Author's response to reviews

Title: Plasma Chemokine Signature Correlates with Lung Goblet Cell Hyperplasia in Smokers with and without Chronic Obstructive Pulmonary Disease

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Author's response to reviews:

Reviews for Plasma GCH Manuscript
Reviewer's Report

This manuscript addresses an important question of how circulating cytokine signatures relate to goblet/mucous cell hyperplasia (GCH) as observed in smokers with and without COPD. The methods followed are appropriate and well described. The data presented clearly shows that smoking upregulates the levels of CXCL8, CCL4 and CCL22, the neutrophil and macrophage chemoattractants in plasma, and the levels strongly correlate with the observed GCH development.

Most of the previous studies had established the role of neutrophils and macrophages in the pathogenesis of COPD following smoke-induced exacerbations based on either in-vitro studies or from various animal models. There is a minor concern with regards to the sample size and more importantly, about the racial variation in the cohort, which dampens the enthusiasm and authors too share this concern. Nonetheless, the readers of this journal will be able to appreciate the merits of the reported findings if the following concerns are addressed. The title and abstract to aptly convey the reported findings.

Major Compulsory Revisions

1. The neutrophils and macrophage counts from patient blood or BAL should be presented to ascertain the correlation of these inflammatory cells to the observed chemokine profiles in this cohort.

Response: While we agree with the reviewer that neutrophil and macrophage counts from both BAL and plasma would have been ideal for this analysis, BAL and plasma cell counts were not performed as a part of this study.

2. Please discuss your results in the context of findings from ECLIPSE studies (Agusti et al., PLOS One 2012: 7(5):e37483); and the study reported by from
Brozyn et al., (COPD 2009; 6: 4-16) on Th1 type chemokines in smokers and COPD patients.

Response: The discussion was expanded to include a discussion of our results in comparison to the data from the ECLIPSE study, and we have incorporated these references.

Minor Essential Revisions
1. Is it possible to analyze the biopsies from small airways and compare that with large airways reported in this manuscript?

Response: The images obtained from the biopsy specimens were done so in a blinded fashion, without differentiation between distal or proximal airways. However, given the depth by which endobronchial biopsies were obtained (not more distal than the segmental level), these airways all represent large airway epithelium.

Discretionary Revisions
None

Level of interest: An article of importance in its field
Quality of written English: Acceptable
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: Maor Sauler

Reviewer's report:
Victor Kim et. al., in their manuscript, utilize bronchoscopically obtained lung tissue and plasma samples from a modestly sized patient population to determine the association between systemic chemokines and goblet cell hyperplasia. This study wishes to address the question of what circulating chemokines are associated with goblet cell hyperplasia. The study utilizes human tissue samples, an approach that is the major strength of this study. It is well written and I have no reason to doubt the accuracy of the data being presented.

However, I have the following methodologic concerns and questions regarding the relevance of this study.

Major Criticisms:
1) the authors do not clearly justify studying the association between circulating chemokines and goblet cell size. More than "down-stream" consequences of disease, is there a patho-mechanistic hypothesis that is being addressed? For example, can the authors show a relationship between recruited airway cells and goblet cell hyperplasia? At minimum, can the authors offer a more clear rationale
for why the study was undertaken?

Response: We added the following sentence to the last paragraph of the introduction, which states the hypothesis: “We hypothesized that elevations in systemic cytokines would be associated with increased GCH as a result of immune trafficking to the lung and subsequent mucin production.” In addition, we analyzed cytokines that have documented activity in enhancing Th2-type responses, and would be expected, in-turn, to promote mucin expression. Our objective was to more fully evaluate the systemic and local inflammatory environment present in patients with lung goblet cell hyperplasia.

2) Given that bronchoscopically obtained biopsies are taken with forceps, with variable tissue compliance and applied force, can the authors provide some evidence that ex vivo goblet cell size is related to in vivo size?

Response: To our knowledge, there is no study that compared bronchoscopically obtained samples to samples of epithelium in a surgical lung biopsy. For this study, however, there was a single bronchoscopist (VK) who performed all of the procedures and used the same technique for retrieval of the endobronchial mucosal biopsies, using the same applied force for all biopsies.

3) Why did the authors not perform any multivariate adjustment for confounding variables? At minimum, there is ample patient size to adjust for smoking status (current/former).

Response: There were only 3 former smokers among the 27 subjects in the study, so these subjects were combined into the non- or ex-smoker group. A sentence was added to the results section to clarify this.

4) Given the small sample size, why did you use a Pearson correlation? Would consider non-parametric correlation testing.

Response: We re-analyzed the data using Spearman’s correlation and found a stronger correlation coefficient of 0.552, p=0.003. This change is now a part of the statistics section, results, and Figure 4.

Minor Criticism:

1) I would minimize discussion of immune cell trafficking in COPD. This study does not address those question. This study looks at the association between systemic chemokines and goblet cell hyperplasia – it does not evaluate neutrophil or monocyte/macrophage migration.

Response: We agree with the reviewer that discussion regarding immune trafficking is beyond the scope of this manuscript. We therefore put in the limitations paragraph that nothing about the results can speak to immune trafficking, as this was not evaluated.

2) Why is Goblet Cell Hyperplasia not included in Table 2?

Response: The measure of goblet cell hyperplasia, mucin volume density, is
included in the last line of Table 2.

3) I would show at least 2-3 examples of goblet cell hyperplasia/condition.

Response: Figure 1 has been changed to reflect 2 examples of each condition, healthy nonsmokers, healthy smokers, and COPD subjects, respectively.

4) Ensure that all statements in the conclusion are clear in suggesting that any positive findings are purely an association.

Response: The reviewer makes a valid point, and we agree that these findings are associations, and that this fact should be emphasized. We changed the middle sentence of the conclusions to the following: “These associations suggest that smoking has a systemic effect on circulating cytokine levels that lead to the development of GCH.” In addition, we noted in the limitations paragraph that these findings are purely associations.

5) The study would greatly benefit from more patients. Where power calculations used to determine the sample size?

Response: We agree with the reviewer that the study would benefit greatly from more subjects. Power calculations were not used to determine sample size.

5) Could the author detail where/when a pre- and post- test adjustment for multiple comparisons was used. From the description in the text, it seems as if this did not occur routinely.

Response: In the statistics section, it states: “Differences between the three groups (nonsmokers, healthy smokers, COPD) were assessed using one-way ANOVA or Chi squared test. Post hoc tests after ANOVA were performed using Bonferroni correction.”

Discretionary Revision:

1) As the authors point out, goblet cell size (as opposed to function) is not strongly associated with patient’s symptoms. Did the author obtain information related to symptoms or exacerbations?

Response: We agree with the reviewer that it would have been more informative to obtain data regarding the subjects’ symptoms and exacerbations. Unfortunately, this information was not collected.

Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
I declare I have no competing interests.
Reviewer: Venkateshwar Mutyam
Reviewer's report:
General Comments
This article briefly highlights the correlation between increased systemic chemokine levels in current active smokers compared to nonsmokers and COPD subjects and its association with goblet cell hyperplasia. The relative depth of the manuscript in terms of the study of inflammatory cytokine levels being influenced by smoking leading to GCH is a reasonable contribution to the literature.

However, one of the main conclusions of GCH being associated with current smoking in this study has been an important finding in author's recent previous publication, which should be addressed and acknowledged in this study.

Response: We changed the first sentence of the discussion to reflect that this corroborates findings from our prior study.

Discretionary Revisions:
1. Page 4: Line 23: “See Figure 2” wording should be moved to line 20
Response: This was changed in the manuscript.

2. Page 5: Line 19: findings suggest that smoking (delete these), Line 22 – add subjects after COPD
Response: done. Thanks to the reviewer for pointing out these errors. The suggestions were incorporated into the manuscript.

3. The small sample size in this study should also be acknowledged up front
Response: “In this small cohort” was added to both the abstract and the first paragraph of the discussion to acknowledge the small sample size.

Minor Essential Revisions:
1. In Figure 1, a mucosal biopsy image of COPD subject should be added for comparison.
Response: 2 examples of each group, healthy nonsmokers, healthy smokers, and COPD subjects, are now displayed in Figure 1.

2. In Figure 3, the bottom of graph bars should be labeled as non-smokers and current smokers for easier understanding of the reader.
Response: We have added abbreviations NS and CS with explanations in the figure legend.

3. The moderate correlation between plasma CXCL8 levels and MVD should be
elaborated in the discussion section.
Response: We included more discussion regarding the correlation between CXCL8 and MVD in the discussion, as per the reviewer’s suggestion.

4. Elaborate the abbreviation EGFR in the abstract section.
Response: We wish to thank the reviewer for picking up this error. This was corrected in the abstract.

5. Page 7: Line 10: Authors talked about higher counts of eosinophil in chronic bronchitic group. Were the eosinophil counts done? If so a brief mention in methods section would be sufficient.
Response: BAL or plasma cell counts were not done in this study.

6. Page 4: Line 20-23: The significant higher levels of plasma chemokine CCL7 in COPD group compared to nonsmoker and healthy smoker group should be addressed in discussion.
Response: A brief discussion about CCL7 was added to the discussion.

7. Page 6: Line 18-19: Authors cited a publication which showed higher levels of sputum CXCL8 in COPD subjects compared to healthy smokers and nonsmoking controls. The present study shows significantly higher levels of
Response: We acknowledged that our findings are in contradistinction with prior literature in the discussion section.

Major Compulsory Revisions:
1. Authors should consider acknowledging their recent publication (PMC4315442) in the current study, since it is closely related.
Response: Thank you, this manuscript is now acknowledged in the first paragraph of the discussion.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

The study is an important contribution to the field. However below is the major concern.
The author of the manuscript has publication (PMC4315442) in PLoS One journal with title “Chronic bronchitis and current smoking are associated with more goblet cells in moderate to severe COPD and smokers without airflow
obstruction” in the month of Feb 2015. The Figure 1 of this Publication has 4 panels A) a healthy nonsmoker, B) a healthy smoker, C) a COPD subject without chronic bronchitis, and D) a COPD subject with chronic bronchitis, taken at 40x.

In the current manuscript the author has used the same figures as Figure 1, A) Healthy nonsmoker and B) Healthy smokers. However the major concern is misrepresentation of Figure 1B, as the healthy smoker in the present manuscript, which is in fact a COPD subject with chronic bronchitis (Figure 1D) from previous publication. Please see below.

These discrepancies and use of similar figures in present study should be addressed before considering for publication.

Response: The reviewer is correct. This is an embarrassing error on the corresponding author’s part. We re-reviewed the images obtained and have provided two images each for nonsmokers, healthy smokers, and COPD subjects, respectively.