Arch Rheumatol 2023;38(1):148-155
doi: 10.46497/ArchRheumatol.2023.9974

ORIGINAL ARTICLE

Anti-tumor necrosis factor alpha treatment does not influence serum levels of the markers associated with radiographic progression in ankylosing spondylitis

Ali Erhan Özdemirel[1], Serdar Can Güven[2], Alper Doğancı[3], Zühre Sarı Sürmeli[4], Ayla Özyuvalı[5], Mehmet Kurt[6], Diana Rüstemova[7], Selin Hassan[8], Ayşe Peyman Yalçın Sayın[9], Hüseyin Tutkak[10], Şebnem Ataman[9]

[1]*Department of Rheumatology, Liv Hospital, Ankara, Türkiye*
[2]*Department of Rheumatology, Ankara City Hospital, Ankara, Türkiye*
[3]*Department of Physical and Rehabilitation Medicine, Erzurum Regional Training and Research Hospital, Erzurum, Türkiye*
[4]*Department of Rheumatology, Aydın State Hospital, Aydın, Türkiye*
[5]*Department of Physical and Rehabilitation Medicine, HFM Beyazpınar Physical Medicine And Rehabilitation Centre, Ankara, Türkiye*
[6]*Department of Physical and Rehabilitation Medicine, Dr. Ergun Özdemir Görele State Hospital, Giresun, Türkiye*
[7]*Department of Physical and Rehabilitation Medicine, Can Private Hospital, Manisa, Türkiye*
[8]*Department of Physical and Rehabilitation Medicine, Başkent University Medical School, Ankara, Türkiye*
[9]*Department of Rheumatology, Ankara University Medical School, Ankara, Türkiye*
[10]*Department of Immunology and Allergy, Ankara University Medical School, Ankara, Türkiye*

ABSTRACT

Objectives: The study aimed to determine the levels of change of the markers related to radiographic progression, such as Dickkopf-1 (DKK-1), sclerostin (SOST), bone morphogenetic protein (BMP)-2 and -4, and interleukin (IL)-17 and -23, in ankylosing spondyloarthritis (AS) during anti-tumor necrosis factor alpha (TNF-α) treatment.

Patients and methods: Fifty-three anti-TNF-α naïve AS patients (34 males, 19 females; median: 38 years; range, 20 to 52 years) refractory to conventional treatments meeting the modified New York criteria or Assessment of SpondyloArthritis International Society classification criteria were enrolled to this cross-sectional, controlled study between October 2015 and January 2017. Fifty healthy volunteers (35 males, 15 females; median: 36 years; range, 18 to 55 years) with similar age and sex characteristics were recruited. Serum DKK-1, BMP-2, BMP-4, SOST, IL-17, and IL-23 levels were measured in both groups. The serum levels of the markers were measured again after about two years (mean follow-up duration of 21.7±6.4 months) in AS patients who started anti-TNF-α treatment. Demographic, clinical characteristics, and laboratory parameters were recorded. The disease activity at the time of inclusion was assessed through the Bath Ankylosing Spondylitis Disease Activity Index.

Results: Serum DKK-1, SOST, IL-17, and IL-23 levels in the AS group before anti-TNF-α treatment were significantly higher compared to the control group ($p<0.01$ for DKK-1, $p<0.001$ for others). There was no difference regarding serum BMP-4 levels, whereas BMP-2 levels were significantly higher in the control group ($p<0.01$). Forty (75.47%) AS patients had serum marker levels measured after anti-TNF-α treatment. No significant change was observed in the serum levels of these 40 patients measured 21.7±6.4 months after the initiation of anti-TNF-α treatment ($p>0.05$ for all).

Conclusion: In AS patients, there was no change in DKK-1/SOST, BMP, and IL-17/23 cascade with anti-TNF-α treatment. This finding may suggest that these pathways act independently of each other, and their local effects are not influenced by systemic inflammation.

Keywords: Ankylosing spondylitis, bone morphogenetic protein, Dickkopf-1, sclerostin.

Received: October 17, 2022 ***Accepted:*** November 21, 2022 ***Published online:*** February 01, 2023

Correspondence: Ali Erhan Özdemirel, MD. Liv Hospital, Romatoloji Bölümü, 06680 Çankaya, Ankara, Türkiye.
E-mail: erhanozdemirel@hotmail.com

Citation:
Özdemirel AE, Güven SC, Doğancı A, Sarı Sürmeli Z, Özyuvalı A, Kurt M, et al. Anti-tumor necrosis factor alphatreatment does not influence serum levels of the markers associated with radiographic progression in ankylosing spondylitis. Arch Rheumatol 2023;38(1):148-155.

Ankylosing spondylitis (AS) is a chronic inflammatory disease affecting primarily sacroiliac joints, the spine, other joints, and entheses. Bone erosions, new bone formations, and ankylosis may occur due to chronic inflammation.[1] Syndesmophytes, which result from abnormal bone formation, have been stated to play a key role in radiographic damage and progression in AS patients. Syndesmophytes contribute to AS-related symptoms and findings and quicken deterioration in functional status.[2]

Syndesmophytes are assumed to develop secondary to the reparative process, which develops in response to inflammation and involves cartilage metaplasia.[3] Anti-tumor necrosis factor alpha (TNF-α) agents are effective in the treatment of AS; however, despite the rapid suppression of inflammation, the absence of a clear effect on the deceleration of radiographic progression in the short term (<2 years) aroused different hypotheses.[3] Consequently, the importance of local pathogenetic factors (e.g., altered gene expression and biomechanical factors) has been revealed. It is still unclear how the new bone formation that develops after inflammation gains autonomy and how it is triggered.[3]

It is extremely important to halt or delay ankylosis and control the regulation of new bone formation at the molecular level in AS. Wnt proteins are known to be potent inducers of new bone formation.[4] Natural inhibitors of Wnt, such as Dickkopf-1 (DKK-1) and sclerostin (SOST), neutralize Wnt activation and prevent new bone formation.[4] In a study, the blockage of DKK-1 led to the activation of the Wnt signaling pathway, resulting in large-scale growth in peripheral osteophytes and fusion in sacroiliac joints.[5] In the literature, there are studies indicating that DKK-1 and SOST levels are lower in AS patients in comparison to control subjects.[6,7]

Bone morphogenetic proteins (BMPs) are members of the TGF-β (transforming growth factor-β) family, which play a critical role in osteoblast differentiation by binding to mesenchymal cell surface receptors.[4] A study reported that autoantibodies that developed against noggin, a BMP inhibitor, were at higher levels in AS patients; thus, BMP function was enhanced, and new bone formation was induced.[8] Chen et al.[9] observed that AS patients with high levels of BMP-2, -4, and -7 were more prone to radiographic progression.

The interleukin (IL)-17/23 axis plays a significant role in the pathogenesis of AS, and increased levels have been demonstrated.[10] It is known that the inhibition of these cytokines effectively suppresses inflammation in AS patients. It has been stated that IL-23 is overexpressed in the entheses of AS patients and induces IL-17 and -22 production.[4] IL-22 has been reported to induce osteoblast differentiation and new bone formation through STAT (signal transducer and activator of transcription) 3 in entheses.[11] Additionally, *in vitro* studies have revealed that IL-23 and -17 induce osteoclastogenesis through receptor activator of nuclear factor kappa B (RANK) expression.[12] Therefore, the IL 17/23 axis seems to have pleiotropic effects on bone formation in AS patients.

In this study, we aimed to research whether both non-inflammatory (Wnt, BMP) and inflammatory (IL 17/23 axis) pathways, which are effective on bone formation in AS patients, are influenced by anti-TNF-α treatment, and to our best knowledge, this is the first study to concurrently investigate the effect of anti-TNF-α treatment on these three pathways.

PATIENTS AND METHODS

A cross-sectional study was conducted on AS patients who consecutively attended to the rheumatology outpatient clinic of the Ankara University Medical School, Department of Physical and Rehabilitation Medicine, Division of Rheumatology between October 2015 and January 2017. A total of 53 anti-TNF-α naïve AS patients (34 males, 19 females; median: 38 years; range, 20 to 52 years) with active disease (Bath Ankylosing Spondylitis Disease Activity Index [BASDAI] score of ≥4) who were refractory to conventional treatment (nonsteroidal anti-inflammatory drugs [NSAIDs] or sulfasalazine) and accepted to use anti-TNF-α treatment were enrolled. Patients who did not meet the modified New York criteria or the Assessment of SpondyloArthritis International Society (ASAS) classification criteria, who had previously received a TNF-α inhibitor or other anticytokine therapy,

used any immunosuppressive agents other than NSAIDs, sulfasalazine, or steroids (excluding local use) within the last three months, had a history of bone fracture within the last two years, received medical treatment for osteoporosis, were pregnant, had malignancy, acute infection, secondary amyloidosis, severe hepatic, renal, or cardiac disease, and any concomitant other rheumatic disease were excluded.[13-15] A control group was formed from 50 healthy volunteers (35 males, 15 females; median: 36 years; range, 18 to 55 years) with similar age and sex characteristics. After the inititation of the anti-TNF-α treatment, their previous medical treatments were discontinued, reduced, or continued during the treatment process according to the clinical course. The demographics, clinical characteristics, and BASDAI scores were recorded.

All laboratory assays were carried out in the same biochemical laboratory. Erythrocyte sedimentation rates (ESRs) and C-reactive protein (CRP) values were recorded. To evaluate DKK-1, SOST, IL-17, IL-23, BMP-2, and BMP-4 levels, venous blood samples were obtained after a minimum of 8 h of fasting. Samples for these biomarkers were collected in sterile containers and centrifuged within a maximum of 120 min at 4000 rpm for 10 min and then stored at –80°C until examination. The serum concentrations of these markers were assessed using commercial enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions (AviseraBioscience, Santa Clara, CA, USA for SOST; Boster Biological Technology, Fremont, CA, USA for IL-17, DKK-1, BMP-2, and BMP-4; eBioscience, Vienna, Austria for IL-23). The sensitivity of the DKK-1, SOST, BMP-2 and -4, and IL-7 and -23 kits was <15.6 pg/mL, 0.39 pg/mL, <2 pg/mL, and <1 pg/mL, respectively.

Statistical analysis

Statistical analyses were conducted using the IBM SPSS version 21.0 software (IBM Corp. Armonk, NY, USA). The variables were examined by visual (histograms and probability plots) and analytical (Kolmogorov-Smirnov test) methods to determine normal or nonnormal distributions. Continous variables were presented either by means ± standard deviations or medians (min-max) according to normality. The Mann-Whitney U test was performed for group comparisons. Categorical variables were presented by number and percentages and compared by the chi-square test. A p value <0.05 was considered statistically significant in all analyses.

RESULTS

The mean disease duration was 7.2±6.0 years. Median CRP and ESR values were 7.8

Table 1. Demographic and clinical characteristics of AS patients and healthy controls

	AS patients (n=53)					Healthy controls (n=50)					
	n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median	Min-Max	p
Age (year)				38	20-52				36	18-55	0.077
Sex											
Male	34	64.15				35	70				0.201
Female	19	35.85				15	30				
Disease duration (year)			7.2±6.0								
CRP (mg/L)				7.8	1-61.5						
ESR (mm/h)				11.5	1-51						
BASDAI (range 0-10)			5.3±3.1								
Using NSAIDs	48	90.56									
Using sulfasalazine	12	22.64									
Using NSAIDs and sulfasalazine	11	20.75									

AS: Ankylosing spondylitis; SD: Standard deviation; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; NSAIDs: Non-steroidal antiinflammatory drugs.

Table 2. Baseline biomarker levels in AS patients and healthy controls

	AS patients (n=53)		Healthy controls (n=50)		
	Median	Min-Max	Median	Min-Max	*p*
Dickkopf-1 (pg/mL)	5402.2	1666-23201.1	4362.7	928.3-9494.4	<0.01
Sclerostin (pg/mL)	145.3	33.2-1454.3	79.0	15-690.8	<0.001
Bone morphogenetic protein 2 (pg/mL)	51.5	2-1556.4	204.0	2-1326.7	<0.001
Bone morphogenetic protein 4 (pg/mL)	541.4	4-7203.7	501.6	3-2924.4	0.585
Interleukin 17 (pg/mL)	613.6	1-2324.0	288.2	35.7-1764.0	<0.001
Interleukin 23 (pg/mL)	54.7	12-401.9	14.9	4-102.7	<0.001

AS: Ankylosing spondylitis.

Table 3. Biomarker levels before and after treatment in AS patients who received anti-TNF-α treatment

	AS patients who received anti TNF-α (n=40)				
	Baseline		After treatment (21.7 months average)		
	Median	Min-Max	Median	Min-Max	*p*
Dickkopf-1 (pg/mL)	5278.4	1432-23201.1	5050.7	1354-21134.4	>0.05
Sclerostin (pg/mL)	141.6	39.2-1278.3	130.4	21-960.5	>0.05
Bone morphogenetic protein 2 (pg/mL)	59.5	2-1003.4	52.7	2-1244.6	>0.05
Bone morphogenetic protein 4 (pg/mL)	534.6	4-7764.2	578.0	4-8128.7	>0.05
Interleukin 17 (pg/mL)	567.6	1-2543.5	288.2	1-2202.6	>0.05
Interleukin 23 (pg/mL)	6	4-401.9	4	0-267	>0.05

TNF-α: Tumor necrosis factor alpha; AS: Ankylosing spondylitis.

(1-61.5) mg/L and 11.5 (1-51) mm/h, respectively. Most of the patients were under NSAIDs prior to anti-TNF-α treatment (n=48, 90.56%). The mean BASDAI score was 5.3±3.1. Demographics of AS patients and control subjects, as well as clinical characteristics of AS patients, are presented in Table 1.

Serum DKK-1, SOST, IL-17, and IL-23 levels before anti-TNF-α treatment in the AS group were significantly higher compared to the control group ($p<0.01$ for DKK-1, $p<0.001$ for others). There was no difference regarding serum BMP-4 levels, whereas BMP-2 levels were significantly higher in the control group ($p<0.01$, Table 2).

Of 53 AS patients, 40 had serum samples collected again after a mean of 21.7±6.4 months after anti-TNF-α treatment was initiated. The treatment of six out of the remaining 13 patients was changed due to ineffectiveness. The treatment of two patients was discontinued due to side effects (pneumonia in one patient, deep neutropenia in another patient), and five patients were lost to follow-up at the last visit. When all parameters (DKK-1, SOST, BMP-2, BMP-4, IL-17, and IL-23) were compared before and after anti-TNF-α treatment for 40 patients, no statistical difference was observed ($p>0.05$, Table 3).

DISCUSSION

The DKK-1, SOST, IL-17, and IL-23 levels of the AS patients were significantly higher compared to the control group, whereas BMP-2 levels were significantly lower and BMP-4 levels were similar to those of the control group in our study. The levels of these parameters did not

seem to be affected significantly by anti-TNF-α treatment.

In AS, inflammation in the vertebral entheses leads to erosions in the cartilage and bone followed by fibrous and adipose tissue infiltration and finally ossification, resulting in abnormal bone formation (syndesmophytes).[16] However, the relationship between these intertwined processes comprises very complex pathogenetic processes and has not been fully clarified. Focal fatty change at vertebral corners is one of the most significant markers for the development of syndesmophytes. Several studies revealed that there was no significant difference in radiographic progression after the use of anti-TNF-α in AS patients for two years, but starting treatment in the early period of the disease and receiving anti-TNF-α treatment for more than four years were observed to ameliorate radiographic progression.[3,4] This can be explained by activation of osteoclasts by TNF-α together with IL-17. Right afterward, the differentiation of TNF-α preadipocytes into adipocytes is accelerated, forming a basis for the development of syndesmophytes. However, the rapid administration of anti-TNF-α treatment may halt this transformation and prevent the development of fat infiltration after inflammation.[3] Furthermore, Descamps et al.[17] reported that SOST and BMP-7 levels increased in time in accordance with disease activity in axial spondyloarthritis, yet, the increase was less pronounced with anti-TNF-α treatment. Briefly, the earlier and more effectively the inflammation is suppressed at the vertebral corner, the more the radiographic progression is suppressed.

The Wnt pathway and natural inhibitors of this pathway, DKK-1 and SOST, have been investigated extensively in terms of syndesmophyte development in AS. Syndesmophyte formation has been shown to be higher in AS patients who have lower levels of DKK-1 and SOST.[16] Despite contradictory reports on serum levels of DKK-1, a meta-analysis stated that DKK-1 levels were generally higher in AS patients compared to the control group, as in our study.[18] On the other hand, it has also been reported that high DKK-1 levels are associated with increased AS risk and progression.[19] However, a reduction in the development of syndesmophytes would be expected in AS patients in this case, which is explained by the concept of functional DKK-1. Accordingly, total serum DKK-1 and functional DKK-1 levels are not correlated, and functional DKK-1 levels are lower in AS patients.[19,20] Another study reported that LRP (low-density lipoprotein receptor-associated protein) 5/6 binding of DKK-1 was reduced, resulting in the accelerated development of syndesmophytes due to the decrease in Wnt inhibition, and this decrease in binding was not influenced by the serum DKK-1 level.[21] All these findings indicate the importance of local factors in AS. In our study, the non-measurement of the functional level can be considered a limitation.

The changes in the radiographic progression related markers after anti-TNF-α treatment have also been investigated. Kwon et al.[6] reported no changes in serum DKK-1 levels after anti-TNF-α treatment in established AS. In another study, serum DKK-1 (not functional DKK-1) and radiographic progression (in the long term) were shown to decrease after anti-TNF-α treatment in patients with early AS.[22] Consequently, in this study, the DKK-1 level did not change in the long term, even though it was somehow affected by inflammation in the early period; however, its function at the local level was not investigated. This situation is noteworthy in terms of showing the importance of early and effective treatment and indicating a distinction between inflammatory and noninflammatory pathways in the long term. In our study, the patients' mean disease duration was 7.2±6.0 years, and the change in DKK-1 could not be examined in terms of disease duration in patients in the early period.

Our results demonstrated higher serum SOST levels in AS patients than those of the control group and were not affected by anti-TNF-α treatment. Theoretically, a high level of SOST is against radiographic progression, but the importance of local factors is visible in SOST, as in DKK-1. It has been reported that there is almost no SOST expression in AS patients' periarticular bones, and this can even be a condition specific to AS patients, which will reduce Wnt inhibition, resulting in the development of syndesmophytes.[23] In a study investigating whether the SOST level was affected by anti-TNF-α in AS patients, in which patients who received and did not receive anti-TNF-α therapy were cross-sectionally evaluated, the SOST level did not differ, similar to our results.[7] In a study investigating whether

the SOST level was affected by anti-TNF-α in AS patients, in which patients who received and did not receive anti-TNF-α were evaluated cross-sectionally, the SOST level did not differ, similar to our results.[7] In another study, SOST levels were observed to be low in AS patients, unlike in our study, and it was asserted that this might be associated with persistent inflammation and poor response to anti-TNF-α treatment.[24] However, since the mean disease durations of the patients included in this study are not known, it did not seem possible to make a direct comparison with our study, considering the possible changes in these biomarkers with disease duration.

Bone morphogenetic proteins pathway is another important pathway contributing to radiographic progression in AS. In a mouse model, BMP-2, -6, and -7 were shown to be immunohistochemically overexpressed in entheses.[25] In a study by Bleil et al.,[26] BMP-2 and -7 were contrarily shown to be at low levels in facet biopsies of AS patients. In a study conducted by Chen et al.,[9] serum BMP-2, -4, and -7 levels were increased and associated with radiographic progression. In our study, serum BMP-4 levels were similar to those of the control group, but BMP-2 levels were lower in the AS group. In another study on AS patients, fibroblasts cultured with BMP-2 exhibited more osteoblastic effects than osteoarthritis.[27] Thus, in pathogenetic understanding, the low serum BMP-2 level may not mean a reduced local osteoinductive effect in our study, contrary to overall findings. Another study demonstrated that inflammatory cytokines had an inducing effect on BMP-2 and -6 in the early period of the disease, which might indicate the beginning of the bone formation process that started after inflammation.[28] In our study, the absence of any change in BMP-2 and -4 levels before and after anti-TNF-α treatment can be explained, as in DKK-1, by the fact that our patients were not in the early disease period. In another study, increased levels of BMP-2 and -6 were observed in the synovium of AS patients.[29] In the same study, however, increased levels of the same BMPs were found in the synovium of patients with rheumatoid arthritis characterized by erosion in the joints, which was an interesting finding.[29] All these contradictory results indicate the importance of local factors, disease duration (early or late period), and local functionality of the markers that may contribute to progression rather than their serum levels and that these disease pathogenesis-based pathways may have a dual local effect.

Similar to the literature, serum IL-17 and -23 levels were found to be higher than the control group in our study.[4,30,31] The IL-17/23 axis has dual effects on radiographic progression in AS. Generally, IL-17 stimulates osteoclastogenesis, whereas IL-23 induces osteoblastogenesis through IL-22.[30] However, IL-23 indirectly contributes to osteoclastogenesis by inducing IL-17 and both IL-23 and -17 can contribute to osteoblastogenesis by elevating prostaglandin E2 (PGE2).[30,32] It was also reported that IL-17 activity downregulates DKK-1, which can potentially induce new bone formation.[33] The IL-23/17 axis seems to have quite pleiotropic effects on bone formation, and it is not clear yet which aspects outweigh at which stages. As in our study, it has previously been reported that this axis is not affected by anti-TNF-α treatment.[10]

Although a relatively homogeneous group in terms of treatment was tried to be formed before the initiation of anti-TNF-α treatment, anti-TNF-α treatment cannot be said to be fully effective on the markers we have checked since the use of NSAIDs has an effect on PGE2, PGE2 exhibits a synergistic effect with BMP-2, and the IL-23 axis affects PGE2. The small sample size is a major limitation, and due to the low number of patients, the local effects of the markers seem to be more important than their serum levels. Furthermore, we could not perform subgroup analyses regarding the effects of different anti-TNF-α agents. Our study also lacks a group including conventionally treated AS patients. Another limitation is that the effect of the markers on radiographic progression (with radiographic scoring) was not investigated according to a certain cut-off value (low *vs.* high), even at the serum level. Lastly, we think that the study could have provided more solid data if it had included patients in the early disease period.

In conclusion, new structural bone formation is the most important cause of functional limitation in AS. There are many inflammatory and noninflammatory pathways that contribute to this formation, and the relationship of these pathways with each other is extremely

complicated. Both the data in the literature and the results of our study indicate that these pathways are probably independent of each other, despite having a relative relation, particularly in the early disease period. Although the transition to the osteoproliferative process following inflammation in the entheses is difficult to clarify, particularly due to the prominence of local factors, early and effective treatment may be a prominent option to stop this process.

Ethics Committee Approval: The study protocol was approved by the Ankara University Medical School Ethics Committee (date: 11.03.2013, IRB: 04-160-13, 11.03.2013). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Concept and design: Özdemirel AE, Tutkak H, Yalçın Sayın AP, Ataman Ş. Supervision: Tutkak H, Yalçın Sayın AP, Ataman Ş, Data collection: Özdemirel AE, Doğancı A, Sarı Sürmeli Z, Özyuvalı A, Kurt M, Rüstemova D, Hassan S, Data analysis: Özdemirel AE, Güven SC. Interpretation of the data: Özdemirel AE, Güven SC. Writing the manuscript: Özdemirel AE, Güven SC. All authors approved the final version of the manuscript.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: This research received bursary from Ankara University The Scientific Research Projects Coordination Unit.

REFERENCES

1. McVeigh CM, Cairns AP. Diagnosis and management of ankylosing spondylitis. BMJ 2006;333:581-5. doi: 10.1136/bmj.38954.689583.DE.
2. Baraliakos X, Listing J, Rudwaleit M, Haibel H, Brandt J, Sieper J, et al. Progression of radiographic damage in patients with ankylosing spondylitis: Defining the central role of syndesmophytes. Ann Rheum Dis 2007;66:910-5. doi: 10.1136/ard.2006.066415.
3. Chiowchanwisawakit P, Lambert RG, Conner-Spady B, Maksymowych WP. Focal fat lesions at vertebral corners on magnetic resonance imaging predict the development of new syndesmophytes in ankylosing spondylitis. Arthritis Rheum 2011;63:2215-25. doi: 10.1002/art.30393.
4. Baum R, Gravallese EM. Bone as a target organ in rheumatic disease: Impact on osteoclasts and osteoblasts. Clin Rev Allergy Immunol 2016;51:1-15. doi: 10.1007/s12016-015-8515-6.
5. Uderhardt S, Diarra D, Katzenbeisser J, David JP, Zwerina J, Richards W, et al. Blockade of Dickkopf (DKK)-1 induces fusion of sacroiliac joints. Ann Rheum Dis 2010;69:592-7. doi: 10.1136/ard.2008.102046.
6. Kwon SR, Lim MJ, Suh CH, Park SG, Hong YS, Yoon BY, et al. Dickkopf-1 level is lower in patients with ankylosing spondylitis than in healthy people and is not influenced by anti-tumor necrosis factor therapy. Rheumatol Int 2012;32:2523-7. doi: 10.1007/s00296-011-1981-0.
7. Ustun N, Tok F, Kalyoncu U, Motor S, Yuksel R, Yagiz AE, et al. Sclerostin and Dkk-1 in patients with ankylosing spondylitis. Acta Reumatol Port 2014;39:146-51.
8. Tsui FW, Tsui HW, Las Heras F, Pritzker KP, Inman RD. Serum levels of novel noggin and sclerostin-immune complexes are elevated in ankylosing spondylitis. Ann Rheum Dis 2014;73:1873-9. doi: 10.1136/annrheumdis-2013-203630.
9. Chen HA, Chen CH, Lin YJ, Chen PC, Chen WS, Lu CL, et al. Association of bone morphogenetic proteins with spinal fusion in ankylosing spondylitis. J Rheumatol 2010;37:2126-32. doi: 10.3899/jrheum.100200.
10. Milanez FM, Saad CG, Viana VT, Moraes JC, Périco GV, Sampaio-Barros PD, et al. IL-23/Th17 axis is not influenced by TNF-blocking agents in ankylosing spondylitis patients. Arthritis Res Ther 2016;18:52. doi: 10.1186/s13075-016-0949-6.
11. Kang YK, Zhang MC. IL-23 promotes osteoclastogenesis in osteoblast-osteoclast co-culture system. Genet Mol Res 2014;13:4673-9. doi: 10.4238/2014.June.18.10.
12. Yago T, Nanke Y, Kawamoto M, Furuya T, Kobashigawa T, Kamatani N, et al. IL-23 induces human osteoclastogenesis via IL-17 in vitro, and anti-IL-23 antibody attenuates collagen-induced arthritis in rats. Arthritis Res Ther 2007;9:R96. doi: 10.1186/ar2297.
13. van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361-8. doi: 10.1002/art.1780270401.
14. Rudwaleit M, van der Heijde D, Landewé R, Listing J, Akkoc N, Brandt J, et al. The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis (part II): Validation and final selection. Ann Rheum Dis 2009;68:777-83. doi: 10.1136/ard.2009.108233.
15. Rudwaleit M, van der Heijde D, Landewé R, Akkoc N, Brandt J, Chou CT, et al. The Assessment of SpondyloArthritis International Society classification criteria for peripheral spondyloarthritis and

for spondyloarthritis in general. Ann Rheum Dis 2011;70:25-31. doi: 10.1136/ard.2010.133645.
16. Cici D, Corrado A, Rotondo C, Cantatore FP. Wnt signaling and biological therapy in rheumatoid arthritis and spondyloarthritis. Int J Mol Sci 2019;20:5552. doi: 10.3390/ijms20225552.
17. Descamps E, Molto A, Borderie D, Lories R, Richard CM, Pons M, et al. Changes in bone formation regulator biomarkers in early axial spondyloarthritis. Rheumatology (Oxford) 2021;60:1185-94. doi: 10.1093/rheumatology/keaa296.
18. Zhang L, Ouyang H, Xie Z, Liang ZH, Wu XW. Serum DKK-1 level in the development of ankylosing spondylitis and rheumatic arthritis: A meta-analysis. Exp Mol Med 2016;48:e228. doi: 10.1038/emm.2016.12.
19. Daoussis D, Liossis SN, Solomou EE, Tsanaktsi A, Bounia K, Karampetsou M, et al. Evidence that Dkk-1 is dysfunctional in ankylosing spondylitis. Arthritis Rheum 2010;62:150-8. doi: 10.1002/art.27231.
20. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, et al. Dickkopf-1 is a master regulator of joint remodeling. Nat Med 2007;13:156-63. doi: 10.1038/nm1538.
21. Cortes A, Maksymowych WP, Wordsworth BP, Inman RD, Danoy P, Rahman P, et al. Association study of genes related to bone formation and resorption and the extent of radiographic change in ankylosing spondylitis. Ann Rheum Dis 2015;74:1387-93. doi: 10.1136/annrheumdis-2013-204835.
22. Haroon N, Inman RD, Learch TJ, Weisman MH, Lee M, Rahbar MH, et al. The impact of tumor necrosis factor α inhibitors on radiographic progression in ankylosing spondylitis. Arthritis Rheum 2013;65:2645-54. doi: 10.1002/art.38070.
23. Appel H, Ruiz-Heiland G, Listing J, Zwerina J, Herrmann M, Mueller R, et al. Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. Arthritis Rheum 2009;60:3257-62. doi: 10.1002/art.24888.
24. Saad CG, Ribeiro AC, Moraes JC, Takayama L, Goncalves CR, Rodrigues MB, et al. Low sclerostin levels: A predictive marker of persistent inflammation in ankylosing spondylitis during anti-tumor necrosis factor therapy? Arthritis Res Ther 2012;14:R216. doi: 10.1186/ar4055.
25. Lories RJ, Derese I, Luyten FP. Modulation of bone morphogenetic protein signaling inhibits the onset and progression of ankylosing enthesitis. J Clin Invest 2005;115:1571-9. doi: 10.1172/JCI23738.
26. Bleil J, Sieper J, Maier R, Schlichting U, Hempfing A, Syrbe U, et al. Cartilage in facet joints of patients with ankylosing spondylitis (AS) shows signs of cartilage degeneration rather than chondrocyte hypertrophy: Implications for joint remodeling in AS. Arthritis Res Ther 2015;17:170. doi: 10.1186/s13075-015-0675-5.
27. Yang M, Yuan H, Miao M, Xu W. The osteogenic potential of ligament fibroblasts is greater in ankylosing spondylitis patients than in patients with osteoarthritis. Z Rheumatol 2015;74:340-5. doi: 10.1007/s00393-014-1394-z.
28. Lories RJ, Derese I, Luyten FP. Modulation of bone morphogenetic protein signaling inhibits the onset and progression of ankylosing enthesitis. J Clin Invest 2005;115:1571-9. doi: 10.1172/JCI23738.
29. Kuratani T, Nagata K, Kukita T, Hotokebuchi T, Nakasima A, Iijima T. Induction of abundant osteoclast-like multinucleated giant cells in adjuvant arthritic rats with accompanying disordered high bone turnover. Histol Histopathol 1998;13:751-9. doi: 10.14670/HH-13.751.
30. Rossini M, Viapiana O, Adami S, Idolazzi L, Fracassi E, Gatti D. Focal bone involvement in inflammatory arthritis: The role of IL17. Rheumatol Int 2016;36:469-82. doi: 10.1007/s00296-015-3387-x.
31. Appel H, Maier R, Bleil J, Hempfing A, Loddenkemper C, Schlichting U, et al. In situ analysis of interleukin-23- and interleukin-12-positive cells in the spine of patients with ankylosing spondylitis. Arthritis Rheum 2013;65:1522-9. doi: 10.1002/art.37937.
32. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 1999;103:1345-52. doi: 10.1172/JCI5703.
33. Daoussis D, Kanellou A, Panagiotopoulos E, Papachristou D. DKK-1 is underexpressed in mesenchymal stem cells from patients with ankylosing spondylitis and further downregulated by IL-17. Int J Mol Sci 2022;23:6660. doi: 10.3390/ijms23126660.