Author's response to reviews

Title: Early increase in serum-COMP is associated with joint damage progression over the first five years in patients with rheumatoid arthritis

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Author's response to reviews: see over
Author's response to reviews

Title: Early increase in serum-COMP is associated with joint damage progression over the first five years in patients with rheumatoid arthritis

Authors: Maria LE Andersson, Björn Svensson, Ingemar F Petersson, Ingåld Hafström, Kristina Albertsson, Kristina Forslind, Dick Heinegård and Tore Saxne

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Author's response to reviews: We thank the reviewers for their comments and suggestions. The responses are given below the reviewers’ respective comments. Appropriate changes have been made in the manuscript which are indicated in the response and marked in the new version of the manuscript “Early increase in serum-COMP is associated with joint damage progression over the first five years in patients with rheumatoid arthritis – 1910173365912155”
Reviewer's report

**Title:** Early increase in serum-COMP is associated with joint damage progression over the first five years in patients with rheumatoid arthritis

**Version:** 2  **Date:** 16 April 2013

**Reviewer:** Peter Junker

**Answer to reviewer's report:**
This is an interesting and clinically relevant study showing, that radiographic progression can be predicted in newly diagnosed rheumatoid arthritis from S-COMP changes between baseline and 3 months after initiation of synovitis suppressive treatment. By this approach, inter-individual variations in S-COMP can be partially overcome. The study was conducted on the well-known BARFOT Early RA Cohort, and the prediction model is well described with due reference to current laboratory standards and clinical studies.

I have the following minor comments:

1. **P6:** Reasons for non-availability of serum samples/radiographs should be mentioned considering that this subset comprises around 25% of the total number of candidate cases. It is stated, that these 124 patients did not differ from those included upon inclusion – were there any differences regarding treatment or subsequent course of the disease including X-ray findings at 5 years?

   The information regarding treatment, disease specific measurements and X-ray findings at 5 year, of the 124 patients excluded from the study, are included and now somewhat extended in the text p. 6. The main reasons for non-availability was that either serum samples at the 3 month follow up or some essential clinical information or radiographs were missing. This is an inherent problem in a longitudinal study of this type.

2. **Was blood sampling standardized with respect to e.g. physical activity?**

   The blood sampling was not standardized with respect to physical activity. However, the sampling was made during day-time when variations in serum-COMP in individual patients performing normal daily activities are low (Andersson MLE, Petersson IF, Karlsson K, Jonsson EN, Månsson B, Heinegård D, Saxne T. Diurnal variation in serum levels of cartilage oligomeric matrix protein (COMP) in individuals with knee osteoarthritis or rheumatoid arthritis. *Ann Rheum Dis* 2006;65:1490-1494)

3. **In order to better assess the possible significance of the different Prednisolone doses between groups, please consider to calculate cumulated Prednisolone dose at the individual level in all 3 groups.**

   There was no difference between the groups in cumulative prednisolone dose over five years, however this result should be interpreted with caution because
we have not data on the prednisolone treatment between the follow-ups. Thus we have refrained from including this in the manuscript.

4. P5: The authors have previously reported that single high COMP S-levels are associated with progressive disease in RA. Were there any associations between baseline COMP and radiographic progression in the subsets defined in this study?

We found no association between baseline S-COMP and radiographic progression assessed according to Sharp van der Heijde at 1, 2 or 5 years. Added to the discussion p.12. This may seem contradictory to other studies, however one reason for performing the present study was that despite having found statistical correlations on group level in previous studies, the utility of a single value in individual cases in a clinical setting seems quite low and we wanted to see whether a different approach might increase the prognostic utility.

5. P7 and P10: Any clinical or laboratory/X-ray associations with follow-up COMP at 6, 12 and 24 months should be described in a little more detail or at least be commented upon in the discussion.

There was no significant associations between follow-up S-COMP at any time point and any clinical or I radiographic outcome variables. This information has been added to the discussion at page 13.

6. Normal range of S-COMP should be included.

In the recommendations from the manufacturer each laboratory should define their own expected range of values. In the directions for use from the manufacturer expected values from blood donors are reported. In fact these data were generated in our laboratory with individuals recruited from roughly the same area as the RA patients in the present study. In a group of 256 blood donors (117 female (age 20-73), 139 male (age 19-73)) the mean value was 10.6 U/L, median 10.4, 95 percentile 15.1 Since we are dealing with changes in serum-COMP in this study in individual patients we feel that the normal values are less important for interpretation of the results, but the point is well taken. As can be seen in the figures there are quite a few patients with serum-COMP within the "normal range" as expected. This supports the efforts to find alternative ways of using the biomarker than using single values at a fixed time point.

7. The authors rightly discuss the somewhat surprising finding, that COMP change was associated with erosion score rather than joint space narrowing. Since RA is a systemic disease and since cartilage is a major source of circulating COMP, the authors should consider also presenting joint counts in addition to DAS28. Associations between COMP and total joint counts may allow for a more detailed interpretation of the COMP-X-ray findings.

There was no significant difference in joint count (swollen or tender) between the groups at any time point. Information added in results, p. 11
8. P11: It seems reasonable to add a brief comment on the discrepancy between inter-group COMP and radiographic changes vs. comparable clinical outcomes.

S-COMP has a rather short half-life in serum, and is reflecting the cartilage turnover at the time point for sampling. New research suggests that the increased turnover of biological markers starts years before the cartilage damage is visible at radiographs, probably even before symptoms. Thus, biomarkers reflect the on-going process in cartilage and clinical outcomes and radiographs the results of this process. Therefore, it is difficult to assign predictive implications to single COMP-values and correlations between single COMP values and radiographic or clinical variables obtained at the same time points should not be expected.

10. The introduction could be somewhat shortened.

We have considered to shorten the introduction, but decided to keep it in its present form, since we feel that the non-specialized reader needs this information to appreciate the message of the paper.
Reviewer's report
Title: Early increase in serum-COMP is associated with joint damage progression over the first five years in patients with rheumatoid arthritis

Version: 2 Date: 9 May 2013

Reviewer: Xu Shengqian

Answer to reviewer's report:
I think major compulsory revisions in this paper should be made before a decision on publication can be reached.

1# Why didn’t the paper have group of control (normal) in research design? Differences of serum COMP between RA and control should be studied at inclusion, after 1, 2 and 5 years.

   We are not sure what the reviewer means since we are studying changes of serum-COMP in individual patients, not absolute values. We cannot think of a proper way to include normal controls in this setting. Furthermore, we know from previous unpublished work that serum-COMP in healthy individuals are very stable over time e.g. over a 6 month period, each individual has his/her own level. We refrained from including this information in the manuscript but instead referred to our study of the genetic influence of serum-COMP in the introduction (reference 24). See also answer to reviewer Peter Junker, point 6.

2# It is well-known that changes in serum levels of COMP in arthritis reflect processes in the cartilage. Were alterations of serum-COMP in RA at 1, 2 and 5 year follow-up observed by author associated with damage of cartilage or joint damage progression? It was not interpretated in this paper and there were not any detection involved loss and damage of cartilage, either.

   We found no association between the absolute values of S-COMP at follow-up and radiographic findings assessed according to Sharp van der Heijde. This is not expected, see also response to reviewer Peter Junker, point 8.

3# Some NASIDs such as diclofenac can influence cartilage in RA, so category of NSAID should be listed in this paper and associations between changes of serum-COMP and suspicious NSAID should be discussed.

   All patients except 12 patients in this study were treated with DMARD and/or prednisolone, which should have more effect on cartilage/inflammation than NSAIDs in patients with rheumatoid arthritis. The 12 patients not treated with DMARDS and/or prednisolone was evenly distributed in the three groups. We have no exact data on the NSAIDs used by each patient, indeed nearly all patients used NSAIDs. However, NSAIDs are not likely to influence serum-COMP (unpublished work). Indeed, since most patients used such drugs we are not able to discern any influence of these drugs and if so, it should be similar in all groups.
Comment 1: Clarify sample handling. The serum samples have been stored for nearly 20 years. It must be clarified if the have previously been thawed/refrozen or used for other purposes. This because stability of any measured protein always is a concern.

COMP is stable in serum thawed and refrozen (such experiments have been done both in our laboratory and by the manufacturer). Some of the samples are not thawed before and some is thawed and refrozen. Added in to methods p. 7

Comment 2: The radiografic progressors had the highest baseline ESR. ESR at baseline have previously been linked to radiografic progression in other studies e.g. the Euridiss study. These are the patients most likely to have the greater influx of inflammatory cells into the joint, the most severe synovitis and the greater induction of various proteolytic enzymes which degrade both the cartilage and the bone. Thus the logistic regression analyses should also be corrected for ESR.

ESR is included in the new analysis (p.11), which did not change the conclusion.
Reviewer's report

Title: Early increase in serum-COMP is associated with joint damage progression over the first five years in patients with rheumatoid arthritis

Version: 2 Date: 17 May 2013

Reviewer: Rong Mu

Answer to reviewer's report:

This is an interesting paper and the topic of this paper is of potential clinical importance. The authors have identified the relevance of alteration of serum concentrations of COMP in three months as an indicator for progressive joint destruction in RA. There are several papers about COMP and RA, but has limited paper on change of COMP as a dangerous signal such as this paper. Therefore it is novel in this respect. I would recommend the authors respond to several items.

1. A larger number of patients in group DeCr were treated with prednisolone from inclusion to the 24 month follow-up and fewer patients in group InCr from the 3 months to the 2 year follow-up. Because prednisolone is also benefit for joint destruction, it is possible that it was the usage of prednisolone instead of alteration of COMP predicting the joint damage. The influence of prednisolone should be excluded.

Prednisolone is shown to reduce radiographic and morphologic joint destruction and this effect on the cartilage is probably reflected by the decreased S-COMP levels. Despite this difference in prednisolone treatment between the groups there is an increased risk for radiographic progress in the InCr group controlled for medical treatment i.e. prednisolone and DMARD.

Se p 11 “The association remained after controlling for gender, age, disease duration, ESR, anti-CCP, RF, DMARD and prednisolone treatment at inclusion (OR 3.15 (95% CI 1.23-78.8, p=0.019).”

The use of analyzing early change in S-COMP may help to decide on which patients you should treat aggressively in order to prevent joint destruction. The point raised by the reviewer is well taken, however this report tries to focus on a novel principle of using a biomarker and we realize the problem with prednisolone use, but have controlled for it in the statistical analyses. It would have been impossible to recruit an RA patient group today not being exposed to prednisolone.

2. What is the best interval for monitoring COMP? 3 months? 6 months? Or longer? The authors mentioned the data at 6 months, 1 and 2 years. More clarity on this and the implications of your data would be very helpful and give the paper more meaning.
To our knowledge this is the first study of early changes in S-COMP. We had only access to samples at baseline and after 3 months. For clinical purposes a shorter interval would have been better, but this needs to be tested in a new study. Thus the ideal interval for clinical and or scientific purposes remains to be determined. The change in S-COMP could e.g. be influenced by disease duration, a problem which is common to many other variables in studies of "early RA". Further studies, designed to decide the optimal interval, are thus needed to answer this question.

3. Why the authors use 20% as a borderline? Please just be more specific about it.

In a study by Bresica et al (2007) the reference change value of S-COMP is calculated. The reference change value is a way to handle the significant within-subject biological variation in biomarkers described by Ricos et al (2004 and 2007). We chose this cut off based on this article, but we realize that this is somewhat arbitrary, however it should be stated that we didn’t try any other cut off, i.e. we didn’t choose the one that suited the hypothesis best.

4. The Group InCr had shorter disease duration of 3.5 ((1-12) months, while the other two groups had longer disease duration of 6 months, although no significant differences were found. Is the level of COMP related to the disease duration?

See answer above, no. 2. Thus in all studies of "early RA" the question of disease duration in relation to most variables is a difficult one.