of 3.33±1.97; the number of painful joints average of 4.39±3.25, median of 5; and the value in the visual analog scale (VAS) of the disease determined by the patient was of 6.52±1.94.

72.2% had positive rheumatoid factor and 83.3% had anti-citrullinated peptide antibodies. 72.2% of the patients initiated biological therapy with anti-TNFα drugs, 22.2% with abatacept and only 5.6% with tocilizumab. 66.7% of the patients included in the study took disease modifying drugs (DMARDs) associated with biological therapy. The average dose of prednisone was 4.86±4.65 mg, median of 5.

Of the 18 patients who completed the 6 months of the study, 44% had an EULAR response at 6 months, and of the 15 patients who completed the study, 7 (46.7%) presented an EULAR response at 12 months.

Analytical variables and related to bone metabolism

The serum levels of DKK1 were of 35.96±36.25 pg/ml, and those of sclerostin 56.09±36.46 ng/ml. Only 6.3% of patients had osteoporosis according to DXA. The values of the rest of the variables are shown in table 2.

Variables related to cardiovascular risk

The right middle c-IMT was 0.55±0.15 mm and the left one was 0.62±0.20 mm. 33.3% of the patients presented carotid plaques. 61.1% of patients had a low SCORE; none presented a high or very high SCORE. However, when applying the modified SCORE for RA, 11.1% of the patients presented a high SCORE, and when performing the carotid ultrasound, 38.9% of the patients were classified with a very high SCORE and 5.6% high.

After 12 months of treatment, no statistically significant changes were found in these variables.

Correlation between bone remodeling, BMD, DKK1, disease activity and c-IMT

No statistically significant correlation was observed between the disease activity measured by DAS-28-VSG and the levels of bone alkaline phosphatase, osteocalcin, CTX, 25-OH vitamin D3, DKK1, sclerostin, BMD (g/cm^2) or the thickness of the intima media. However, we found a correlation between the thickness of the intima media and levels of bone alkaline phosphatase ($p=0.01$, $r=0.6$) and sclerostin ($p=0.05$, $r=0.5$).

Disease activity, measured by DAS-28-ESR, and cardiovascular risk, assessed by means of SCORE, modified SCORE and SCORE by carotid ultrasound, were not related.

Changes in DKK1, sclerostin and markers of bone remodeling after treatment

In the subgroup of patients who presented an EULAR response at 12 months of treatment, we found an increase in DKK1 levels at 12 months of treatment with biological therapy (baseline: 20.55±8.13 pg/ml vs 12 months: 31.20±4.88 pg/ml, p=0.03) (Figure 1), there were no changes in the levels of sclerostin (Figure 2). Regarding markers of bone remodeling, only an increase in osteocalcin levels was detected (baseline: 11.25±3.28 ng/ml vs 12 months 15.78±4.11 ng/ml, p=0.01).

In the subgroup of patients who did not present EULAR response at 12 months of treatment, no changes were found in the levels of DKK1, sclerostin, or markers of bone remodeling.

Changes in bone metabolism and cardiovascular risk after treatment

No statistically significant changes were detected after 12 months of treatment in the BMD (g/cm^2), in the thickness of the intima media (mm) or in the presence of carotid plaques.

Discussion

Our study found a statistically significant increase in DKK1 levels not associated with BMD changes or cardiovascular risk in the subgroup of patients who presented EULAR response at 12 months of treatment.

Different epidemiological studies have shown an association between the loss of bone mineral density, vascular calcification and cardiovascular morbidity and mortality[15-17]. The Wnt pathway is involved in the regulation of vascular calcification and in the differentiation of smooth muscle cells to osteoblasts[18]. Thus, an increase in the expression of DKK1 in carotid atherosclerotic plaques has been resported[19,20], as well as an increase in serum concentrations of sclerostin in patients with atherosclerotic disease and type 2 diabetes[21].

Furthermore, elevated circulating levels of DKK1 have been demonstrated in patients with RA, which were related to radiological damage[8,22-25], and the expression of sclerostin seems to correlate positively with DKK1 levels[9]. However, this is the first prospective study in which the effect of biological therapy on the inflammatory burden of the disease is analyzed, and its relationship with cardiovascular risk and bone metabolism taking into account Wnt pathway inhibitors (DKK1 and sclerostin).

Our preliminary study showed a decrease in DKK1 levels after 6 months of treatment[26], which was in agreement with that published by Briot et al.[13]. In this article, patients with active RA treated with tocilizumab experienced a decrease in DKK1 concentrations, as well as a decrease in markers of bone formation at 3 and 12 months of treatment, not finding changes in the levels of sclerostin. However, our 12-month results showed a statistically significant increase in the levels of DKK1 and ostecalcin in the subgroup of patients who achieved an EULAR response to treatment. These discordant results could be due to the sample size of our study. However, it has recently been reported that anti-TNFα produces in the short term (6 months) an increase in PTH levels and a decrease in DKK1, and that this increase in PTH could promote bone resorption and attenuate the normalization of serum levels of DKK1 in AR[27]. The authors suggest a direct relationship between TNFα and PTH, suppressing TNFα production of PTH. The anti-TNFα, therefore, would prevent this suppression and would lead to an increase in the levels of PTH and, secondarily, of DKK1. In this sense, in our study we found a decrease in serum levels of DKK1 at 6 months, without changes in PTH levels. In contrast, a non-significant PTH increase was detected at 12 months in the subgroup of patients with an EULAR response, which could explain the increase in DKK1 levels. In the case of the study by Briot et al., PTH levels were not analyzed, which could have influenced the levels of DKK1. In addition, most of the patients included in our study initiated treatment with anti-TNFα and only 5% with tocilizumab, so this difference in results could be due to a class effect of the drugs.

It should be noted as limitations of our study, the absence of control group and multivariate analysis, which could have limited the detection of a variable with confusing effect on the results found. However, it should be noted that the demographic, clinical and biological variables were similar both in the group of patients with an EULAR response to treatment at 12 months and in the group that did not present such response.

The follow-up time of patients could have influenced not finding changes in c-IMT and BMD, since published studies have found changes in c-IMT in patients with RA after 2 years of treatment with biological therapy[28]. In the case of BMD, despite studies in which changes were found at one year of treatment, most showed results at 2 years[29].

Table 1. Sociodemographic and clinical characteristics

N	18
Age, mean ± SD	45.22±14.47
Woman, n (%)	13 (72.2)
BMI, mean ± SD	30.39±7.85
HT, n (%)	2 (11.1)
DLP, n (%)	5 (27.8)
Drinking alcohol, n (%)	2 (11.1)
Smoking habit, n (%)	8 (44.4)

SD: standard deviation; BMI: body mass index; HT: arterial hypertension; DLP: dyslipidemia.

Table 2. Biochemical variables related to bone metabolism, disease activity and baseline cardiovascular risk, at 6 and 12 months

	Basal	**6 months**	**12 months**
Total cholesterol (mg/dl), mean ± SD	206±44.36	212.22±54.59 p=0.34	208.13±47.4 p=0.50
HDL-cholesterol (mg/dl), mean ± SD	53.22±11.72	52.40±19.34 p=0.27	51.20±16.4 p=0.72
LDL-cholesterol (mg/dl), mean ± SD	150.44±42.21	155.40±49.40 p=0.44	156±23.88 p=0.54
Triglycerides (mg/dl), mean ± SD	123.72±45.63	138.27±58.62 p=0.23	136.21±37.79 p=0.54
CRP (mg/l), mean ± SD Median	9.98±10.97 5	7.30±8.11 p=0.12 5	8.60±7.68 p=0.57 6
PTH-i (pg/ml), mean ± SD	41.58±17.23	45.23±12.12 p=0.86	49.36±15.89 p=0.12
25-OH vitamin D3 (ng/dl), mean ± SD	19.11±7.94	22.91±15.63 p=0.18	20.68±8.31 p=0.74
Bone alkaline phosphatase (µg/dl), mean ± SD	12.25±4.89	12.22±2.71 p=0.81	12.40±3.83 p=0.78
Osteocalcin (ng/ml), mean ± SD	12.83±5.51	15.59±8.99 p=0.18	17.72±6.52 p=0.002
CTX (ng/ml), mean ± SDE	0.27±0,11	0.32±0.16 p=0.19	1.83±5.80
DKK1 (pg/ml), mean ± SD Median	35.96±36.25 26.56	28.79±17.32 p=0.53 24.71	36.27±20.43 p=0.07 31.28
Sclerostin (ng/ml), mean ± SD Median	56.09±36.46 45.65	89.97±177.68 p=0.31 43.61	59.60±62.47 p=0.4 42.03
BMD (g/cm^2) - L2-L4, mean ± SD - Femoral neck, mean ± SD	 1.19±0.17 0.99±0.13		 1.19±0.22 p=0.22 0.93±0.15 p=0.5
DXA central - Normal, n (%) - Osteopenia, n (%) - Osteoporosis, n (%)	 14 (87.5) 1 (6.3) 1 (6.3)		 10 (83.3) 1 (8.3) 1 (8.3)

Table 2. (*cont.*)

	Basal	6 months	12 months
DAS-28-VSG, mean ± SD	4.51±0.99	3.39±1.36 p=0.001	3.31±1.24 p=0.001
VAS-disease, mean ± SD	6.52±1.94	3.66±2.74 p=0.001	3.20±3.12 p=0.002
Median	7	4	3
SCORE			
- Low, n (%)	11 (61.1)	11 (61.1)	9 (60)
- Moderate, n (%)	7 (38.9)	6 (33.3)	5 (33.3)
- High, n (%)	0 (0)	1 (5.6)	1 (6.7)
- Very high, n (%)	0 (0)	0 (0)	
SCORE modified			
- Low, n (%)	10 (55.6)	10 (55.6)	9 (60)
- Moderate, n (%)	6 (33.3)	5 (27.8)	3 (20)
- High, n (%)	2 (11.1)	2 (11.1)	3 (20)
- Very high, n (%)	0 (0)	1 (5.6)	
SCORE-ultrasound			
- Low, n (%)	8 (44.4)	9 (50)	7 (46.7)
- Moderate, n (%)	2 (11.1)	1 (5.6)	0 (0)
- High, n (%)	1 (5.6)	1 (5.6)	2 (13.3)
- Very high, n (%)	7 (38.9)	1 (38.9)	6 (40)
c-IMT right (mm), mean ± SD	0.55±0.15	0.55±0.16 p=0.92	0.57±0.17
c-IMT left (mm), mean ± SD	0.62±0.20 p=0.10	0.59±0.18	0.61±0.15
Carotid plates, n (%)	6 (33.3)	5 (27.8)	5 (33.03)

SD: standard deviation; PCR: C-reactive protein; c-IMT: carotid intima-media thickness.

In conclusion, we can say that in patients with active RA treated with biological therapy we have observed a significant increase in serum concentrations of DKK1 and osteocalcin, not finding any association with changes in BMD or cardiovascular risk. Therefore, studies with a larger sample size are needed to confirm these results, and to help define the role of DKK1 and sclerostin in RA and in the response to treatment with biological therapy.

Conflict of interests: The authors declare no conflict of interest.
The authors state they have observed the precepts of the Helsinki declaration on clinical studies.

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Figure 1. Changes in DKK1 concentrations after biological treatment in patients who achieved EULAR response

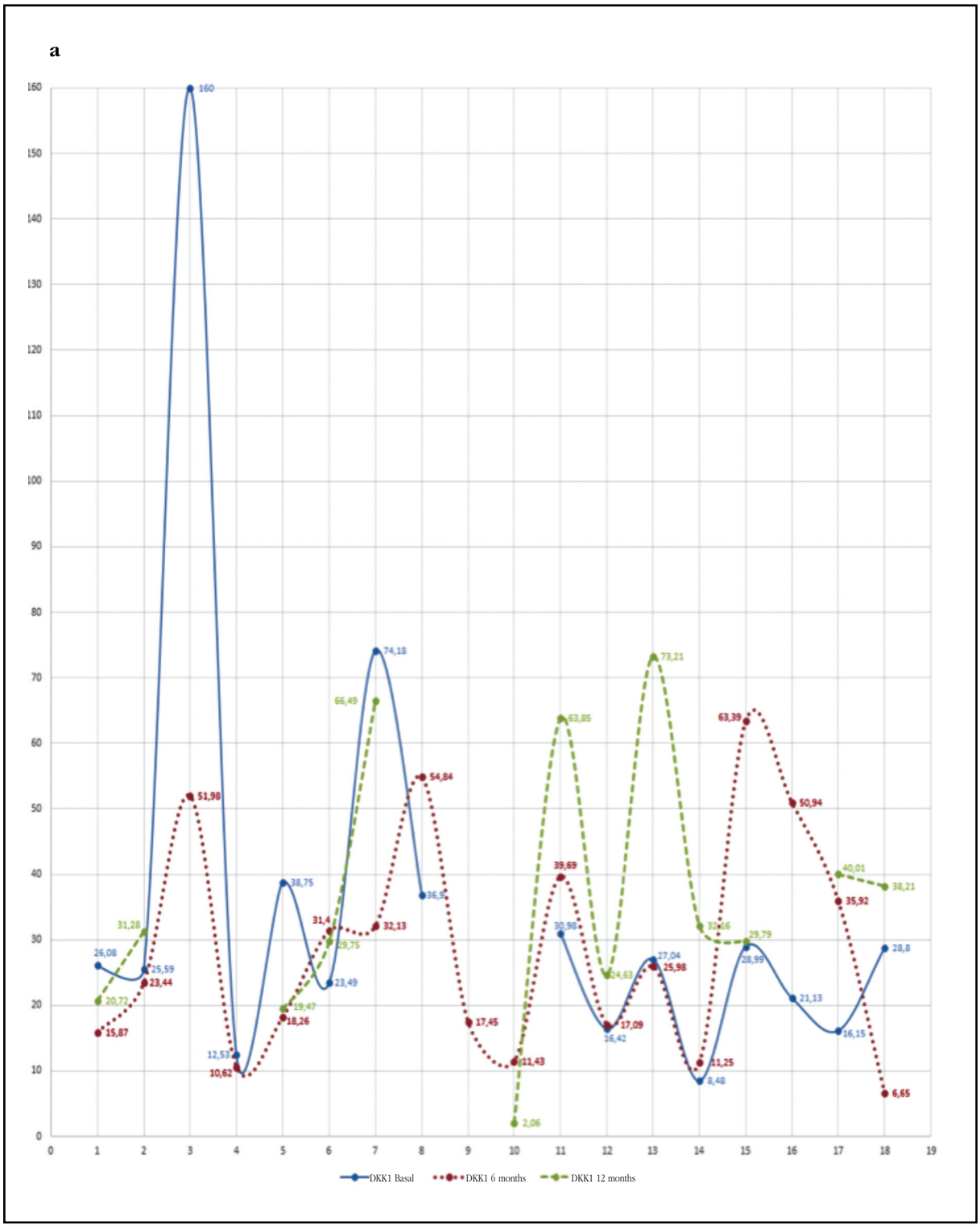

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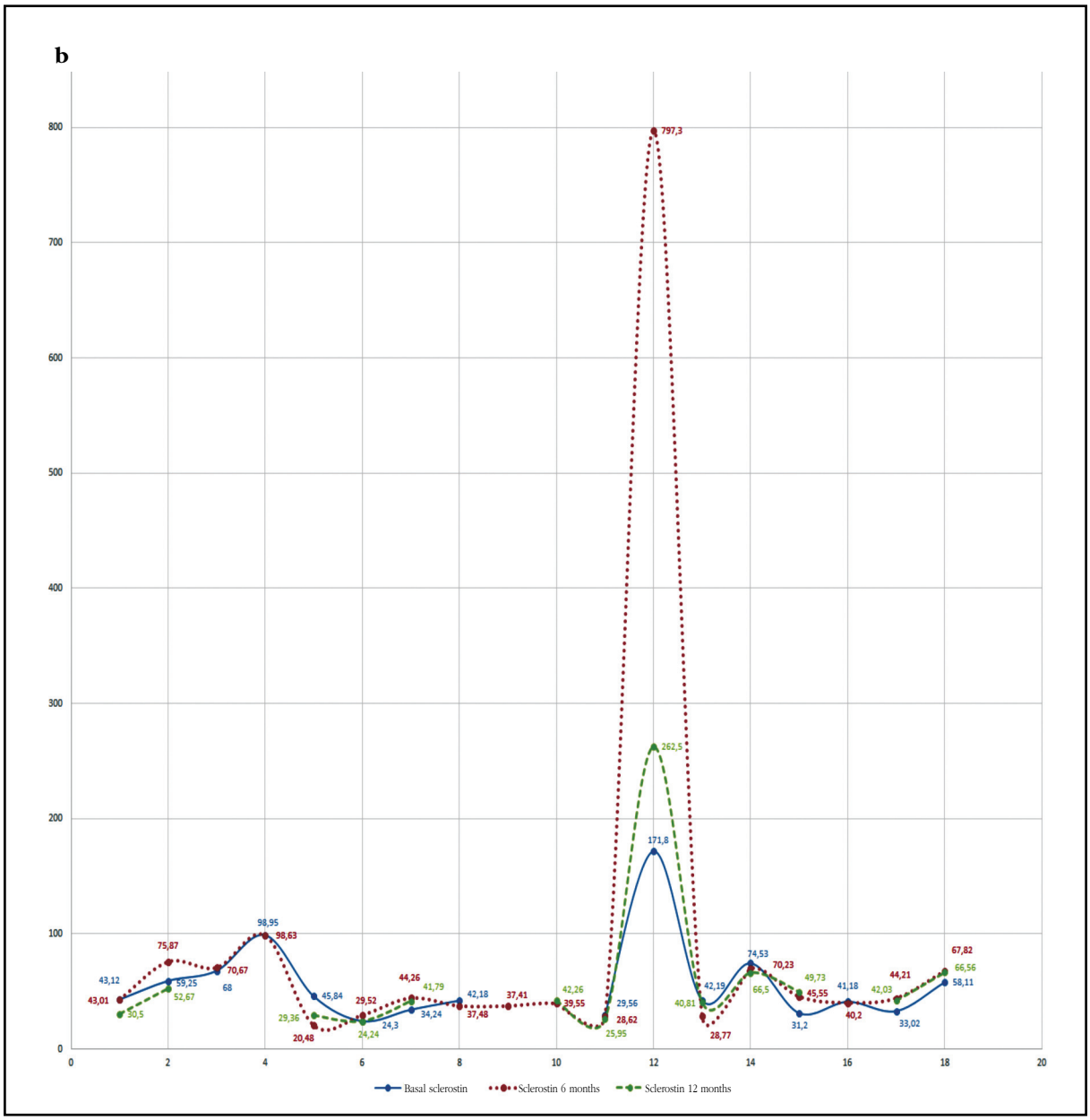
Figure 2. Changes in sclerostin concentrations after biological treatment in patients who achieved EULAR response



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