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Rapid changes in bone formation regulator biomarkers in early axial spondyloarthritis

Authors

Elise Descamps, MD (1), Anna Molto, MD (1), Didier Borderie, MD, PhD (2), Rik Lories, MD, PhD (3), Corinne Miceli Richard, MD, PhD (1), Marion Pons, MD (1), Christian Roux, MD, PhD (1), Karine Briot, MD (1)

Affiliations

(1) Department of Rheumatology, Cochin Hospital, and Epidemiology and Biostatistics Unit, Sorbonne Paris Cite Research Center, Paris Descartes University, INSERM U1153, Paris

(2) Department of Biology, Cochin Hospital, Paris Descartes University

(3) Skeletal biology and Engineering Research Center, KU Leuven and Division of Rheumatology, University Hospitals Leuven, Belgium.

Corresponding author:

Dr Karine Briot, MD, Department of Rheumatology, Hôpital Cochin, 2

27 rue du Faubourg Saint-Jacques, 75014 Paris, France. E-mail : karine.briot@aphp.fr

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Key messages:

- Serum BMP-7 and serum sclerostin levels significantly increased over 5 years in early axial spondyloarthritis patients whereas serum DKK-1 did not change.
- Serum BMP-7 had a rapid increase over 5 years with a median increase of 223.7% (0%, 10700%). The proportion of patients with undetectable values was 48% at baseline, 20.2% at two years and 13.3% at five years.
- The increase in serum BMP-7 was more pronounced in patients with active disease (ASDAS-CRP ≥ 1.3) but less with pronounced TNFi use
- Serum sclerostin was correlated at baseline with bone formation and negatively with CRP level.

ABSTRACT

Objectives: The hallmark of advanced axial spondyloarthritis (axSpA) is spine ankylosis, due to excessive ectopic bone formation. This prospective study aimed to describe the changes in serum levels of different regulators (sclerostin, dickkopf-1 (DKK-1)) and markers of bone formation (bone morphogenetic protein 7 (BMP-7)) over 5 years in early axSpA patients, and to assess determinants of such changes.

Methods: The DESIR cohort is a prospective, multicenter French study of 708 patients with early (> 3 months and < 3 years) inflammatory back pain suggestive of axSpA. Serum levels of BMP-7, sclerostin and DKK-1 were assessed at baseline, after two and five years. Changes in bone formation regulators over time were analysed using mixed linear models. Causal inference was done by multivariable analysis.

Results: Serum BMP-7 significantly increased over time with a median relative change of 223.7% (IQR: 0%, 10100%) i.e. 0.17 pg/mL per month ($p < 0.001$). Serum sclerostin significantly increased over time with a median relative change of 14.8% (-7.9%, 41.4%) i.e. 0.001 ng/mL per month ($p < 0.001$). Serum DKK-1 did not significantly change over time. Serum BMP-7 changes increased over time in active disease (ASDAS-CRP ≥ 1.3 , $p = 0.01$) and increased less over time with a dose effect of TNF inhibitors (TNFi) ($p < 0.001$). No determinant was associated with serum sclerostin change.

Conclusion: Serum BMP-7 levels significantly increased over time and changes were related to TNFi use and disease activity. Serum sclerostin levels significantly increased over time but to a lesser degree than serum BMP-7.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease with symptoms related to sacroiliitis, spinal inflammation, peripheral enthesitis and arthritis. In advanced axSpA, the hallmark of structural damage is ectopic new bone formation leading to the formation of syndesmophytes potentially resulting in spine ankylosis. AxSpA raises a paradox: patients have both systemic and local bone loss secondary to inflammation, and local new bone formation[1]. Local inflammation appears to be crucial to trigger the new bone formation process in rodent models of disease [2]. However, the relationship between inflammation assessed by Magnetic Resonance Imaging (MRI) and bone formation in radiographs remains unclear as new bone formation develops at sites with and without prior identified inflammation[3]. For a long time, Tumour necrosis factor inhibitors (TNFi) have failed to prove their efficacy to inhibit radiographic progression[4–6]. Recent studies have shown the benefit of TNFi on structural damage in sacroiliac joints (SIJ)[7] and on spinal radiographic progression in axSpA with early initiation of TNFi and a long duration of follow up[8–10]. Recent data also suggest that mechanical strain drives both inflammation and new bone formation in SpA [11,12].

Local bone formation likely results from an endochondral ossification process. The pathways underlying this phenomenon include the wingless-type like (Wnt) signalling and bone morphogenetic proteins (BMP). Sclerostin and Dickkopf-1 (DKK-1), potent inhibitors of bone formation, bind Wnt co-receptors to directly inhibit Wnt binding and signalling[13]. BMPs are members of the transforming growth factor- β superfamily and were originally identified for their bone-inductive capacity. Many BMPs are suggested play a role in different stages of the ankylosis process of a mouse model: BMP-2 in early stage, BMP-7 (in pre-hypertrophic

chondrocytes) and BMP-6 (in hypertrophic chondrocytes) in advanced stages[14]. These processes are highly regulated at different stages[15].

In axSpA, serum sclerostin levels were low in patients with new syndesmophytes[16]. In an animal model of SpA, blockade of DKK-1 promoted ankylosis of the sacroiliac joints[17]. In axSpA, low levels of serum DKK-1 were associated with new bone formation[18]. In a mouse model of SpA and in human enthesal biopsies, activation of the BMP signalling cascade has been demonstrated in peripheral enthesal lesions[14].

Our hypothesis was that modulators of pathways associated with bone formation may change over time and that these changes could be related to inflammation or use of “anti-inflammatory” drugs (such as non-steroidal anti-inflammatory drugs (NSAIDs) and/or TNFi).

The aim of our study was to describe the 5-year changes (relative to baseline) in bone formation modulators (sclerostin, DKK-1) and markers (BMP-7) in early axSpA. We also further analysed parameters associated with changes of each biomarkers.

MATERIALS AND METHODS

Patients

DESIR is a large national multicenter cohort of patients with early inflammatory back pain (IBP) highly suggestive of axSpA[19]. Patients included had IBP of recent onset (> 3 months and < 3 years) with symptoms suggestive of SpA according to the rheumatologist's assessment (score ≥ 5 on a 0-10 numerical rating scale, where 0 = not suggestive and 10 = very suggestive). Patients were followed every 6 months during the first 2 years then yearly; the follow-up of the 10th year is ongoing and a 20-year follow-up is planned. For this study, we used data of the first 5 years of follow-up.

Bone formation regulator assessment

The serum samples were prospectively collected and aliquoted in the Biological Resources Center at Bichat Hospital (Certificate number 34457). Serum levels of BMP-7, sclerostin and DKK-1 were assessed at baseline, 2 and 5 years in all serum available. Serum BMP-7 level was assessed with a human BMP-7 Quantikine ELISA Kit (R&D Systems). Results are expressed in picogram per millilitre (pg/mL), normal reference range is inferior to 31.3 pg/mL. Sclerostin serum level was assessed with the human sclerostin HS EIA kit (TECO medical). Results are expressed in nanogram per millilitre (ng/mL). Normal reference ranges are 0.48 ± 0.14 ng/mL and 0.64 ± 0.15 ng/mL in premenopausal women and men respectively [20]. Serum DKK-1 level was assessed with the human DKK-1 Quantikine sandwich ELISA kit (R&D Systems), results are expressed in pg/mL.

Imaging assessment

All imaging scoring were evaluated by three trained central readers, who were blinded to any other data (details of this reading has been described elsewhere[7]). MRI of cervical, dorsal

and lumbar spine and SIJ were available at baseline (N = 692) and (per protocol) in 9 centers at 2 (N = 209) and 5 years (N = 173). Inflammatory lesions were assessed using the SPARCC[21] (SPondyloArthritis Research Consortium of Canada) at spine and at SIJ. This score quotes inflammatory lesion in the spine on T2-STIR (weighting, depth, intensity) on the 6 most inflammatory vertebrae, each divided into quadrants, ranging from 0 to 108. For the SIJ SPARCC, bone marrow oedema on T2-STIR (depth, intensity), enthesitis and capsulitis are scored on each SIJ divided into quadrants, ranging from 0 to 48. Patients had plain radiographs of the cervical, dorsal, lumbar spine and pelvis at baseline, 1, 2 and 5 years. Structural damages of cervical and lumbar spine were assessed with the radiographic score mSASSS[22] (modified Stoke Ankylosing Spondylitis Spine Score), ranging from 0 to 72. Structural damage of the SIJ was assessed according to the modified New York criteria[23].

Per protocol, 14 of the 26 centers performed bone mineral density (BMD) measurements with dual-energy X-ray (DXA) at hip and lumbar spine at baseline (N = 342), 2 (N= 276) and 5 (N = 210) years. BMD (g/cm²) was determined at the lumbar spine (second-fourth vertebrae) and the upper part of the left femur. In view of the age of the studied population, the Z-score was used. Low BMD was defined as Z score ≤ -2 (at at least at one site) according to International Society of Clinical Densitometry[24].

Statistical analysis

Variables for the statistical analysis were demographic characteristics of the patients (age, gender, weight (kg), body mass index (kg/cm²), cigarette smoking status, disease characteristics (HLA B27 status, ASAS criteria), disease activity (BASDAI[25], ASDAS-CRP[26], CRP (mg/L)), imaging (mSASSS, modified New York grading, SPARCC at spine and SIJ), BMD at lumbar spine and hip, Z score, treatments (NSAIDs e.g. the ASAS-NSAID score[27] and TNFi).

Quantitative data were expressed in mean [95% confidence interval] or median (interquartile range (IQR)). Qualitative data were expressed in number (%). The correlation between serum level of bone markers and variables was evaluated by Pearson's correlation coefficient (r) or Spearman's correlation coefficient (r_s).

We used mixed linear model to describe the distribution of each serum marker over the 5 years of follow-up and to assess the longitudinal relationship between biomarkers and other disease and treatment data, as described previously. At first, we evaluated whether serum biomarkers changed significantly over time in complete cases; if no significant change was observed, no further analyses were performed. Then, in all data available, we evaluated the interaction between time and the variables associated with significant change of serum markers over time. Variables identified as significantly associated with biomarkers serum levels on univariate analysis ($p < 0.10$) were entered into multivariate linear mixed model (with patient and center as random effects). In multivariate analysis, $p < 0.05$ was considered statistically significant. Goodness of fit of the different models was determined by the Akaike information criterion (AIC), and the models with the lowest AIC were chosen. All analyses were performed using the free software R version 3.4.2 (2017-09-28).

RESULTS

708 patients were included in DESIR cohort of which 327 were men (46.2%). The mean age at baseline was 33.7 [33.1, 34.4] years. Overall, 410/707 (58.0%) of the patients were HLA-B27 positive, 425/707 (60.1%) fulfilled the ASAS criteria for axSpA and 92/684 (13.5%) the modified New York criteria. At baseline, ASDAS-CRP was 2.7 [2.6, 2.7], mean mSASSS was 0.41 [0.29, 0.53] (Table 1). During the first five years of the cohort, 258 patients (36.4%) were exposed to at least one TNFi with a mean duration of 4 [3.9, 4.2] years.

Baseline values of regulators of bone formation

Serum BMP-7

At baseline (N = 702), mean serum BMP-7 was 11.0 [8.9, 13.0] pg/mL. Median serum BMP-7 was 2.1 (0, 15.7) pg/mL. In 337 (48%) patients, BMP-7 serum levels were below the detection limit. Serum BMP-7 was weakly correlated with weight ($r_s = 0.10$, $p = 0.008$), inversely with ASDAS-CRP ($r_s = -0.08$, $p = 0.047$) but not with CRP. Serum BMP-7 was higher in patients with sacroiliitis in radiograph (14.9 [4.1, 25.6] pg/mL vs 10.3 [8.5, 12.0] pg/mL, $p < 0.001$) or at least with one syndesmophyte (12.8 [3.8, 21.7] pg/mL vs 10.8 [8.7, 12.9] pg/mL, $p < 0.001$). Serum BMP-7 was correlated with BMD in lumbar spine and hip ($r = 0.16$, $p = 0.003$ and $r = 0.16$, $p = 0.002$, respectively) and was lower in low BMD (6.2 [4.3, 8.2] pg/mL vs 9.2 [7.5, 10.8] pg/mL, $p = 0.03$).

Serum sclerostin

At baseline (N = 702), mean serum sclerostin was 0.45 [0.44, 0.46] ng/mL. Median serum sclerostin was 0.43 (0.33, 0.54). Serum sclerostin was correlated with age ($r = 0.28$, $p < 0.001$), weight ($r = 0.10$, $p = 0.007$), mSASSS ($r = 0.08$, $p = 0.03$), syndesmophytes ($r = 0.15$, $p < 0.001$),

CRP level ($r_s = -0.14$, $p < 0.001$), BMD in lumbar spine and hip ($r = 0.18$, $p = 0.001$ and $r = 0.19$, $p < 0.001$, respectively).

Serum DKK-1

Serum DKK-1 was 5413.0 [5237.8, 5588.4] pg/mL at baseline ($N = 702$). Median serum DKK-1 was 4936 (3871, 6656). Serum DKK-1 was weakly correlated with CRP level ($r_s = 0.09$, $p = 0.01$) and BMD in femoral neck ($r = 0.12$, $p = 0.03$).

Biomarkers correlation

Serum DKK-1 was slightly correlated with serum sclerostin ($r_s = -0.10$, $p = 0.008$) and serum BMP-7 ($r_s = 0.14$, $p < 0.001$).

Five-year changes in biomarkers

Serum BMP-7

At 2 ($N = 550$) and 5 ($N = 443$) years, serum BMP-7 was 20.3 [18.7, 21.9] pg/mL and 20.9 [19.3, 22.6] pg/mL and proportion of patients with undetectable value was 20.2% and 13.3% respectively. Serum BMP-7 significantly increased over 5 years, median increase was 223.7% (0%, 10700%), i.e. of 0.17 pg/mL per month ($p < 0.001$) (Figure 1).

In univariate analysis, 5-year increase of BMP-7 was more pronounced in males ($p = 0.03$), in patients with ASDAS-CRP ≥ 1.3 ($p = 0.01$) but less in patients treated with TNFi ($p = 0.007$) with a time effect of TNFi use ($p = 0.007$). In multivariate analysis, serum BMP-7 increased more in patients with ASDAS-CRP ≥ 1.3 (0.19 pg/mL per month vs 0.06 pg/mL per month in inactive disease, $p = 0.01$) but the increase was less pronounced in TNFi use (decrease of 0.03 pg/mL per month of TNFi use, $p < 0.001$) (Figure 2).

Serum sclerostin

At 2 (N = 556) and 5 (N = 443) years, serum sclerostin levels were 0.46 [0.45, 0.47] ng/mL and 0.51 [0.49, 0.53] ng/mL, respectively. Serum sclerostin increased over time, median increase was 14.8% (-7.9%, 41.4%), i.e. 0.001 ng/mL per month ($p < 0.001$) (Figure 3).

In univariate analysis, serum sclerostin increased with age ($p < 0.001$), more in men ($p = 0.03$) but less in smokers ($p = 0.04$). In multivariate analysis, no determinant was associated with serum sclerostin change.

Serum DKK-1

At 2 (N=556) and 5 years (N=442) respectively, serum DKK-1 was 5621.3 [5407.6, 5835.0] pg/mL and 4998.2 [4728.9, 5267.6] pg/mL. Serum DKK-1 decreased over 5 years with a median decrease of 4.7% (-33.3%, 29.2%), i.e. 5.9 pg/mL per month but not significantly ($p = 0.09$). Thus, as defined a priori, no further analysis were performed.

5-year SPARCC changes and 5-year BMD changes according to bone formation markers

SPARCC changes were associated with gender, smoking status, sacroiliitis but not with bone markers. BMD in lumbar spine changes were associated with gender, weight, mSASSS and sacroiliitis and for BMD in hip with inflammatory bowel disease, sacroiliitis and TNFi use but neither with bone markers.

DISCUSSION

This study shows a significant increase in BMP-7 over 5 years in a large cohort of early axSpA patients. Changes in serum BMP-7 were impacted by disease activity and use of TNFi, as a high increase in active disease and a low increase were observed in treated patients. Sclerostin serum increased over time but changes were not clinically relevant. Serum DKK-1 did not change over time. We did not show a significant association between bone markers and SPARCC as for BMD.

In other cohorts of advanced axSpA (mean disease duration of 12.7 ± 11.3 years), serum BMP-7 were higher in axSpA than controls (61.5 ± 33.0 pg/mL vs 36.7 ± 12.1 pg/mL) [28,29]. But there are some discrepancies about the value of serum BMP-7 in healthy controls. In a study of patients with pulmonary arterial hypertension, using the same kit, in healthy controls, median serum BMP-7 was 2.5 (0.9, 6.6) pg/mL[30]. In our study, median serum BMP-7 at baseline was 2.1 (0, 15.7) pg/mL close to that reported in healthy controls. Over 2 years, we showed that serum BMP-7 had a rapid median increase of 308% and reach a median value of 223.7% at 5 years. In a cohort of advanced axSpA, serum BMP-7 was correlated with sacroiliitis, Bath Ankylosing Spondylitis Radiology Index (BASRI), mSASSS and disease duration but not with CRP[28] as seen in our cohort.

Appel *et al.* demonstrated that in axSpA, sclerostin expression was significantly lower than controls, patients with rheumatoid arthritis (RA) and osteoarthritis[16]. In our study, sclerostin serum levels were lower than the values reported in other studies (due to different characteristics of population, different kits) and below the normal threshold value of the laboratory[20]. We confirmed previous studies which showed that serum sclerostin increased with age[31], was associated with CRP level and structural changes[33].

In a cohort of advanced axSpA patients, serum DKK-1 was correlated with mSASSS ($r = 0.24$) and lumbar spine Z score ($r = -0.25$) [32] whereas in our cohort serum DKK-1 was correlated with BMD in femoral neck and systemic inflammation.

There are few studies that assessed prospectively change in serum BMP-7, sclerostin and DKK-1 in axSpA. We showed a significant increase in BMP-7 over 5 years, changes were related by use of TNFi and disease activity. In a cohort of 40 recent axSpA, after a mean follow up of 51.7 ± 19.7 months, serum BMP-7 significantly increased, changes were correlated with BASRI, use of TNFi was not analysed [33]. Korkosz *et al.* studied changes of serum BMP-7 over 6 months in 40 non-recent (8.3 ± 5.6 years) axSpA treated with TNFi or NSAIDs[34]. In TNFi use, serum BMP-7 significantly increased from 1.4 pg/mL to 20.3 pg/mL whereas in NSAIDs use, it did not change. Wendling *et al.* demonstrated in 13 non-recent axSpA, after 10 weeks of TNFi use, serum BMP-7 did not change[35].

In recent axSpA naive of TNFi, serum sclerostin mean value at 2 years was significantly higher in axSpA without syndesmophyte growth than in axSpA with new syndesmophytes[16]. Saad *et al.* compared serum sclerostin over 1 year between 30 non-recent (11.9 ± 9.3 years) axSpA after initiation of TNFi and 30 controls[36]. Serum sclerostin was lower in axSpA (60.5 ± 32.7 pmol/L) than in controls (96.7 ± 52.9 pmol/L) and significantly increased over time (mean was 16.8% in 1 year) after initiation of TNFi. Our study showed different results with a low increase on serum sclerostin over 5-year and no effect of TNFi. Korkosz *et al.* showed that serum sclerostin remained unchanged over 6 months in TNFi or NSAIDs use.

In axSpA naive of TNFi, serum DKK-1 mean value at 2 years was significantly higher in axSpA without syndesmophyte growth than in axSpA with new syndesmophytes[18]. In advanced

axSpA, serum DKK-1 significantly decreased after 6 months of TNFi use[34]. In our cohort, serum DKK-1 did not change over time.

Studies demonstrated that NSAIDs use prevent new bone formation[37,38]. We did not show change of serum biomarkers according to NSAIDs. This difference could be related to the intermittent use of NSAIDs and the low NSAIDs intake.

In our study, disease activity and TNFi were associated with change of serum BMP-7 over 5 years. Experimental studies demonstrated the link between bone formation and inflammation. In entheses of patients with axSpA, Laloux *et al.* described persistent oedema with an inflammatory infiltrate of lymphocytic CD8+[39]. Lories *et al.* demonstrated in arthritic synovium from SpA and RA patients that proinflammatory cytokines (IL-1 and TNF α) were associated with an increase of BMP-2 and BMP-6[40]. However, in a mouse model of SpA, inhibition of TNF α was not associated with bone formation[41]. Bone formation regulator pathways seemed to be involved with systemic inflammation and thus can explain positive effect of TNFi to prevent bone formation. However, change of serum BMP-7 was very low in TNFi use and the effect of TNFi was weak.

Our study had several strengths. First, we used data from a large cohort of early axSpA, with a prospective follow up and assessment of bone formation regulators over 5 years. Secondly, in this longitudinal study, in order to overcome the non-independency of observations related to repeated measurements over time, we used mixed linear model, taking patient and center effect as random effects in our models.

However, our study had limitations. Since DESIR is an early axSpA cohort, structural damages were minor, especially for formation of syndesmophytes at 5 years, which was very low. Thus,

might explain the absence of significant association of bone markers according to structural damages in multivariate analysis. Furthermore, assessment of serum bone had limits. The varied results of the studies with these biomarkers may be due to different study populations or the use of different ELISA kits. Moreover, serum levels are not reflects of the local expression of the protein in entheses, or bone [42]. Finally, we did not study effect on mechanical loads on bone formation as we had no data concerning the physical activity level.

To summarize, this study showed that serum BMP-7 significantly increased over time and these changes are significantly associated with the use of TNFi and disease activity. More studies with a longer follow-up will be needed to confirm these results and to assess the structural impact of the bone formation markers changes.

REFERENCES

- 1 Roux C. Osteoporosis in inflammatory joint diseases. *Osteoporos Int* 2011;22:421–33. doi:10.1007/s00198-010-1319-x
- 2 Schett G, Stolina M, Dwyer D, et al. Tumor necrosis factor alpha and RANKL blockade cannot halt bony spur formation in experimental inflammatory arthritis. *Arthritis Rheum* 2009;60:2644–54. doi:10.1002/art.24767
- 3 Baraliakos X, Listing J, Rudwaleit M, et al. The relationship between inflammation and new bone formation in patients with ankylosing spondylitis. *Arthritis Res Ther* 2008;10:R104. doi:10.1186/ar2496
- 4 van der Heijde D, Landewé R, Einstein S, et al. Radiographic progression of ankylosing spondylitis after up to two years of treatment with etanercept. *Arthritis Rheum* 2008;58:1324–31. doi:10.1002/art.23471
- 5 van der Heijde D, Landewé R, Baraliakos X, et al. Radiographic findings following two years of infliximab therapy in patients with ankylosing spondylitis. *Arthritis Rheum* 2008;58:3063–70. doi:10.1002/art.23901
- 6 van der Heijde D, Salonen D, Weissman BN, et al. Assessment of radiographic progression in the spines of patients with ankylosing spondylitis treated with adalimumab for up to 2 years. *Arthritis Res Ther* 2009;11:R127. doi:10.1186/ar2794
- 7 Dougados M, Maksymowych WP, Landewé RBM, et al. Evaluation of the change in structural radiographic sacroiliac joint damage after 2 years of etanercept therapy (EMBARK trial) in comparison to a contemporary control cohort (DESIR cohort) in recent onset axial spondyloarthritis. *Ann Rheum Dis* 2018;77:221–7. doi:10.1136/annrheumdis-2017-212008
- 8 Molnar C, Scherer A, Baraliakos X, et al. TNF blockers inhibit spinal radiographic progression in ankylosing spondylitis by reducing disease activity: results from the Swiss Clinical Quality Management cohort. *Ann Rheum Dis* 2018;77:63–9. doi:10.1136/annrheumdis-2017-211544
- 9 Haroon N, Inman RD, Leach TJ, 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
- 10 van der Heijde D, Baraliakos X, Hermann K-GA, et al. Limited radiographic progression and sustained reductions in MRI inflammation in patients with axial spondyloarthritis: 4-year imaging outcomes from the RAPID-axSpA phase III randomised trial. *Ann Rheum Dis* 2018;77:699–705. doi:10.1136/annrheumdis-2017-212377
- 11 Jacques P, Lambrecht S, Verheugen E, et al. Proof of concept: enthesitis and new bone formation in spondyloarthritis are driven by mechanical strain and stromal cells. *Ann Rheum Dis* 2014;73:437–45. doi:10.1136/annrheumdis-2013-203643
- 12 Lories RJU, Luyten FP, de Vlam K. Progress in spondylarthritis. Mechanisms of new bone formation in spondyloarthritis. *Arthritis Res Ther* 2009;11:221. doi:10.1186/ar2642
- 13 Bonewald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. *Bone* 2008;42:606–15. doi:10.1016/j.bone.2007.12.224

- 14 Lories RJU, 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
- 15 Lories RJU, Daans M, Derese I, et al. Noggin haploinsufficiency differentially affects tissue responses in destructive and remodeling arthritis. *Arthritis Rheum* 2006;54:1736–46. doi:10.1002/art.21897
- 16 Appel H, Ruiz-Heiland G, Listing J, 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
- 17 Uderhardt S, Diarra D, Katzenbeisser J, 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
- 18 Heiland GR, Appel H, Poddubnyy D, et al. High level of functional dickkopf-1 predicts protection from syndesmophyte formation in patients with ankylosing spondylitis. *Ann Rheum Dis* 2012;71:572–4. doi:10.1136/annrheumdis-2011-200216
- 19 Dougados M, Etcheto A, Molto A, et al. Clinical presentation of patients suffering from recent onset chronic inflammatory back pain suggestive of spondyloarthritis: The DESIR cohort. *Joint Bone Spine* 2015;82:345–51. doi:10.1016/j.jbspin.2015.02.006
- 20 TECOmedical Group, Sclérostine ELISA, <https://www.tecomedical.com/fr/kits-et-ractifs-de-laboratoire-pour-diagnostic-in-vitro/paramtres-osseux/mtabolisme-osseux/Sclroistine-ELISA>, 2019
- 21 Maksymowych WP, Inman RD, Salonen D, et al. Spondyloarthritis Research Consortium of Canada magnetic resonance imaging index for assessment of spinal inflammation in ankylosing spondylitis. *Arthritis Rheum* 2005;53:502–9. doi:10.1002/art.21337
- 22 Creemers MCW, Franssen MJ a. M, van't Hof MA, et al. Assessment of outcome in ankylosing spondylitis: an extended radiographic scoring system. *Ann Rheum Dis* 2005;64:127–9. doi:10.1136/ard.2004.020503
- 23 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.
- 24 Lewiecki EM, Gordon CM, Baim S, et al. Special report on the 2007 adult and pediatric Position Development Conferences of the International Society for Clinical Densitometry. *Osteoporos Int* 2008;19:1369–78. doi:10.1007/s00198-008-0689-9
- 25 Garrett S, Jenkinson T, Kennedy LG, et al. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol* 1994;21:2286–91.
- 26 van der Heijde D, Lie E, Kvien TK, et al. ASDAS, a highly discriminatory ASAS-endorsed disease activity score in patients with ankylosing spondylitis. *Ann Rheum Dis* 2009;68:1811–8. doi:10.1136/ard.2008.100826
- 27 Dougados M, Simon P, Braun J, et al. ASAS recommendations for collecting, analysing and reporting NSAID intake in clinical trials/epidemiological studies in axial spondyloarthritis. *Ann Rheum Dis* 2011;70:249–51. doi:10.1136/ard.2010.133488

- 28 Liao H-T, Lin Y-F, Tsai C-Y, et al. Bone morphogenetic proteins and Dickkopf-1 in ankylosing spondylitis. *Scand J Rheumatol* 2018;47:56–61. doi:10.1080/03009742.2017.1287305
- 29 Yuan T-L, Chen J, Tong Y-L, et al. Serum Heme Oxygenase-1 and BMP-7 Are Potential Biomarkers for Bone Metabolism in Patients with Rheumatoid Arthritis and Ankylosing Spondylitis. *Biomed Res Int* 2016;2016:7870925. doi:10.1155/2016/7870925
- 30 Liu D, Wu B-X, Sun N, et al. Elevated Levels of Circulating Bone Morphogenetic Protein 7 Predict Mortality in Pulmonary Arterial Hypertension. *Chest* 2016;150:367–73. doi:10.1016/j.chest.2016.03.007
- 31 Mödder UI, Hoey KA, Amin S, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. *J Bone Miner Res* 2011;26:373–9. doi:10.1002/jbmr.217
- 32 Rossini M, Viapiana O, Idolazzi L, et al. Higher Level of Dickkopf-1 is Associated with Low Bone Mineral Density and Higher Prevalence of Vertebral Fractures in Patients with Ankylosing Spondylitis. *Calcif Tissue Int* 2016;98:438–45. doi:10.1007/s00223-015-0093-3
- 33 Park M-C, Park Y-B, Lee S-K. Relationship of bone morphogenetic proteins to disease activity and radiographic damage in patients with ankylosing spondylitis. *Scand J Rheumatol* 2008;37:200–4. doi:10.1080/03009740701774941
- 34 Korkosz M, Gąsowski J, Leszczyński P, et al. Effect of tumour necrosis factor- α inhibitor on serum level of dickkopf-1 protein and bone morphogenetic protein-7 in ankylosing spondylitis patients with high disease activity. *Scand J Rheumatol* 2014;43:43–8. doi:10.3109/03009742.2013.805241
- 35 Wendling D, Cedoz J-P, Racadot E. Serum levels of MMP-3 and cathepsin K in patients with ankylosing spondylitis: effect of TNF α antagonist therapy. *Joint Bone Spine* 2008;75:559–62. doi:10.1016/j.jbspin.2008.01.026
- 36 Saad CGS, Ribeiro ACM, Moraes JCB, 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
- 37 Poddubnyy D, Rudwaleit M, Haibel H, et al. Effect of non-steroidal anti-inflammatory drugs on radiographic spinal progression in patients with axial spondyloarthritis: results from the German Spondyloarthritis Inception Cohort. *Ann Rheum Dis* 2012;71:1616–22. doi:10.1136/annrheumdis-2011-201252
- 38 Wanders A, Heijde D van der, Landewé R, et al. Nonsteroidal antiinflammatory drugs reduce radiographic progression in patients with ankylosing spondylitis: a randomized clinical trial. *Arthritis Rheum* 2005;52:1756–65. doi:10.1002/art.21054
- 39 Laloux L, Voisin MC, Allain J, et al. Immunohistological study of entheses in spondyloarthropathies: comparison in rheumatoid arthritis and osteoarthritis. *Ann Rheum Dis* 2001;60:316–21.
- 40 Lories RJU, Derese I, Ceuppens JL, et al. Bone morphogenetic proteins 2 and 6, expressed in arthritic synovium, are regulated by proinflammatory cytokines and differentially modulate fibroblast-like synoviocyte apoptosis. *Arthritis Rheum* 2003;48:2807–18. doi:10.1002/art.11389

- 41 Lories RJU, Derese I, de Bari C, et al. Evidence for uncoupling of inflammation and joint remodeling in a mouse model of spondylarthritis. *Arthritis Rheum* 2007;56:489–97. doi:10.1002/art.22372
- 42 Garnero P. The Utility of Biomarkers in Osteoporosis Management. *Mol Diagn Ther* 2017;21:401–18. doi:10.1007/s40291-017-0272-1

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Contributors

All authors contributed to the manuscript.

All authors were responsible for the interpretation of data.

ED and KB wrote the first version of the manuscript. All authors critically revised and approved the final version of the manuscript.

Ethical approval information

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Competing Interests

R Lories : Speaker/Consultancy fees to KU Leuven from Abbvie, Celgene, Eli-Lilly, Janssen, Merck, Novartis, Pfizer, UCB

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Data sharing statement

Data are available on demand by emailing the corresponding author.

Table 1 : Characteristics of whole population at baseline

	Patients (n = 708)
Men, n (%)	327 (46.2)
Age (years)	33.7 [33.1, 34.4]
Disease duration (months)	18.2 [17.4, 19.0]
Weight (kg)	68.8 [67.8, 69.8]
Body mass index (kg/cm²)	23.9 [23.6, 24.2]
Cigarette smoking status, n (%)	257/701 (36.7)
HLA B27 positive, n (%)	410/707 (58.0)
ASAS criteria positive, n (%)	425/707 (60.1)
BASDAI	44.7 [43.2, 46.1]
ASDAS-CRP	2.7 [2.6, 2.7]
CRP (mg/L)	7.9 [6.9, 8.9]
mSASSS	0.41 [0.29, 0.53]
Modified New York criteria, n (%)	92/684 (13.5)
SPARCC score at spine	2.1 [1.6, 2.6]
SPARCC score at sacroiliac joint	3.1 [2.6, 3.5]
Bone mineral density at lumbar spine (g/cm²)	1.08 [1.06, 1.1]
Bone mineral density at hip (g/cm²)	0.99 [0.98, 1.0]
Z-score ≤ - 2 (at at least one site), n (%)	103/324 (31.8)
Current use of oral NSAIDs, n (%)	653/707 (92.4)
NSAIDs intake (6 months)	44.7 [41.7, 47.7]
Serum BMP-7 (pg/mL)	11.0 [8.9, 13.0]
Serum sclerostin (ng/mL)	0.45 [0.44, 0.46]
Serum DKK-1 (pg/mL)	5413.0 [5237.8, 5588.4]

Results are expressed as mean [95% confident interval] and number (%)

Figure 1 : Serum BMP-7 changes over 5 years

Serum BMP-7 level significantly increased over 5 years with a median increase of 223.7% (0%, 10700%), i.e. of 0.17 pg/mL per month ($p < 0.001$).

Figure 2: Change of serum BMP-7 levels according to TNFi use

Change of serum BMP-7 was impacted by use of TNFi : serum BMP-7 increased less in treated patients than in naïve patients

Figure 3 : Serum sclerostin changes over 5 years

Serum sclerostin level significantly increased over 5 years with a median increase of 14.8% (-7.9%, 41.4%), i.e. of 0.001 ng/mL per month ($p < 0.001$).

Supplementary

Figure 4 : Serum BMP-7 level at baseline

Mean serum BMP-7 level at baseline was 11.0 [8.9, 13.0]pg/mL and median serum BMP-7 was 2.1 (0, 15.7)

Figure 5 : Serum sclerostin level at baseline

Mean serum sclerostin level at baseline was 0.45 [0.44, 0.46]ng/mL