Author’s response to reviews

Title: S100A4 is elevated in axial spondyloarthritis: a potential link to disease severity

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Author’s response to reviews:

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Dear editorial office,

We would like to thank to the reviewers for the comments and valuable suggestions. We have amended our manuscript accordingly and we believe it has improved the manuscript. Reviewing the manuscript carefully, we found out that we have mistaken the units for S100A4 concentration. Therefore, we have replaced the pg/ml by ng/ml in the revised version.

Below, we have summarized our responses to reviewers:
Reviewer reports:

Ricardo J. O. Ferreira, PhD student (Reviewer 1):

Being a Health professional and clinical researcher, it was a pleasure to read your manuscript. The relevance of the topic was well supported, the methods are adequate, and it was easy to read this concise report. There are however, some improvements that should be done, as described below.

ABSTRACT

1) It would be good if you could mentioned that the S100A4 is a Ca-binding protein. Also, in the methods you refer to Dickkopf-1, but most readers might need a very brief description of why is this being assessed as well (you do it very well in the manuscript).

As suggested by the reviewer, we have added short description of DKK-1 to the abstract and to the introduction section of the manuscript (similarly for S100A4 being calcium binding protein).

2) You also used CRP as a surrogate for inflammation (disease activity), but his is not mentioned in abstract. I understand if the word limit precludes this.

We have added the information about CRP into the abstract.

3) (a remark not only for the abstract) although the correlations were statistically significant, they are weak and this was never mentioned in the manuscript.

According to the reviewers comment we have mentioned in the manuscript and in the abstract too that some of the correlations were rather weak.

4) In the conclusion, the word "during syndesmophyte formation" may lead the reader to think in a longitudinal/causeal relation. You may consider to rewrite this.

We thank the reviewer for this comment. We have modified the sentence.

BACKGROUND

Well done.

5) I only missed a paragraph about Dickkopf-1. Referring it in the results section (last part of the results, with references, is not correct.

As pointed out by the reviewer above, there was missing a brief introduction of DKK-1. We have added a short text about DKK-1 and its relation to bone remodelling to the introduction of the manuscript. We have removed the reference (8) from the results section.

METHODS

6) You may clearly mention the type of study.

The information about the cross-sectional type of study was added to the methods.

7) You should clearly mention the inclusion and exclusion criteria for both the patients and controls, which you did not. You only presented a sentence (in the end of the paragraph; earlier would be better) about "where" were they recruited.

We have modified the sentence as “58 consecutive patients with axSpA fulfilling the Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axSpA that were recruited from a single centre of the outpatient department of the Institute of Rheumatology in Prague as demonstrated elsewhere …”

8) I prefer when the sample characteristics (and number of recruited participants) is presented in the begging of results rather then in the methods. Not mandatory, however.

We have not modified this point.

9) I would advise you to mentioned all data collected in the methods. Only when reading the table 1 I was aware of the variables collected.

The information about which demographic and clinical parameters of the patients were collected was added to the methods.
10) if the word count allows, you may consider to add a brief description about the BASDAI and mSASSS, at least their range and what is good/bad.

Thank you, we have described the BASDAI and mSASSS in more details as follows: “Disease activity was determined using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) that consists of a 0 – 10 scale measuring fatigue, spinal and joint pain, enthesitis and duration and severity of morning stiffness. Radiographic damage was assessed using the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS), a well-validated scoring method measuring lumbar and cervical spine chronic structural changes including erosions, sclerosis, squaring, non-bridging and bony bridging syndesmophytes (range 0-72).”

11) You may want to make more clear that the Mann-whitney was used to compare X with Y (main aim)

As suggested by the reviewer, we have clarified the statistical analysis in the methods.

RESULTS

12) as mentioned previously, please mention the weak correlations.

13) MAIN POINT: the associations reported in the end of the second section of the results (association with clinical manifestations, e.g. uveitis) were not mentioned as secondary (or exploratory objectives), neither reported in the methods.

We have corrected the points 12) and 13) according to the reviewers comment.

DISCUSSION

very well done. Consider again the "weak correlation".

- The explanation about DKK-1 may be moved into introduction (or methods)

14) Do you consider that the sample size (not previously determined) and the cross-sectional design were the only limitations

Figures

It would be good in the Figure 2 the disease sub-types could be in different colours. We have modified the Figure 2 accordingly.

Wish you good work to improve this manuscript.

Savino Sciascia, MD, PhD (Reviewer 2):

Main comments

- The study could benefit of the inclusion of other controls with Rheumatic Diseases (e.g. RA)

Our research group has previously reported significant up-regulation of S100A4 levels in plasma of patients with RA or SLE compared to healthy individuals (Klingelhöfer et al. 2007, Arthritis Rheum; Sumova et al. 2019, Rheumatol Int.). This was also included in the discussion.

- It would be informative to stratify patients according to exposure to different drugs at the time of the serum sampling, mainly steroids (also stratifying for low-medium-high doses).

In general, glucocorticoids are not recommended (not effective) for the treatment of axial SpA. Nevertheless, when splitting the patients into three groups according to the treatment (NSAIDs, csDMARDs and TNF inhibitors), no significant differences in the level of S100A4 were observed among the groups. We have added this graph as Supplementary Figure.
- Statistical analysis needs further clarification. In the results the authors refer to "when adjusted for disease duration, sex, age, BASDAI and CRP levels, the p-value reached the border of the statistical significance". The methods section needs to be implemented to clarify these aspects.

As mentioned by the reviewer, this point was missing in the methods. We have implemented this aspect to the section of methods/statistics.

Minor comments

Some language editing is required (e.g., we were curious...) …?
The paper went through the language editing by AJE (American Journal Experts).

It is out of the scope of the paper to analyse pathogenic aspects. The following sentence should be omitted or re-phrased "We thus hypothesized that S100A4 protein may participate in the formation of syndesmophytes in patients with axSpA."
As suggested by the reviewer, the abovementioned sentence was omitted from the paper.

We believe that we were able to modify the paper accordingly that it could be reconsidered for publication.

Sincerely Yours
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