Author’s response to reviews

Title: Tissue metabolite of type I collagen, C1M, and CRP predicts structural progression of rheumatoid arthritis

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Tissue metabolite of type I collagen, C1M, and CRP predicts structural progression of rheumatoid arthritis Anne Bay-Jensen, PhD; Adam Platt, PhD; Martin Jenkins, PhD; Michael Weinblatt, MD; Inger Byrjalsen, DMSc; Kishwar Musa, MSc; Mark Genovese, MD; Morten A Karsdal, PhD BMC Rheumatology

Rebuttal letter.

Dear Editor,

I appreciate the change for revising the manuscript. Please find below point to point address of the reviewers comments.

Kind regards

Dr. Bay-Jensen
Response to reviewers

Reviewer reports:

Mohammed Sharif (Reviewer 1):

Bay-Jensen et al investigated the relationship between serum concentrations of two relatively new biomarkers (C1M and C3M) to radiographic progression in rheumatoid arthritis using two established biomarkers (CRP and RF) as reference markers. The study is well designed and for most part the manuscript is well written, and the data presented clearly. However, the manuscript lacks clarity in some places and there are some typing errors.

- Thank you for the overall comment. We have been through the manuscript and made general corrections (see document with track-changes).

Lines 12-22 in page 7, the authors indicate that biomarkers were measured in all patients of the placebo arm but later (page 11, lines 17-30) suggest that biomarkers were measure at baseline in escape and non-escape sub-groups. A simple flow chart is required to clearly show patient sub-groups, their origin and which biomarkers are being measured. This is an important manuscript as there is a lot of interest in identifying biomarkers that can be used for targeting treatments and enrichment of patient cohorts for clinical trials of potential new drugs. Accordingly, providing clarity for patients' sub-groups, data collection and analysis is very important.

- Thank you for that constructive comment. We have made a flow chart of the study design of this biomarker sub-study (see below and new figure 1).

Please correct the typing errors in page 8, lines 9 and 23/24; page 13, line 25, add "phase" after "acute". Also, it would be useful to have a brief description in the method section of how C1M and CRP was combined to check their additive effect for predicting progression.

a) Thank you for noticing this. The errors have been corrected.

b) Following sentence was added to the statistical section of the methods: “In addition, investigation of the additive predictive value of the biomarkers were likewise tested by logistic regression.”

c) Also see update of table 4.
Janet L Huebner, M.S. (Reviewer 2): The study by Bay-Jensen, et al reports on the utility of high serum levels of biomarkers C1M and C3M, compared to CRP and RF, for their prognostic value of structural disease progression and the potential to be used as tools for enrichment of clinical trials. This elegant assessment of biomarkers reflecting different aspects of disease pathogenesis (inflammation and autoimmunity vs tissue metabolism) suggest that C1M may be a useful tool for the much-needed enrichment of clinical populations for disease progression, resulting in significant reductions of patients needed and costs of conducting the trial.

Major concerns:

1) C1M and CRP appear to have performed similarly and there was no indication that C1M provided any additional prognostic value over that of CRP for the purposes of enrichment of clinical trials. The authors themselves state (p12, line 36) that "very similar patterns for C1M and CRP which could indicate that the markers would perform equally well in enrichment for patients with a structural progressive disease." However, since these two markers are indicative of different processes (inflammation vs tissue metabolism), is it possible for the authors to provide some additional discussion about the value of C1M as a biomarker of type I collagen metabolism and how that relates to RA disease progression? What unique information can C1M provide?

a. Thank you for the comment, which really getting to the point.

b. Following was added to the discussion:” However, the two markers are distinct in their molecular origin. CRP is released mainly from the liver and act as an acute reactant, whereas C1M is released from the inflamed tissue and therefore a direct measure of tissue turnover [25]. The strong dependency of the markers may be explained by the common connection to inflammation and that CRP is an upstream modulator of tissue turnover. Hints to the difference between the two markers can be found in aforementioned phase III clinical study LITHE, testing the efficacy of tocilizumab. Tocilizumab completely and instantly suppressed the level of CRP. In contrast, C1M is gradually suppressed over time in response to treatment underlining that the two markers are somewhat differentially modulated [26]. An interesting observation is that C1M is elevated and associated with synovitis in osteoarthritis, which is normally not considered as an inflammatory disease [27]. Although C1M and CRP are equally predictive of progression in this study, they provide independent information.”

2) There is a bias in the patient selection that would result in enrichment for structural progressors since the OSK clinical trials included RA patients who were not only selected
partly based on high CRP levels, as the authors indicate, but who also had an inadequate response to treatments (MTX, DMARDS, anti-TNF). Please include this as an additional limitation.

a. Point taken. This has been added to the limitation

b. Following was added to the limitations in the discussion:” Lastly, the patients were all inadequate responders to either MTX, DMARDs or anti-TNF, which are very common phase III populations, however may provide shewed progression ratio as compared to overall RA population.”

3) The power calculation is based only on C1M, but one would assume, as the authors stated, that similar reductions would be possible by using CRP. It would be powerful tool to know how patients selected based on high C1M vs high CRP differ and how individual biomarkers may be useful in trials assessing different therapeutic treatments focused on different disease processes.

a. Thank you for that comment.

b. We have looked at the combination of biomarkers and updated table 4. The result is that a better prediction can be achieved by combining the biomarkers. Especially the combination of C1M(high) with CRP (Very High) has a markedly high OD ratio.

<table>
<thead>
<tr>
<th>n (%)</th>
<th>Progression</th>
<th>Rapid progression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>C1MH</td>
<td>116 (44.4)</td>
<td>2.05</td>
</tr>
<tr>
<td>C1MV</td>
<td>51 (19.5)</td>
<td>1.29</td>
</tr>
<tr>
<td>CRPH</td>
<td>116 (44.4)</td>
<td>2.08</td>
</tr>
<tr>
<td>CRPV</td>
<td>50 (19.2)</td>
<td>1.33</td>
</tr>
<tr>
<td>C1MH + CRPH</td>
<td>92 (35.2)</td>
<td>2.51</td>
</tr>
<tr>
<td>C1MH + CRPV</td>
<td>46 (17.6)</td>
<td>3.82</td>
</tr>
<tr>
<td>C1MV + CRPH</td>
<td>51 (19.5)</td>
<td>3.14</td>
</tr>
<tr>
<td>C1MV + CRPV</td>
<td>36 (13.8)</td>
<td>3.64</td>
</tr>
</tbody>
</table>
c. Following was added to the discussion:” However, we did observe an improvement of the OR by combining C1M and CRP reaching ORs up to 13. The two markers are distinct in their molecular origin. CRP is released mainly from the liver and act as an acute reactant, whereas C1M is released from the inflamed tissue and therefore a direct measure of tissue turnover [25]. The strong dependency of the markers may be explained by the common connection to inflammation and that CRP is an upstream modulator of tissue turnover. Hints to the difference between the two markers can be found in aforementioned phase III clinical study LITHE, testing the efficacy of tocilizumab. Tocilizumab completely and instantly suppressed the level of CRP. In contrast, C1M is gradually suppressed over time in response to treatment underlining that the two markers are somewhat differentially modulated [26]. An interesting observation is that C1M is elevated and associated with synovitis in osteoarthritis, which is normally not considered as an inflammatory disease [27]. Although C1M and CRP are equally predictive of progression in this study, they provide independent information.”

Minor Concerns:

Thank you for those comments

1) P6, line 10 and p11, line 37: Although type I and type III collagen may be among the most abundant in the body, type III collagen is not the most abundant in the joint.
   a. corrected

2) P7, line 33: please clarify the method by which radiographic progression was defined specifically, '…changes >0.23 units linear extrapolated from >0.5 units/year'.
   a. clarified

3) There are several grammatical errors that require correction
   a. Proof-read an extra time

4) P8, line 41: "A pooled dataset of 474 placebo and MTX-treated patients.." does this mean that only some of the placebo patients were MTX-treated or all of them? If all, I would suggest re-writing as follows: 474 placebo (MTX-treated).
   a. All patients were MTX treated. Text clarified
5) P8, line 46: please clarify by changing to the following - "Of the 474 patients, 181 patients with inadequate response to the therapy went into escape at week 12…"
   a. A study low has been added (figure 1) and text revised

6) P10, line 13. The authors state that the overlap between patients identified by C1M and CRP was 80%, but it would be useful to know what percentage, if any, were uniquely identified by C1M.
   a. Added to the text. 24 patients were identified by C1M and 24 patients with CRP.

7) P13, line 10. Insert (see supplement) after 'reduce patient numbers by 50%.'
   a. Inserted

8) In tables, please include the units of measure for biomarker concentrations
   a. added

9) In methods or supplement - please include metrics of ELISA based assay for C1M and C3M (inter- and intra-assay CVs; percent measurable, etc)
   a. Added to the method section of the main manuscript file.