Reviewer's report

Title: Early and similar matrix alterations in alveolar and small airway walls of COPD patients.

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Reviewer: Riitta Kaarteenaho

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The manuscript of Eurlings and co-authors presents histochemical analyses of elastin, collagen and hyaluronan, and immunohistochemical analysis of pSMAD2 in the lung tissue of the patients with COPD stage II (n=16), stage IV (n = 19) and in smoking controls (n=14). Although the topic is both interesting and important, some corrections and additions would essentially benefit presentation of the study.

Major Compulsory Revisions

Introduction:

In the fifth paragraph in the Introduction it has been mentioned “So far, none of these previously mentioned studies examined matrix remodelling simultaneously in different compartments of the lung and in different severities of COPD.” Some recent publications, however, about the topic of ECM in COPD would be exemplifying to present in more detail in the Introduction, and also to debate in the Discussion. Annoni and co-authors evaluated by histo- and immunohistochemistry elastic fibers, collagens type I, III and IV, versican, decorin, biclycan, lumican, fibronectin and tenascin in different compartments of lung i.e. in large and small airways and lung parenchyma in COPD, smokers without COPD and nonsmoking controls (Annoni et al, Eur Respir J 2012). Karvonen HM et al (Respir Res 2013) studied by immunohistochemistry 101 patients including non-smokers, smokers with normal lung function, ex-smokers with normal lung function, current smokers with COPD and ex-smokers with COPD for alpha-smooth muscle actin, tenasin-C and EDA-fibronectin, which factors were quantified in large and small airways and in alveoli.

Study subjects:

Demographic data of the manuscript does not include the information how COPD was defined, i.e. the diagnostic criteria for COPD, which information would be important to add. In the current form of the manuscript only COPD stages have been informed. Furthermore, the data of the medication for COPD including systemic and inhaled corticosteroid of each patient would be significant to add. In the Table 1 and in the Discussion it was informed that most of the control patients were ex-smokers. The non-smoking period of control cases, i.e. period from smoking cessation to lung surgery operation would be needed to include into the demographic data.
Staining:

Picro Sirius Red staining stains collagens I and III, which was not mentioned in the manuscript. In addition, by using polarized light resulting birefringence the collagen fibers can be distinguished between type I and III, which was not mentioned either. Moreover, the manufacturer of the Picro Sirius Red reagent was missing.

It was informed that two blinded independent observers scored semi-quantitatively pSMAD2 expression. Repeatability of the analyses (by using kappa coefficient) would be needed. In addition, the information of the repeatability of the analyses and the number of pathologists that analyzed other staining i.e. elastin, collagen and hyaluronan would be necessary.

Quantification of matrix:

A more detailed description of the quantification system of each factor would be essential. Image examples of quantification analyses might be informative. For alveolar staining, information of the protocol for exclusion of vessels and airways from the total alveolar area analysis would be needed. It was informed that a total of 111 airways were measured from all patients. It would be important, however, to clarify also the numbers of small airways analyzed per patient. If the numbers of small airways would decrease in severe COPD cases, as it could be expected, it would be interesting to evaluate if this effect possibly have some association to the results.

References:

The list of references in its current form is not according to the instructions for the authors of the Journal. In most of the references, only the first author has been listed, and not all. Moreover, name of the journals are written mostly in their total length, and not by using the official abbreviations of the journals. In some journals capital letters have been used, but not in all. Information of volumes, numbers and years of the journals are totally missing in some references. In sum, the reference list needs complete revision according to the instructions of the Journal.

Figures:

Images with higher magnification with scale bars would be more illustrative than the present figures 1, 2, 3 and 6. Hyaluronan images are not very sharp and therefore new images would be needed. The figure 5 shows that SA ratio include quite high distribution which fact could be debated in the Discussion section.

Minor Essential Revisions

Name:

Name of the manuscript would be useful to reconsider especially in the context of a word “early” since COPD stage II, but not stage I, were included.
Abstract:

According to the instructions for the authors of the Journal, the abstract should be structured into separate sections of Background, Methods, Results and Conclusions.

**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