Reviewer's report

Title: Matrix Metalloproteinase-9 Mediated Inflammation Persists up to 6 Months after Smoking Cessation

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Reviewer: George Leikauf

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Major Concerns:

1. Is the question posed by the authors well defined? No.
The scientific question addressed in this study was compromised by the high drop out rates of the subject who attempted to stop smoking. In the introduction, the authors attempt to address the similarities and difference between inflammatory cell types and proteinases/antiproteinases in lung of persons with asthma and COPD. This background seems to suggest that there might be difference in these subgroups in the level of inflammation and proteinase that would be altered during smoke cessation. However, there is not enough members of each group (non-asthmatic/non-COPD, asthmatic, mild COPD and moderately severe COPD) at the end of the study. All the subject groups are combined for the analysis. Therefore, the question posed is “if smoking cessation is associated with changes in numbers of neutrophils, MMP7, MMP8, MMP9 and TIMP1 levels in induced sputum specimens” in persons with and without asthma or COPD. The attempt to obtain data from different clinical subtypes diminishes the power of the study greatly. Thus, the study can only be viewed as preliminary. A case crossover design using only asymptomatic smokers would be a useful improvement. Unfortunately, based on dropout rates, the study probably would need to recruit about 300 individuals to obtain data on 45 individuals at 6 months. Each subject will serve as their own control, however.

2. Are the methods appropriate and well described? No.
The statistical analysis is inappropriate. It is difficult to determine what the mean and standard deviations were for each group. Multiple comparisons are being done, which requires ANOVA with correction. The main comparison of interest in Figure is the smoker 6 month values compared to the smoker baseline. Both MMP7 and MMP8 seem to have improved and it difficult to determine why these value at 6 months are not statistically different from the baseline values for this group. Also it is not clear what the sample size is for each group and this should be added to the figure legend (is it 30 nonsmokers vs. 17 smokers at each time after cessation?)

3. Are the data sound? Yes, but only preliminary.
The sample size is small and statistical methods are of concern. Also the method of measurement of MMP does not include a measure of the enzyme activity, but
only the amount of the enzyme present. Activity could and should be measured by zymography or other commercial methods.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition? No.

Table 1 provides the data on the starting population, but Figure 1 is only a subset of the recruited population. The Subject characteristics for the remaining population should be report systematically.

5. Are the discussion and conclusions well balanced and adequately supported by the data? No.

The variability of the increase in MMP9 at 3 months in the Smoker group does support the strong conclusions: “MMP-9 release was unaffected by smoking cessation. Its elevation in IS even after smoking cessation may contribute to ongoing lung damage typical of COPD.” The overall balance of proteinase to antiproteinase levels could be improved because the largest amount of proteinase in these samples was MMP8, which decreases more than MMP9 increases. The conclusion that cessation does not stop the “ongoing lung damage typical of COPD” also is faulty because the subjects included COPD (n = 7) and non-COPD subjects (n=10). Lastly, what happen to the decline in lung functions of the individual who stopped smoking, did they continue to progress?

7. Do the title and abstract accurately convey what has been found? No.

The title “Matrix metalloproteinase-9 mediated inflammation…” implies that MMP9 is the cause of inflammation. However, inflammation (i.e. neutrophil levels) in the smoker declined at 6 months whereas the MMP9 levels did not. Again, the conclusion of the abstract is too strong.

Minor/Discretionary Concerns

1. It is difficult to see the median line in the solid boxes of the Nonsmoker in Figure 1.

2. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Somewhat

The bibliography is sparse and could be improved by including information on the benefits of smoke cessation. In addition, endogenous activation of MMP9 and MMP14 in non-smoking persons with asthma and COPD due to endogenous acrolein formation has been reported in the literature. This could explain the persist MMP9 levels.

3.6. Are limitations of the work clearly stated? Yes.

However it would nice if the paper stated that 6 months may not be long enough of a follow-up. Most benefits for smoke cessation (reduced lung cancer risk or slowing of loss of lung function) only are measurable after 3 years.

9. Is the writing acceptable? Yes.
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