Author’s response to reviews

Title: Annual decline in forced expiratory volume and airway inflammatory cells and mediators in a general population-based sample

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Cover Letter

We appreciate the efforts of the Editor and Reviewers to evaluate and comment on our manuscript, which we believe has improved the manuscript.

Editor Comments (EC):

EC1:” It does seem that there are still concerns for inadequate description of methods (both statistical, as well as sputum processing”

Authors’ reply:” Regarding description of sputum processing, see our reply to OH and statistical methods see our reply to WL.”

EC2:” It appears that several of the analyses that were conducted in response to reviewer concerns were not reflected in the manuscript adequately.”

Authors’ reply:” Multivariate analyses are now added in to the revised manuscript, see our reply to WL1”.

Olaf Holz (Reviewer 1), (OH):

OH1: 1. “As noted by all reviewers there were numerous mistakes and "typos" in the table of the original manuscript. Unfortunately there are still mistakes in the revised versions of the tables.

In table 2 the numbers for the tertiles were corrected. But these corrected values do not match with the respective numbers in table 3. Which set of values is correct? “

Authors’ reply: “We regret that there were still errors in the table 2 in the revised version and these typos are now corrected.”

OH1:2. In addition, there should be a note to table 2 that the mean packyear level is derived from both current and former smokers and indicated in the comment to OH.

Please also provide a line that indicates the number of sputum samples that were available for the 3 tertiles. As indicated in comment CF3 and on page 9, line 12, 13 "some" samples failed to fulfill the criterion of having 20% squamous cells. Please also delete "some" and state the exact number in the text.

Authors’ reply: “The number of sputum samples having 20 % or more squamous cell is now added to the manuscript, page 9, line 14. The note about packyear and number of sputum samples is added at the bottom of table 2. “

OH2:” The answer to comment OH8 (the description of sputum induction) is not clear. All participants inhaled first 0.9% and all participants then inhaled 3, 4, and 5%? Each inhalation period lasted for 7 minutes? The statement "until a sufficient amount was delivered" suggests that some subject only e.g. inhaled for 5 min others e.g. for 20 min. The danger in doing so is, that it could affect sputum composition, as the first portions produced are generally rich in neutrophils and later portions richer in macrophages) is not clear.

OH2:1. “Apparently, the total inhalation time was not standardized, which is likely to have increased the variability of sputum neutrophil counts. This cannot be corrected at this stage and is already described sufficiently in the method part.”

Authors’ reply: “We thank OH for this important comment. In the revised version we have added our comment in the discussion, page 20 lines 15-17.”

OH2:2. “For the reader it is not clear, if whole expectorate was processed (by definition this is unselected sputum containing large quantities of saliva) or if sputum was selected from saliva
before the processing. The response to this question is not clear: OH9”5. Was the sputum selected from saliva prior to processing? Or was the whole expectorate processed. This is important to interpret the biomarker levels in the supernatant. "Authors’ reply: Sputum was selected from the prior saliva. We have removed the excess sputum from the sample.

I figure that sputum plugs were selected, but then this needs to be clearly stated in the text. Using a criterion of maximal 20% squamous cells as indicated in the comment to CF3 is very strict and would we rather incompatible with using the "whole expectorate" for processing.

Authors’ reply:” Thank you for your comment. It now added in the revised manuscript, see page 9 line 3 and 4. “

Waseem Labaki (Reviewer 2), (WL):

WL1:” I again think that a multivariable model including confounders that could affect the association between cell counts and FEV1 decline should be included in the manuscript. It is fine (even desired) to show the univariate analyses, but it is equally important to determine what happens to the association after accounting for basic confounders. It looks like the author did build a multivariable model based on their reply to my comment. However, I did not see it included in the manuscript. Instead, on page 19, line 20, it is mentioned that "the data did not allow for multivariate analyses." This is confusing and should be clarified as the raw data should be available to conduct such analyses.”

Authors’ reply:” Multivariate analyses are now included in to revised manuscript, see page 15 line 4-8.”

WL2: “I am still not sure about the ACO terminology the way it is used in the manuscript. Although a positive spirometric reversibility test is a common feature of ACO, some smokers with and without COPD may show reversibility and still not have asthma.”

Authors’ reply:” We thank the reviewer for this comment. However, a universal ACO definition does not exist. We chose to use a definition of ACO that is in agreement with GOLD guidelines. We agree with the reviewer these cases may or may not have asthma in addition to COPD. We believe that our definition of ACO simplifies this concept. Actually, none of the other reviewers had any comments to our ACO definition.
WL3:” Minor typo in Table 1: The percentage of never smokers in the no follow-up visit should be 48%, not 52.”

Authors’ reply:” It is now corrected.”

WL4: ”For Table 2, the authors stated in their reply to my comment that they used log-transformed blood counts in a one-way ANOVA test. I recommend they explicitly mention in the manuscript that blood counts were log-transformed and that all other data was normally distributed so the reader does not question their use of an ANOVA test (especially given the overall small sample size).”

Authors’ reply:” It is added in the bottom of table 2.”

WL5:” Typos in Table 2: a) The percentage of males should be 47% (not 45%) in the < 25 ml/yr group and should be 50% (not 53%) in the 25-38 group. b) The percentages displayed between parentheses next to actual counts in the "Smoking History" section of the table are calculated based on row% whereas the rest of the table show column%; this should be fixed for consistency. c) For monocytes, the values for each group are 0.4, 0.4 and 0 respectively. Therefore, it would be expected to be between 0 and 0.4 for the entire cohort, but it is being shown as being 0.4 in the table (unless this is due to rounding). d) The standard deviation for macrophages in the entire group is shown as 1 in the table, which seems to be too low given that standard deviations for each of the subgroups were 20, 18 and 12, respectively. Please clarify.

Authors’ reply:” a) We regret the percentage typos in table 2, and thank the reviewer for clearing up this point. The typos were in the numbers of subjects n, and now they are corrected, hence the percentages of males have also become correct;

b) We meant to report percentages in columns. The errors are now corrected.

c). This is exactly what we have done. It was due to the rounding (0.3488 was rounded to 0.4).

d). It is now corrected

WL6:” Typo in Table 3: The number of individuals in each subgroup is shown as 20, 19 and 23, respectively, whereas it was 19, 20 and 23, respectively, in Table 2.

Authors’ reply:” The number of individuals in table 2 is corrected.”