Author's response to reviews

Title: The neutrophil protein S100A12 is associated with a comprehensive ultrasonographic synovitis score in a longitudinal study of patients with rheumatoid arthritis treated with adalimumab

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Author's response to reviews: see over
Dear Editor,

Please find enclosed a manuscript entitled “The neutrophil protein S100A12 is associated with a comprehensive ultrasonographic synovitis score in a longitudinal study of patients with rheumatoid arthritis treated with adalimumab”.

The calcium binding protein S100A12 is mainly found in neutrophil granulocytes and is released during inflammation. Like the related S100-protein, calprotectin, it is a promising biomarker in several inflammatory disorders. The present study is to our
knowledge the first to show that this protein is associated with ultrasonographic findings in patients with rheumatoid arthritis. The associations found are stronger than between conventional inflammatory markers and ultrasonographic scores. Also, this is a longitudinal and comprehensive study of 78 joints, 36 tendons/tendon groups and 2 bursae, giving the opportunity to reflect the whole synovium.

The authors declare no competing interests.

Appropriate reviewers could be Andreas Diamantopoulos, andreas.diamantopoulos@sshf.no and/or Athan Baillet abaillet@chu-grenoble.fr.

We were encouraged by the editor of “Arthritis Research and Therapy” to transfer our manuscript to “BMC Musculoskeletal Disorders” and we hope that you find the revised article interesting and suitable for your journal.

We thank the reviewers of “Arthritis Research and Therapy” for their useful and important comments and have changed and improved the manuscript according to their suggestions.

Reviewer number 1:

1. The authors state that S100A12 showed higher correlations with US scores than CRP. This seems correct at baseline and after 1 month but not at 3, 6 and 12
months. Is it possible that the very low CRP levels (median 5 mg/l) at baseline could explain this finding?

The median correlation coefficients for S100A12 were higher than for CRP and ESR. In the revised manuscript we include in the discussion that the low CRP levels might explain this finding.

2. In what way is S100A12 superior to calprotectin as a biomarker for RA inflammation?

S100A12 does not seem to be superior to calprotectin in this cohort, but we suggest that these proteins should be compared in further studies of larger cohorts with regard to correlation with clinical parameters, US scores and their ability to predict destruction.

Reviewer number 2:

1. It appears as if this is a retrospective post-hoc analysis of a study that has been published in 2010. It is not clear whether the analyses of serological markers reported here were within the primary aims of that study. It is also not clear whether corrections for multiple comparisons have been applied in statistical analyses. Also, were all tests for significance two-sided? When looking at the figures one gets the impression of a broad overlap of the data.
The analyses of S100 proteins and other potential markers of inflammation were within the secondary objectives of the study published in 2010, but the presently used ELISA method for quantification of S100A12 was not developed at that time.

Corrections for multiple comparisons have not been applied, and we are aware that the results must be interpreted with caution. Even though this was a pilot study, we found the results of major interest and worth publishing.

All the tests for significance were two-sided, and this information has now been included in the manuscript.

2. The authors state that the US examiner was blinded for the US results from previous examinations/time point during the follow-up of the patients. How is that possible if it was the identical examiner each time?

In the revised manuscript we state that the US examiner had no access to the US and other clinical results from previous examinations and was blinded for the results from the clinical joint assessments and laboratory tests completed the same day.

3. How meaningful is the sum score with regard to disease severity? In other words, is it expected that the biomarker is higher when more joints are affected (which appears logical) or also if only a few large joints are extensively affected (still the sum score is higher when a large number of joints is only moderately affected)? With that regard it is remarkable that the correlation to the assessor’s global VAS was at least as strong as with the US score.
Thank you for this comment. We believe that the levels of S100A12 as well as other inflammatory markers are related to the total volume of synovitis. Since this is a pilot study, we have not tried to calculate a form of total synovitis with weighting of the joints reflecting their size. However, we expect that this could have given higher correlations with the inflammatory markers, and we plan to include this in a future and larger study on S100A12 in RA patients.

4. At the end of the result section it says that „Variable degree of correlation was found between the leukocyte proteins S100A12 and calprotectin. “ What does that mean?

We now state, hopefully more clearly, that there were variable degree of correlations between the leukocyte proteins S100A12 and calprotectin; being from moderate to not significant through the follow-up period.

5. Although parts of the study were already published, more details on clinical characteristics of the patients, the response rates and a possible correlation of S100A12 to ACR response rates would be interesting.

We have now analysed for possible associations between the different inflammatory markers and good/moderate responders and non-responders according to the EULAR response criteria. We found ESR higher in the non-responders, but ESR is part of the DAS28 and the EULAR response criteria. In a logistic regression analysis
none of the inflammatory markers could predict response. We found that 15 patients responded and 5 did not after three months. Because of the low number of patients and skewed distribution between responders/non-responders, we think that logistic regression is not appropriate as a statistical method in this material. Therefore this information is not included in the manuscript.

6. The S100A12 levels at the start of the study appear lower than what was reported for patients with active arthritis. Also, the decrease with successful therapy is less pronounced than in the literature. How can that be explained?

The ELISA for S100A12 that is used is not the same as in other studies. The concentrations cannot be directly compared, as specified in the revised manuscript. In addition, the levels of the conventional inflammatory markers are also rather low, even at baseline.

In addition to the revisions mentioned above, some minor changes of the manuscript have been done.

Kind regards,

Hilde Haugedal Nordal