Author's response to reviews

Title: Serum levels of reactive oxygen metabolites at 12 weeks during tocilizumab therapy are predictive of 52 weeks-disease activity score-remission in patients with rheumatoid arthritis

Authors:

Arata Nakajima (a-nakaji@sf7.so-net.ne.jp)
Keiichiro Terayama (kei-tera@sakura.med.toho-u.ac.jp)
Masato Sonobe (masato0609@aol.com)
Yasuchika Aoki (yasuaoki35@sf4.so-net.ne.jp)
Hiroshi Takahashi (hiroshi.takahashi@med.toho-u.ac.jp)
Yorikazu Akatsu (yorikazu.akatsu@med.toho-u.ac.jp)
Junya Saito (junya.saito@med.toho-u.ac.jp)
Shinji Taniguchi (shinji.taniguchi@med.toho-u.ac.jp)
Manabu Yamada (manabu.yamada@med.toho-u.ac.jp)
Ayako Kubota (ayakokubota@med.toho-u.ac.jp)
Koichi Nakagawa (konakag@med.toho-u.ac.jp)

Version: 2 Date: 31 Aug 2019

Author’s response to reviews:

Dear Dr. Liew (Reviewer 1),

Thank you for your comments on our manuscript. According to your comments, we revised the manuscript. The responses to your comments and suggestions are as follows.

Point 1:
Please detail how the cut-off value was determined (e.g. was this an ideal cut point determined from a Youden's J statistic, based on the DAS28-ESR remission data?). The ROC presumably refers to ROM at 12 weeks, please include this in both the body text and figure caption (and also within Table 4). Also do not refer to AUC for ROC as 'not statistically significant' unless it is being compared to another ROC, even if the correlation or the difference between mean scores is not statistically significant. If discussing ROCs for CRP and MMP-3, please mention the AUC, particularly given that the ROC for CRP appears to visually closely match the ROC for ROM.
Response:
The ROC curves for ROM, CRP, and MMP-3 referred to the values at 12 weeks, and the cut-off values for ROC curves were determined by the maximum of a Youden index. This was stated in lines 133-136, 432-434, and Table 4 caption. We removed the description regarding statistical analyses of the AUC for ROC. The AUC for CRP and MMP-3 were stated in lines 191-192.

Point 2:
Please express within the 'Results' section the variables identified by stepwise method in the multivariable logistic regression. Did it include bDMARD naïve status and prednisolone exposure status? If not, it should be explicitly stated that these did not affect the association.

Response:
According to the suggestion, we stated the variables identified by stepwise method in the multivariable logistic regression in lines 195-196 and Table 4 caption. We also stated that the multivariable logistic regression analysis did not include bDMARD naïve status or prednisolone exposure status in lines 198-199.

Point 3:
Does the disconcordance between the associations correlating to CDAI and DAS28-ESR remission affect the generalisability/application of your observations, especially given that your discussion implies that the DAS28-ESR result is in part an artefact of the disease activity estimation? Please include in discussion.

Response:
In the original manuscript, we discussed the reason why the serum level of ROM was not predictive of CDAI-remission, which was also stated in lines 248-253 in this revision. We added another possibility that was stated in lines 254-258.

Point 4:
It appears that ROS may or may not actually represent an index of disease activity, as it may not necessarily be involved causally but may merely be present in patients who respond to disease. It may be worth explicitly highlighting that this is a biomarker predictive of future response rather than an index of disease activity, particularly as in the second part of the background you move quickly from talking about CRP and ESR (traditionally primarily thought of as indices of disease activity) to talking about the need for novel biomarkers, without signposting the difference.

Response:
According to the suggestion, we inserted the sentence in lines 74-75 before talking about the need for novel biomarkers predictive of future response to TCZ therapy.

Point 5:
Minor points:
Background:
Given that the role of ROM (and oxidative stress in general) in the pathogenesis of RA are not commonly known to the lay rheumatology reader, you might consider a hypothesising
introductory sentence at the beginning of the relevant paragraph in the 'Background' (first page of Background, line 51) to explain the biological plausibility behind your study. You might reconsider the wording in the final sentence of the 'Background' in order to not incorporate results prior to the 'Results' (i.e. avoid stating that you "verified that ROM is a useful biomarker to monitor the treatment process during TCZ therapy" prior to describing the study results).

Response: According to the suggestion, we added the introductory sentence to explain the role of oxidative stress in the pathogenesis of RA in lines 78-81. In the final sentence of the ‘Background’, we removed the sentence ‘verified that ROM is a useful biomarker to monitor the treatment process during TCZ therapy’, and described ‘investigated whether ROM could predict future clinical remission during TCZ therapy’ in lines 93-94.

Point 6: Methods: Conventionally, background characteristics of the cohort (and reference to the corresponding Table 1) would be included in the 'Results' - there does not seem to be a necessary rationale for the inclusion of this in 'Methods'.

Response: According to the suggestion, we moved background characteristics of patients to the first part of the ‘Results’ in lines 145-150.

Point 7: Results: The section order makes this harder to follow. Strongly consider moving the discussion about ROM kinetics (which makes up the core part of the section 'Changes in ROM serum levels in the DAS-remission and non-remission groups at 52 weeks of treatment') after the comparisons of parameters at 4 and 12 weeks, to give rationale as to why the kinetics of ROM serum levels by DAS28 remission is of specific interest. Suggest expressing sensitivity and specificity as percentages.

Response: According to the suggestion, we moved the description about ROM kinetics in lines 176-185, after the comparisons of parameters at 4 and 12 weeks. The sensitivity and specificity of the cut-off value for ROM at 12 weeks were expressed as percentages in lines 48 and 191.

Point 8: Tables: With the table captions, make it clear that 'remission' is remission at 52 weeks, and 'non-remission' is non-remission at 52 weeks - this may otherwise be less clear given the time frame titling the tables.

Response: According to the suggestion, we revised the captions in Tables 2 and 3. We also revised Figure 2 legend in lines 426 and 428.
Point 9:
Figures:
Figure 2: please do not stagger the remission/non-remission points so greatly as it is not visually apparent that the data points relate to the same time point (i.e. the 'non-remission' and 'remission' week 0 data point are highly staggered). This would actually make the difference between the two groups more apparent.

Response:
The statistic software we used (SPSS ver. 19) displays automatically the ROM kinetics of the 2 groups as shown in Figure 2. The image was fixed and we were not able to move the data points.

Dear Dr. Humphreys (Reviewer 2),

Thank you for your comments on our manuscript. According to your comments, we revised the manuscript. The responses to your comments and suggestions are as follows.

Point 1:
The background requires greater explanation about why changes in CRP may not reflect true inflammatory burden. As far as I am aware, there is data suggesting IL-6 receptor blockade directly changes CRP, but you are hypothesising this may not be a true change in inflammation. The scientific rationale for this needs to be made more strongly in your introduction in order to justify the study. There should also be justification for why this would not happen with the reactive oxygen metabolites.

Response:
We added scientific rationale, in lines 68-71, that IL-6 receptor blockade might mask the true inflammation which was induced by the other cytokines rather than IL-6, as IL-6 blockade normalizes CRP levels. We believe that these explanations justify our study.

Point 2:
Was there a washout period between patients stopping other biologics and starting IL-6?

Response:
In the switched patients, a washout period of the previous bDMARDs was not provided. This was stated in lines 101-102.

Point 3:
Given that your data are mostly on-parametric and you are using Mann Whitney, it would be more appropriate to report median and inter-quartile ranges. At the moment it looks odd that a tender joint count is reported as 4.7 +/- 6.6, as that implies that you could possibly have a TJC of -1.9

Response:
We understand your point, but we expressed values as mean±SD since the data for the parameters were parametric. We tried to express values as median and IQR, however, our statistic software (SPSS ver. 19) did not show the data as median and IQR, and showed only p-values between the 2 groups, so we keep the data as mean±SD in this revision. If TJS and SJC should be removed since a part of them look odd, we will remove them from Tables 2 and 3.

Point 4:
In tables, you should include what parameters are being reported (e.g. in tables 2 and 3, I think these are means and standard deviation but I don't know).

Response:
According to the suggestion, we stated in the footnotes of Tables 2 and 3 that values were expressed as mean±SD.

Point 5:
Please report what variables were adjusted for in the logistic regression. This should be included in the methods and be documented in table 4. It is important to know what variables were considered for selection by the stepwise model. The numbers in this study are very small, and there is a risk of finding a statistically significant result due to multiple testing rather than a true association.

Response:
According to the suggestion, we stated that the cut-off values for ROM and CRP at 12 weeks were used as variables in lines 138-139 and Table 4 caption.

Point 6:
I think the opening line of your discussion is on overstatement of the results. The numbers are small and you only found an association with one of your outcomes. The data clearly need replicating on a larger scale before definitely being described as "useful".

Response:
We understand your point. According to the comment, we revised the opening sentence of the Discussion as shown in lines 202-203. We also revised sentences as shown in lines 51 and 276-277.

Point 7:
In your discussion you mention that IL-6 levels are a better marker of inflammation in patients on tocilizumab. If that is the case and has been demonstrated, why should the ROM test be developed or measured, why not just use IL6 levels? No justification for this is provided in the discussion.

Response:
According to the comment, we stated why the ROM test was developed and measured in patients with RA. We also stated the points that the measurement of ROM was superior to that of IL-6, in lines 231-236.