Author's response to reviews

Title: Measurement of MMP-9 and -12 degraded elastin (ELM) provides unique information on lung tissue degradation

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Author's response to reviews:

Regarding: Manuscript 1184996429657448

Dear Dr. Emily Crow

Thank you for the positive and constructive review of our manuscript 1184996429657448 entitled “Measurement of MMP-9 and -12 degraded elastin (ELM) provides unique information on lung tissue degradation”

We have carefully read and discussed all of the reviewers comments. Please find below a point-by-point rebuttal to each of individual concerns of the reviewers. This has improved the manuscript in 3 major areas.

1) The suggested literature has been studied carefully and additional references have been added to the manuscript. This has provided further depth to the manuscript.

2) We have improved the statistical representation of the data, as suggested by reviewer 2.

3) We have further elaborated on the rational for choosing the exact sequence and disclosed more sequences. This has provided much more information and more perspective to the manuscript.

4) The style of written English has been edited and the format polished as
requested to conform to the journal style.

The changes to the manuscript are highlighted by using red colored text. We hope that by these important changes that the manuscript is acceptable for publication.

Sincerely,
Helene Skjøt-Arkil

Reviewers' Comments to Author:

Reviewer Farrah Kheramand

“This is an interesting paper and the findings are of significant interest. There is a minor concern regarding the fact that the authors have not carefully examined prior work published in this field. In particular a recent paper entitled "Study of human lung elastin degradation by different elastases using high-performance liquid chromatography/mass spectrometry" by Begona Barroso, Nicolas Abello, Rainer Bischo has extensively examined elastin fragments and Lee et al (NM 2007) has found autoimmune response to the elastin fragments. The authors should use the existing literature to contrast with their current findings in this report.”

The paper by Begona B et al and Lee et al have been examined carefully and commented in the manuscript. Please see page 12.

Reviewer Yong Lin

“In the manuscript, the authors performed in vitro enzymatic degradation of elastin from human aorta by MMPs, cathepsins, aggrecanases and identified elastin derive peptides by proteomic mass spectrometry. An epitope VPGVGISPEA was selected to generate a monoclonal antibody, which was used to develop ELISA and showed the presence of the epitope in plasma of both COPD and IPF patients and claimed to provide unique information on lung tissue degradation. While the study suggests an effective approach to study biochemical elastin degradation relevant to disease process, the experimental methods which lead to the conclusion is not clear and incoherent. The main questions to be asked are:

1. The peptide identifications were studied in three different laboratories. One lab identified 114 peptide using a large group of MMP, cathepsins, and aggrecanases. The results was used to analyze the mode of cleavages in elastin (Table 1). The other labs were working only with MMP-9 and 12 and said to identify 441 peptides, but only few selected peptides are present in Table 2 and 3. There is no explanation to comparison or correlate the identified peptides between the labs.”

Regarding the peptide identification in table 2, it was chosen only to show an example of the observed different cleavage kinetics and not all 284 (416-132)
identified peptides. This was to focus on, that the cleavage products are dependent on incubation time, amount of protease and stability of the peptide rather than showing all 416 identified peptides.

It is correct, that the peptide identification in the different laboratories has not been compared. This would be interesting to perform, but it was deemed out of scope for this manuscript.

“2. The most important question should be asked is the selection of VPGVGISPEA. The peptide is identified in the digestion of vascular elastin by MMP-9 and 12 and selected to generate monoclonal antibody as biomarker for elastin degradation of lung extracellular matrix in COPD and IPF. The elastin derived peptides from lung and vascular system are not likely identical. It has been shown that well studied antigenic elastin peptides VGVAPG isolated from aorta or ligamentum nuchae are not present in the same digestion from lung. (He J etal, Exp Lung Res 2010; 36: 548). In addition neutrophil elastase is the major protease involves in lung elastin degradation in COPD, although macrophage has also partially involve in the disease. Several recent publications have shown desmosine and isodesmosine as a promising biomarker for the lung degradation in COPD. The statement in the manuscript (4th paragraph of Instruction) that “Desmosine and isodesmosine has not proven accurate or precise predictor of COPD” is not accurate.”

It is correct that elastin derived peptides from the lung and vascular system are not likely to be identical, however ELN-441 has been assessed in both tissues and it seems to be present.

Neutrophil elastase is definitely a major protease, but it was chosen not to focus on this protease in the study.

The statement in the manuscript regarding Desmosine has been reformulated and supported by additional references – please see page 4 in the background section.

“3. Soluble lung and soluble aorta have been used to compare the generation of peptide. (Fig3). Definition of soluble lung and aorta and the source or how are they obtained need to be clarified.”

The preparations and sources of soluble and non-soluble elastin from aorta and lung have been further described in the method section. Please see page 5 in the methods section.

Reviewer Raja Abboud

“The authors have used different proteases to degrade human lung and aortic elastin. The elastin fragments were identified by mass spectroscopy. The sequence VPGVGISPEA, named ELN-441 was generated by proteolysis by matrix metalloprotease (MMP)12 and MMP9, and was used to develop monoclonal antibodies against the peptide and to develop an ELISA. The ELISA was used to test serum from 10 subjects with COPD, 29 subjects with idiopathic pulmonary fibrosis (IPF), and 11 healthy controls. Compared with the control
subjects, levels of the elastin peptides were markedly elevated in the COPD patients, and were also elevated in the IPF patients but to lower extent than in COPD. The development of this ELISA specifically for a fragment of elastin appears to be very promising as a marker of elastin degradation in vitro and is likely to be useful blood marker in COPD and emphysema. However, more clinical data are required to evaluate and validate this ELISA, as the authors themselves admit. Specific Comments:

1. Abstract: Conclusions: The statement that this assay is “the first ELISA for serological measurement of elastin degradation “ is not correct and should be amended or deleted (see Discussion: bottom paragraph on page 14)”

The statement has been clarified in the manuscript – please see page 2.

“2. Page 7 last paragraph: Why did you get serum from only 11 controls ? The small number of controls leads to problems defining what is the upper limit of normal, as discussed in the next comment.

3. Page 8: Statistics: Were the data points in the controls normally distributed ? In Fig 4 A, it would be better to show the actual data points and show median and quartile values rather than bars and SD. If the control data were normally distributed, the upper limit of normal would be taken as mean + 1.645 X SD, a level which would be exceeded only by 5% of the normal population, rather than a level of 1 SD above the mean. I have difficulty with the odds ratio and Fig 4C which is an exaggeration of the differences between IPF and COPD compared to controls, and I suggest deletion of that panel.”

We would have liked to assessed the ELN-441 marker in a larger control cohort, but only 11 controls were available in this study.

The data points of the controls were normal distributed, and it is a good point, that the upper limit should be 5% of the normal population. Since the study consists of 11 controls, and not infinity, the upper limit has been calculated as the mean+1.8xSD, which is now reflected in the manuscript.

We believe that the current graph in figure 4A and the improved figure 4C are the most effective and descriptive way of communicating the results, and therefore we have chosen to keep them. However, if it is a prerequisite for the publication of the manuscript that we change these graphs, we will of course reconsider the request.

“DISCRETIONARY REVISIONS

1. Page 3, paragraph 3, sentence before the last: COPD by itself without co-existing emphysema does not result in reduced lung elasticity. Measurement of lung elastic recoil was used to determine the presence of emphysema in COPD before the advent of computerised tomography (CT) scans of the chest.

2. Page 3 line 3 from the bottom; please include inflammation in the small airways and the co-existence of emphysema in a significant proportion of COPD
patients.


4. Page 11: Diagnostic value of ELN-441: I am skeptical about the use of this ELISA in IPF. You can differentiate COPD from IPF by the clinical, pulmonary function data and chest radiographs rather than the ELISA. CT scans of the chest can enhance the diagnosis of IPF and differentiate it from other interstitial lung diseases. Furthermore, the main pathologic problem in IPF is pulmonary fibrosis and not elastin degradation. The ELN-441 ELISA is likely to be helpful to evaluate elastin degradation in COPD, in relation to clinical and radiologic phenotype (predominant airway disease vs emphysema), but you’ll need a greater number of COPD and control subjects than the present ones (10 and 11). Do you have clinical, radiological and pulmonary functional data on the COPD patients?

5. Page 12 paragraphs 2 and 3: You tested in vitro elastin degradation by 10 different proteases to obtain the peptide ELN-441, which was generated by MMPs 9 and 12, but you did not mention neutrophil elastase. Can you please comment on the potential pathogenic role of neutrophil elastase in emphysema, specially that associated with severe alpha1-antitrypsin deficiency. Hopefully, it will be possible in the future to produce a monoclonal antibody to detect elastin degradation by neutrophil elastase in vivo by finding an elastin degradation peptide specific to neutrophil elastase, and producing a monoclonal antibody to it”.

The discretionary revisions have been corrected/clarified in the manuscript. See page 2, 3 and 3. Unfortunately we do not have access to clinical, radiological and pulmonary functional data on the COPD patients. It is correct, that neutrophil elastase is a major protease, but it was chosen not to focus on this protease in the study.