Reviewer's report

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

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Reviewer: Raja Abboud

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MAJOR COMPULSORY REVISIONS

General comments:
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)
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.

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.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I HAVE NO COMPETING INTERESTS