Author’s response to reviews

Title: Ageing and smoking contribute to plasma surfactant proteins and protease imbalance with significant correlations to airway obstruction

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Author’s response to reviews: see over
Dear Chief Editor Professor Norton,

**RE: Manuscript number:** 4061222664737634

Enclosed please find our revised manuscript entitled “Ageing and smoking contribute to plasma surfactant proteins and protease imbalance with significant correlations to airway obstruction” by Helen Ilumets, Witold Mazur, Tuula Toljamo, Noora Louhelainen, Pentti Nieminen, Hideo Kobaysashi, Nobuhisa Ishikawa and Vuokko L Kinnula.

The concerns of both Reviewers were excellent and we have taken all recommendations into careful consideration and modified the manuscript accordingly. We have also consulted our expert statistician, Professor Pentti Nieminen, who was included as one co-author, and reanalyzed all results again. We strongly feel that after the new calculations and corrections, this study has improved significantly. We hope that this manuscript can now be published in BMC Pulmonary Medicine.

All the authors have reviewed the manuscript, and the material submitted for publication is not under consideration for publication anywhere else. No part of the study has been supported by tobacco industry. No author has any competing interests to declare.

Sincerely yours,

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Reviewer’s report
Manuscript number: 4061222664737634
Title: Ageing and smoking contribute to plasma surfactant proteins and protease imbalance with correlations to airway obstruction

Reviewer: Meng-Chih Lin
Reviewer’s report:
1. This is an interesting manuscript that explores the relationship between plasma biomarkers, aging, and smoking. Authors concluded that age has a significant contribution to potential markers related to smoking and COPD. Levels of surfactant protein-A seems to be the best factor in differentiating COPD from the control. However, several points need to be clarified before further decision of acceptance.

We agree, and we have now taken all concerns into careful consideration and modified the manuscript accordingly. We have also consulted our expert in statistics, Professor Pentti Nieminen and reanalyzed the results with him once again.

2. Major compulsory revisions:
   a. The major problem for this study is the lack of a stated clear rationale. Why do authors want to conduct this study? Why were these biomarkers chosen to study? What are the relationships among those biomarkers?

This may be true, why we have clarified the Introduction part of the manuscript as suggested. SP-A and SP-D were chosen since we (by proteomics, SP-A), and others (SP-D) have suggested that these proteins have significant role in the pathogenesis of COPD. MMP-9 was chosen since it represents matrix metalloproteinase which has been linked to COPD in multiple earlier investigations, also those from our laboratory. Additionally there are studies suggesting that surfactants may regulate the balance of proteases/antiproteases, which have now included (Wert et al 2000, Proc Nati Acad Sci USA 97:5972–5977, Ramadas et al 2009, J Immunol 2009, 1;182(3):1560-7, LeVine et al 2000 J. Immunol. 165:3934, Vazquez de Lara et al, Am J Physiol Lung Cell Mol Physiol. 2003 Oct;285(4):L899-906).

b. The second major critique is the subject’s inclusion criteria. How were subjects selected to the study? Randomly or active recruitment? How many subjects were included and upon selection, how many were excluded? It may be helpful to have a diagram to present the study design.

This is a very important concern. Voluntary adult smokers, who agreed to be followed for their health state, were recruited by a newspaper announcement. The inclusion criteria consisted of smokers who considered themselves as healthy but who had smoked median 20 pack-years; none of subjects had been diagnosed with lung or other chronic diseases, allergies, allergic diseases or were taking any prescribed medications A careful clinical history was conducted to exclude previous asthma diagnosis. Originally 620 adult smokers were recruited, this cohort has been described in detail by Toljamo et al Scand J Primary Care 2010 (ref included). The samples were chosen randomly from this cohort. Among young draftees the inclusion criteria consisted of young adults who had smoked mean 5 years and felt themselves healthy without any allergic or other chronic diseases having no daily medications. All voluntary young adults were allowed to participate to the study during their military service. The cohort has been described recently (Hamari et al...
Ann Med 2010), the samples were obtained from those subjects who agreed voluntarily to participate and travel to the hospital area for the additional investigations. These studies have been reviewed and some clarifications included in the manuscript.

c. Authors mentioned that patients received “no other environmental exposures,” was this based on patient self-report or anthropometric measurement?

The information about other environmental exposures was self-reported in the detailed questionnaire in a beginning of the study concerning second-hand smoke, asbestos, arsenic, cadmium, chrome, silica, nickel and lead exposures. This has been included.

d. Study needs to state methods of informed consents. Even though authors stated that this project has obtained an approval from the ethics committee of the hospital, further details in regards to acquiring informed consents was not mentioned. Please clarify this problem.

The study nurse told personally to every participant, both adults as well as military draftees, about the study and gave written information during the baseline visit. Every participant filled then the written informed consent, and this has now been included to the manuscript.

e. Statistical methods need to be clarified. The number of subjects in each group was different and does not seem to have enough power for statistical comparisons. Authors may need a statistics expert to clarify the method

We agree, and all concerns have been taken into careful consideration. We have consulted our expert in statistics, Professor Pentti Nieminen, to analyze some parts of the data. To increase the statistical power we have employed ANOVA and t-test for comparisons between study groups. We now also apply multivariate linear regression method to clarify the effects of age and smoking on all response variables.

3. Minor essential revisions:
a. First appearance of an abbreviation should be replaced be spelled out (ie. Abstract: COPD, TIMP-1, EIA/ELISA, FEV1/FVC. Page 5 line 1: COPD; line 7: SP-D. page 6 line 11: TIMP-1)

We thank the Reviewer for this very careful review, and all the minor errors have also been corrected. After comparison to several corresponding studies ELISA/EIA and FEV1/FVC abbreviations are generally used as abbreviations in corresponding studies.

b. Please check the manuscript carefully by a native English speaker.

The language has been revised by a professional English speaker who has special expertise in revising biochemical/medical texts. The errors concerning English language have been corrected.

Reviewer: Russell Bowler
Reviewer's report:
Major comments:
The COPD group has a very high FEV1% suggesting most are mild or moderate COPD. Please describe how many subjects were GOLD 1,2, or 3.

We have described the COPD stages in the Results part of the study: “In the COPD group, there were 15 patients with stage I, 24 patients with stage II and 5 patients with stage III COPD according to the GOLD classification. Ref GOLD is included in the reference list. All COPD cases were newly diagnosed and were taking no regular medications”.

There is distinct clustering of biomarkers in the young controls (Figure 2A,3A,4A) that likely skew the results. Can the regression curves be calculated without this group? Also, what about multivariate analysis?

We are grateful to the Reviewer for the suggestion to improve the statistical analyses. We agree that the regression slope and correlation coefficient should not be calculated when data includes distinct subgroups. We have now improved the scatter plots to make them easier for readers to evaluate, though we decided to include the cohort of young people as we found it to be very informative. We have consulted our expert in statistics, Professor Pentti Nieminen (also included now as one co-author) and have included the multivariate linear regression analyses to evaluate the possible relevant associations by controlling other variables. All results were recalculated with the statistician.

Minor comments
In the discussion, the authors may want to include a recent BMC publication that clarifies SP-D in BALF of smokers and those with COPD (Moré et al. BMC Pulmonary Medicine 2010, 10:53)
Page 16, last sentence: should be written “included in” not “included into”

We thank the Reviewer for this very careful review and good suggestions. We have included the suggested BMC publication by More et al to discussion (Ref No 41) and corrected all the minor errors.

Figure 1, instead of box plots, actually data points would be useful to display. Also consider adding a table that summarizes all the biomarker data.

We thank the Reviewer for this suggestion. We made the dot plot presentation to visualize the findings. However, these figures appeared to be fussy. After consultation of the statistician, we feel that box plot figures are more helpful for the readers.