Author’s response to reviews

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

Authors:

Natalia Kononova (natalia.kononova@medisin.uio.no; nataliapromenaden@gmail.com)
Liv Ingunn Bjoner Sikkeland (l.i.b.sikkeland@medisin.uio.no)
Faiza Mahmood (Faiza.Mahmood@ahus.no)
Maria Vistnes (maria.vistnes@medisin.uio.no)
Johny Kongerud (johny.kongerud@medisin.uio.no)
Gunnar Einvik (gunnar.einvik@medisin.uio.no)
Vidar Søyseth (vidar.soyseth@medisin.uio.no)

Version: 3 Date: 06 Oct 2018

Author’s response to reviews:

Editor Comments (EC):

EC1: “In response to one of the reviewers comments, the authors described that the sputum induction procedure was considered complete when a "sufficient amount" of sputum was produced. This was included in the discussion, but without any description about the implications for what this would do to the findings of the study. What kind of bias could this potentially introduce? It seems that the result could be an inaccurate representation of neutrophil counts, which may have serious implications for the conclusions of the study. The authors should expand upon this in the discussion. Additionally, this information about the sputum procedure should be reflected in methods.”

Authors’ reply: Olav Holtz mentioned in one of his comments that unstandardised total inhalation time could affect sputum composition, as the first portions produced are generally rich in neutrophils and later portions richer in macrophages. It could be one of possible explanations why we did not find association between sputum cells and dFEV1. However, in a previous study (Sikkeland et al., 2016) our group found predominance of neutrophils in sputum among workers in a similar age group. Subsequently, older age could be an alternative explanation of our finding.”
EC2: “In response to one of the reviewer comments, the authors perform multivariable analysis (not multivariate as written by the authors, which is not accurate). However, when they report these results in the methods section, they comment that the result is a significant association of neutrophils with pack-years smoked, which is NOT the outcome. It would have been assumed that the outcome of interest, or y-variable, would be lung function, in which case this description does not make sense. Therefore, the authors need to reconsider this analysis, possibly reattempt this (y-variable should be fev1, x-variable should be neutrophils and other inflammatory markers, with adjustments for the other covariates noted). They should also consider a table displaying these results

Authors’ reply: "We investigated the associations between dFEV1 as well FEV1 at baseline as outcome variables, and IL-8, IL-6, IL-23 IL-17f, IL-17a, IFN-γ, TNF-α and sputum neutrophils as covariates. None of these relationships reached significant levels. It is likely that the variation in the outcome as well as explanatory variables were too wide, as the residuals of these regressions were normally distributed and in the normal range for FEV1 as well as dFEV1 (figure 1 and figure 2).

It added in page 15, line 4-8.

Figure 1

Figure 2

EC3: The authors response to the query regarding ACO is insufficient. BD reversibility is not an acceptable criterion for ACO nor does GOLD state this. Perhaps the authors should represent the results as related to BD reversibility in lieu of referring to BD reversibility as a proxy for ACO-this would be more accurate and direct. There should be no need to define ACO at all given that this is not necessarily within the scope of the ultimate message of this paper.

Authors’ reply:

Authors’ reply: ACO definition is now replaced by BD reversibility.

EC4:“The response to the reviewers query about log-transformation was to add this information to the footnote of Table 2, however it would be better to include this in the methods section as well.”

Authors’ reply: It is now added in to methods section. See page 10, line 17-18.
EC5: "We would also like to reiterate the importance of ensuring that data published here are
accurate. Therefore we request that the authors double check all data from figures and tables to
ensure that all data are accurate as written."

Authors’ reply: We thank the reviewer’s careful reading of our paper. In the revised version all
the tables are checked again, and the tables as well as the figure are now correct.

EC6: ”Can the authors comment on whether DTT would be expected to degrade any of the
ability to detect levels of the cytokines of interest?"

Authors’ reply: "DTT can affect three dimensional structure of proteins causing to release of
mucus bound molecules or by interfering directly with immunoassay. Previous study of induced
sputum from asthmatic patients has shown that DTT have no effect on IL-8 level and can lower
concentration of myeloperoxidase (MPO) and TNF-α. Another study shows that treatment with
DTT spontaneous sputum from patients with chronic bronchitis and bronchiectasis does not
significantly affect median levels of IL-6, IL-8, but does significantly reduce the median levels
detectable TNF-α, and MPO and produces a small but significant increase in the median MIP-1."

EC7:" Though the authors note that 32% of sputum samples were excluded, this was never
indicated in figure 1. Also, it is not clear what was done analytically with the excluded samples
as the authors appear to be reporting data from the full sample. This needs to be clarified. Is the
cohort of 120 defined for after exclusion of this 32% of the samples? The reviewer attempted to
raise this question which led to the author indicating in the table 2 footnote "Number of sputum
samples was equal to number of participants in all three groups." However, the authors do not
show us how this is possible- what happened to the 32% of samples excluded?"

Authors’ reply: "Again, we thank the reviewer the comment as we admit that it is not clear how
the exclusion of samples with more than 20% squamous cells was performed.

Sputum cell data were used as explanatory variables. Therefore, we restricted the analyses
between the outcome and sputum cells to samples with less than 20% squamous cells."

EC8: "In the discussion, the authors state that "sample selection maybe have biased the results"
of the study but then immediately state "bias is due to chance" because of random selection of
participants from the population. Can the authors elaborate on this concept more. These are
contradictory statements."
Authors’ reply: “We admit that this statement may look contradictory. In the revised manuscript we have rephrased this statement, page 20, lines 4-6”.

EC9 limitations should mention also that 32% of sputum samples were excluded and how this could have impacted the observations of the study.

Authors’ reply: "As mention in EC7, sputum samples with more than 20 % squamous cells were excluded insofar as sputum cells were included as explanatory variables.”

It added page 20, line 8-9.