**Author's response to reviews**

**Title:** High disease activity in ankylosing spondylitis is associated with increased serum Sclerostin level and decreased Wingless protein-3a signaling but is not linked with greater structural damage

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**Author's response to reviews:** see over
Dear Dr Zochling,

Attached, please find our itemized answers to queries of the Reviewers regarding our manuscript titled “Increased serum Sclerostin down-regulates Wingless protein-3a signaling and is linked with lesser structural damage in ankylosing spondylitis patients with high disease activity”. Please note, that in accordance with express request by the Reviewer 1 and with amendments made in response to Reviewer 2’s queries, we slightly changed the title which currently reads: “High disease activity in ankylosing spondylitis is associated with increased serum Sclerostin level and decreased Wingless protein-3a signaling but is not linked with greater structural damage”. Although the Reviewer 1 had no linguistic remarks, in order to meet the request by the Reviewer 2, we re-edited the manuscript from the point of view of English usage.

Author’s response

Reviewer 1 (Evan Romas)

1. The title of the manuscript is misleading because no true biological down regulation is demonstrated, merely correlations. We thank the Reviewer 1 for the critical remark considering the title. It has been changed accordingly. Please note that the title has been also changed in line with the suggestion of the Reviewer 2. The title currently reads: “High disease activity in ankylosing spondylitis is associated with increased serum Sclerostin level and decreased Wingless protein-3a signaling but is not linked with greater structural damage”.
2. The authors use SOST and sclerostin interchangeably in a manner which may confuse a non expert. SOST is the gene and sclerostin is the gene product. It has been corrected, and we consequently use ‘sclerostin’, according to what was measured.

3. para 1 in the discussion concludes that “this confirms lack of connection between inflammatory markers and inhibitors of bone formation in AS”. This conclusion is not supported by the data. We agree. It has been changed accordingly into: “This confirms that interaction between inflammatory markers and inhibitors of bone formation in AS is complex and might not translate into simple correlations”.

4. the authors state, in the penultimate paragraph of the discussion, that “this confirms formerly reported inhibitory effect of SOST on Wnt 3a. However their data, while consistent with these other reports, cannot confirm them because of the descriptive nature of their study. Again we agree with the Reviewer 1. It has been changed into: “...this is in line with formerly reported inhibitory effect of sclerostin on Wnt-3a”.

5. serum sclerostin levels do vary remarkably with age, pubertal growth, gender and menopausal status, as well as the type of assay used. The absence of these key demographic data along with uncertainty of the true underlying disease activity or severity makes the data difficult to interpret. Although we agree that these variables might influence the results we would like to point out that population studied was rather homogeneous. Many others were recruiting patients representing different spondyloarthritis phenotypes. Ours were all adults with dominating axial disease and HLA B27 antigen, with comparable disease duration between groups. They also were not different with regard to drug usage. On the other hand we cannot imagine the study that would take into account all these variables – probably large clinical trials would manage. We must disagree with the Reviewer 1 that data is difficult to interpret due to uncertainty of the true underlying disease activity or severity. Both BASDAI (activity) and mSASSS (severity) were shown. These were patients screened for anti-TNF therapy, so the activity of the disease must have been substantial during 6 month prior to enrollment according to our state reimbursement policy. Severity measure – mSASSS – fluctuates slowly and we need 2 years to observe least significant change. So taken together, although study was not prospective, we have confidence that the reader may have appropriate information
about activity and severity of population studied. Nevertheless, we retrospectively calculated ASDAS-CRP which is believed to be more objective measure of AS activity – according to suggestion of the Reviewer 2 (please refer to our response to Reviewer 2, point 2 and 3).

Reviewer 2 (Xenofon Baraliakos)

1. The Authors state that more patients with high BASDAI vs. patients with low BASDAI had mSASSS lower than the median. However, it would be more useful to give the median/mean mSASSS in the group of patients with high vs. low BASDAI in order to show this relationship and not the percentage of patients who were lying above these cut-offs. We thank the Reviewer 2 for this remark. We compared the median mSASSS values in high and low BASDAI groups and found that they were not statistically different. We amended our table 1 accordingly. We also amended the Results and Discussion. In line with the Reviewer 2’s suggestion we slightly changed the title which now reads: ‘High disease activity in ankylosing spondylitis is associated with increased serum Sclerostin level and decreased Wingless protein-3a signaling but is not linked with greater structural damage.’

2. It is said that disease activity as assessed by the BASDAI was higher in patients with high SOST levels, making the authors conclude that high disease activity is not necessarily correlating with a predictor of radiographic progression, such as SOST. This finding is interesting. However, in this context, it would be also necessary to show the aspect of objective measures of disease activity, such as CRP. So, the question is: did the analyses based on CRP show the same relation to SOST? The Authors touch on this in their discussion but no data on this context are shown in the Results section of the manuscript. In our analysis we found no correlation between BASDAI and Sclerostin level. When, as suggested by the Reviewer 2, BASDAI was substituted with ASDAS-CRP, this was not materially changed. Moreover, when comparing Sclerostin levels in groups with very higher (>3.5) and high (<3.5) ASDAS-CRP score, we found no difference in Sclerostin level. Likewise, we found no correlation between CRP and Sclerostin level. Following the suggestion, we added sentences in the Results and
Discussion concerning these issues. It now reads: To check whether a novel, more objective measures of disease activity would have more bearing on the results, we retrospectively substituted BASDAI with the Ankylosing Spondylitis Disease Activity Score incorporating the CRP level (ASDAS-CRP) [13]. We found no correlation between ASDAS-CRP and sclerostin level. Moreover, we found no difference in sclerostin levels when ASDAS-CRP 3.5 cut-off was used instead of BASDAI 4.0 cut-off. We also compared the mSASSS scores in these groups and we found no statistically significant difference between median values of mSASSS (P=0.13), a finding reproducing our main results where BASDAI was used as clinical measure of AS activity.

3. Similarly it would be interesting to show the relationship between disease activity as assessed by the ASDAS and the laboratory biomarkers. I assume that the ASDAS can be calculated in this study. The two latter issues are important since both CRP and ASDAS have shown a good correlation to MRI activity (Machado P et al), and since MRI activity is known to be linked to new bone formation in AS. According to the suggestion, we compared the mSASSS scores using 3.5 cut-off ASDAS-CRP. We implemented a test for comparison of medians as supplied in the PROC NPAR1WAY procedure of SAS system. We found that there was no statistically significant difference between median values of mSASSS (P=0.13). Accordingly we added a sentence to the Discussion; also see point 2.

4. Discussion: It is wrong to state that untreated patients with low CRP are comparable to patients in a post-TNF state. These are two completely different patients, since untreated patients with low CRP are candidates for different disease course and are not under the influence of anti-inflammatory/disease modifying treatment as compared to patients with completely different disease activity background! This message needs to be deleted from the Discussion. We agree with the Reviewer. The statement about the similarity between our low activity group with low CRP and the post anti-TNF -treatment patients is erroneous and was changed into: “However, it needs to be mentioned that our low-CRP/low AS activity group cannot be directly compared with the findings in the post anti-TNF treatment patients reported by Daoussiss et al.”

5. We re-edited the manuscript to enhance its readability.
We are grateful for the opportunity to amend our manuscript and sincerely hope that after revision it can be considered further. We look forward to hearing from you before long.

Sincerely,

Mariusz Korkosz, MD, PhD