Author's response to reviews

Title: Soluble receptor for advanced glycation end-products and progression of airway disease

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Author's response to reviews:

General comment to editor and reviewers

One of the most salient features of this study was the fact that all of the participants came from a population-based cohort of individuals who had no apparent comorbidity and, at the start of the study, were not receiving any therapeutic interventions. Thus, the degree of airflow limitation was relatively mild in the smokers who were diagnosed with COPD after enrolment. The details of the project, including the inclusion and exclusion criteria, have been published in Toljamo et al. [Ref #16 in the manuscript].

After carefully reading the comments of the reviewers, we have realized that the two groups of smokers needed to be classified more specifically, i.e. as smokers without COPD and smokers with COPD, to ensure that readers would understand the key difference between the two groups of smokers who were included in the study. We have introduced this important change in our revised manuscript.

Reviewer's report

Title: Soluble receptor for advanced glycation end-products and progression of airway disease

Version: 3 Date: 20 December 2013

Reviewer: Gaetano Caramori

Reviewer's report:

Major Compulsory Revisions

1. There is a huge discrepancy within the number of control smokers with normal lung function compared with control non-smoking subjects and COPD patients. The number of these 2 groups should be significantly increased.
All of the participants, smokers and non-smokers, were recruited from a population-based cohort of individuals who considered themselves healthy. The smokers were divided into non-COPD and COPD groups (please see above) according to the presence/absence of airflow limitation at spirometry (post-BD FEV1/FVC < 0.7). Therefore, the number of participants in the smokers with COPD group turned out to be considerably smaller than the number of smokers without COPD. This longitudinal follow-up study began over 10 years ago, and at present, we cannot increase the number of study participants by any means.

2. The clinical details of the subjects presented in table 1 are largely incomplete. Please provide pre and post-BD FEV1, FVC and their ratio.

In accordance with the reviewer’s suggestion, we have added the pre-BD spirometry results to Table 1.

3. Please provide your definition of current smoker and the number of current vs former smokers.

We have added the smoking status to Table 1. The definition of a current smoker is any individual who was presently smoking every day or on some days. (Ref:http://dhds.cdc.gov/guides/healthtopics/indicator?i=smokingstatus)

4. The recent literature in the field of the role of RAGE and HMGB1 in the pathogenesis and COPD should be carefully reviewed and appropriately quoted.

On the basis of the reviewer’s suggestion, we have revised the 1st and 2nd paragraphs of the Introduction and paragraphs 2–4 of the Discussion and have cited recent papers on RAGE and HMGB1 (new Ref# 15, 26, 27).

5. Please provide chest CT score for pulmonary emphysema of your smoker subjects to investigate if there is any correlation between these blood biomarkers and the presence and/or severity of emphysema.

Since the assessment of emphysema by CT scan has not been included in the protocol of the study, we were not able to describe the prevalence of structural emphysema in the two groups of smokers. We have mentioned this limitation in the Discussion.

6. Presence and absence of chronic bronchitis should be described as well as its correlation with the biomarkers.

Since the assessment of chronic bronchitis has not been included in the protocol of the study, we were not able to describe the prevalence of chronic bronchitis in the two groups of smokers. We have mentioned this limitation in the Discussion.
We do not have the data for symptoms compatible with the definition of chronic bronchitis, i.e. chronic cough or sputum production for at least three months in two successive years. However, we do have data on the presence and absence of chronic cough and sputum at the baseline visit. The presence of cough and/or sputum was not associated with circulatory RAGE or HMGB1 levels. The updated data and results were added to Table 1, and accordingly, the Results section has been revised.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests: I declare that I have no competing interests

Reviewer's report
Title: Soluble receptor for advanced glycation end-products and progression of airway disease
Version: 3 Date: 18 February 2014
Reviewer: S. Vamsee Raju
Reviewer's report:
Summary
The authors of this study compared plasma concentrations of sRAGE and HMGB1 in healthy non-smokers, smokers, and COPD patients and evaluated their predictive value in estimating pulmonary function. They have analysed baseline demographic data and a longitudinal decline of pulmonary function during a 4-year follow-up period. Baseline plasma sRAGE levels were significantly lower in healthy smokers and COPD patients than in non-smokers. Moreover, plasma sRAGE concentrations were significantly associated with longitudinal declines of FEV1/FVC independent of demographics and baseline lung function. Surprisingly, there was no significant difference in plasma HMGB1 levels across the study groups contradicting what was recently published.
Critiques:
1. As duly noted by the authors, this study is not large enough to make the assertion that sRAGE might be a diagnostic marker for early stage detection of COPD. Based on what was learnt in this study authors are recommended to list the required parameters for a conclusive larger study (required N for COPD patients, duration of follow up study, spirometric characteristics, additional
In accordance with the reviewer’s suggestion, we proposed additional parameters, i.e. CT scan and diffusion capacity, which could be included in future studies on sRAGE as a biomarker for early detection of COPD, and this is now mentioned in the 3rd paragraph of the Discussion section. Additionally, we have revised the limitations section and added a postulate for a larger sample size of COPD patients that might help clarify the association between sRAGE and the decline of lung function in COPD.

2. Authors proposed to (look at intro) study the relationship between HMBG1 and sRAGE. However, the data figures suggest that these 2 important molecules were studied independent of one another. This is also complicated by the lack of any significant differences in HMBG1. It is recommended that the data for sRAGE and HMBG1 is compared within each study group and discuss their interrelationships, if any.

RESPONSE:

There was no significant correlation between plasma sRAGE and HMGB1 levels in any of the subjects, nor within any of the groups in the present study. We have added this information to the Results section of our revised manuscript.

3. The authors have presented a surprising data set where they contradicted significant differences in HMBG1 in COPD patients. It is important that authors compare the ELISA method used for analysis with earlier work and also discuss how their study differs in terms of inclusion criterion, patient demographics and spirometric differences.

RESPONSE:

The HMGB1 levels in the control subjects were obtained using the same ELISA method described by others (Ref #13), and were comparable with those in the present study. On the other hand, elevated HMGB1 levels have previously been reported in advanced-stage COPD patients and/or in those with lung cancer (Ref #13, 15, 27). We have discussed these differences in disease severity and comorbidity among the various groups of COPD patients in the other studies in the 4th paragraph of Discussion section of our revised manuscript.

4. The HMGB1 values obtained were very low and were not too distant from the detection limit of the assay. Were their sample processing issues associated?

RESPONSE:

The plasma samples were appropriately centrifuged and stored at #80# until analysis. Haemolysis might cause elevated levels of HMGB1, but we did not observe this during our procedure. Although the HMGB1 values were low, they were still within the detection area of the standard curve. Moreover, the plasma
HMGB1 levels of non-smokers and smokers without airflow limitation, i.e. those without COPD, were of the same magnitude when our results were compared to those already published for healthy volunteers (Ref #13).

5. At baseline, there were differences in smokers but not in COPD patients. Discuss in detail how small sample size of COPD patients contributed to this discrepancy.
RESPONSE:
The difference in disease severity in COPD groups might have been responsible for the inconsistencies in sRAGE results between the present study and the previous reports. This point is now clearly discussed in the 3rd paragraph of Discussion section of the revised manuscript.

6. Subgroup analysis for COPD patients shows a significant association b/w sRAGE and lung function decline. Were there any sex based differences within this group? Fewer females in this group might limit such analysis.
RESPONSE:
There was no significant difference in the rate of lung function decline between male and female COPD patients. The small sample size of female COPD patients might have limited the statistical analysis. We have added this point to the limitations outlined in the Discussion section.

7. Place reference published work for alternate variables like age, BMI, smoking status and baseline FEV1/FVC that were also significantly associated with changes in lung function decline (and also sRAGE in some).
RESPONSE:
As far as we know, there has been no previous study on the association between plasma levels of sRAGE and HMGB1 and lung function decline in a longitudinal setting. That also means that, at least to the best of our knowledge, no longitudinal research has been published on the relationship between these molecules and alternate variables such as age, BMI, smoking status, and various lung function parameters, apart from the cross-sectional studies that we present and discuss in our manuscript. If the reviewer means in general the relationship between COPD (i.e. irreversible obstruction by FEV1/FVC in spirometry), there have been a great number of epidemiological studies, probably since the late 1970s, with the most cited being Fletcher C & Peto R: The natural history of COPD. BMJ, 1977;1:1645-48. There have also been numerous studies in which alternate variables such as age, BMI, and smoking status, have been incorporated into different predictive models for COPD patients, i.e. to predict mortality, hospitalisation, exacerbation, etc. Nevertheless, none of these studies have dealt with plasma levels of sRAGE and HMGB1.
In accordance with the reviewer’s suggestion, we have added new references in the Methods section (new Ref # 20–22).

8. One of the great features of this study is that COPD patients were not on any
medications prior to this study. Pls state what medications these patients were on during the 4-yr follow up period and how those medications influenced the lung functions analyses.

RESPONSE:

Among smokers with COPD (n = 51), there were 13 who had started an inhaled medication, i.e. a bronchodilator and/or corticosteroids, at the follow-up visit. The results for the remaining 38 smokers with COPD still showed a significant correlation between baseline sRAGE levels and rate of decline of FEV1/FVC (r = 0.362, p = 0.025). We have added this finding in the Result section of our revised manuscript.