Author's response to reviews

Title: Moraxella catarrhalis acquisition, airway inflammation and protease-antiprotease balance in chronic obstructive pulmonary disease

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Author's response to reviews: see over
Responses to Reviewers’ comments and suggestions on “Moraxella catarrhalis, airway inflammation and protease-antiprotease balance in chronic obstructive pulmonary disease”
G Iyer Parameswaran, Catherine T Wrona, Timothy F Murphy, Sanjay Sethi.

Reviewer’s report
Title: Moraxella catarrhalis acquisition, airway inflammation and protease-antiprotease balance in chronic obstructive pulmonary disease
Version: 1 Date: 26 May 2009
Reviewer: Hortense Slevogt

Reviewers report:
Comment: ‘Moraxella catarrhalis acquisition, airway inflammation and protease-antiprotease balance in chronic obstructive pulmonary disease'
Ganapathi I Parameswaran, Catherine T Wrona, Timothy F Murphy and Sanjay Sethi BMC Infectious Diseases Research article
Overall an interesting study focussing on the influence of Moraxella catarrhalis colonization and infection for the protease-antiprotease balance in patients with COPD. The manuscript is well written and in general, data are sound and the manuscript adhere to the relevant standards for reporting and data deposition. However. The study has some weaknesses mentioned in the following:
Major compulsory revisions:
1.) Page 6 Line 5: The authors should specify: what criteria determined “baseline” and what criteria does imply the increase of respiratory symptoms from baseline (eg. Dypnea to what extent, cough etc.) Was a score system used for the severity of signs and symptoms demonstrated by the patients?
Response: We have altered the text to indicate that “baseline” meant patient’s usual level of respiratory symptoms without any increase in dyspnea, cough or sputum production. Change in respiratory symptoms (increased dyspnea, cough, sputum volume or change in sputum color) that prompted the patient to seek medical attention was considered to be an exacerbation. We do use a scoring system to assess the severity of change in symptoms. This system has previously been described in a 2002 paper which has been cited for more details (NEJM 2002).

Comment: 2.) Fig 2: Is the increase in TNF a significant? The authors should include this in the figure and the figure legend (p19)
Response: The difference in the changes for TNF-α (from baseline) was not significant between colonization and exacerbation. We have altered the Figure and legend to show this.

Comment: 3.) Fig.3 and 4 and result part of these figures on page 10:
In Figure 3 the authors are demonstrating that the neutrophil elastase (NE) is increased in COPD patients with an exacerbation compared to "colonized" patients. However, as shown in Fig. 4 there is no difference in the SLPI between these groups. This should more precisely described in the result part (page 10).

Response: We have revised the text emphasizing this point as suggested (Page 11).

Comment: Moreover on page 10 line 8 the authors are stating: “Colonisation, as well as exacerbation was associated with reciprocal changes in NE and SLP”. This sentence is misleading. It would be clearer to say that as shown in Table 2 M. catarrhalis acquisition was leading to an inverse relationship between NE and SLPI triggered by an increase of NE and a decrease of SLPI.

Response: NE did increase in colonization and exacerbation; SLPI did decrease in colonization and exacerbation, as compared to pre-acquisition values. We have added a table (Table 3) showing this and clarified this point in the main text (Page 11). Therefore, there were inverse relationships between NE and SLPI for colonization, as well as exacerbation.

Comment: However the increase of NE was dependent on whether patients were “colonized” or had an exacerbation (fig 3). Regarding SLPI there was no difference in the decrease of SLPI in colonized patients when compared to exacerbated patients (Fig 4). Thus, the decrease in SLPI shown in Tabl. 2 seemed to be dependent on the acquisition of M. catarrhalis and seemed to be independent from colonization vs exacerbation as shown in fig 4. The authors should more precisely describe and discuss these results.

Response: Please see response to above comment. SLPI did decrease from pre-acquisition values in colonization and exacerbation (Table 3). The mean decrease for SLPI was larger in exacerbation compared to colonization (-0.55 vs. -0.47), but did not reach statistical significance (shown in Fig 4).

Comment: In the discussion part the authors should add an assessment of how M. catarrhalis-induced inflammation might be or is related to the protease-antiprotease imbalance since they show in Tabl. 2, in Fig 1-2 as well as in Fig 3 that the M. catarrhalis-related increase of NE can be related to the concomitant increase of M. catarrhalis-induced IL-8 and TNF.

Response: We agree that airway inflammation and changes in NE are probably related, since IL-8 chemo-attracts neutrophils, which produce NE. Prior studies have shown this relationship between IL-8 and NE during exacerbation (cited). We have extended these observations exploring the relation of IL-8 and TNF-α levels with NE during colonization (Figures 8 and 9). The text has been amended to show this (Page 11).
Comment: 4.) (Fig 5) To distinguish between the protease:antiprotease balance in patients without vs with M. catarrhalis infection and colonized with or having an exacerbation due to M. catarrhalis the linear regression for the relationship between SLPI and NE should also be differentially provided for # Samples from patients in the preacquisition state alone , # The acquisition state and with the additional differentiation between a.) with M. catarrhalis colonization and b.) exacerbation. Furthermore a correlation coefficient for each of the correlations should be provided. 
Response: This has been done (Figures 5,6,7).

Minor compulsory revisions:
Comment: 1.) Page 5: In the introduction it is important to specifically introduce active neutrophil elastase (NE) and secretory leukocyte protease inhibitor (SLPI) for there potential role in the course of COPD alone as well as for emphysema development. The authors should introduce to what extent measurement of the SLPI and NE is representative for assessing the protease-antiprotease balance in the airways. 
Response: This has been done (Page 5).

Comment: 2) In figure 2 and 4 a p value should be provided demonstrating that data are not significant. 
Response: Done.

Comment: 3.) The data presented in Fig 3 and 4 demonstrate that M. catarrhalis exacerbation is associated with an increase in NE when compared to “colonized” patients, whereas no difference is seen between those two groups for the decline in SLPI.
Activation of neutrophils with IL-8 and other chemoattractants has previously been shown to result in an increased expression of NE (Nadel, Chest 2000). Fig 1-3: Thus, it would also be interesting to correlate the NE data with the IL-8 and TNFα in colonized vs exacerbated patients by linear regression to point out this relationship. The authors should state if the increase in NE seems to be associated with M. catarrhalis-induced inflammation since they can be linked with the increase in IL-8 (and TNF α, if significant). 
Response: The relationship between IL-8 and NE has been previously described (cited in Discussion). We have added a similar analysis for NE and IL-8/ TNF-α (Figures 8,9). Reference added.

Comment: In addition the authors should add to the discussion part a statement whether they interpretate the data as M. catarrhalis-specific effect or as an unspecific effect due to the M.catarrhalis-induced inflammation. 
Response: This has been added to the discussion (Page 13).
Comment: 4) Page 5 line 14 as well as later on in the manuscript: acquisition of a new strain of M. catarrhalis implicates a differentiation of different M. catarrhalis strains. In this work only the presence of M. catarrhalis is documented. Therefore it would be more concise to just say “acquisition of M. catarrhalis”.
Response: Acquisition was defined as a new strain of M. catarrhalis for the patient, as shown by Pulsed field gel electrophoresis. This has been described in previous papers (cited). The text has been amended to clarify this (Page 6).

Comment: 5.) Page 11 line 11: Previous studies… This statement should be accompanied by appropriate citations.
Response: Done.

Discretionary Revisions
Comment: 2.) Page 11 and 12: Downregulation of SLPI was also described to e.g. be due to overexpression of TGF beta (Jaumann et al. Eur Res J 2000; 15: 1052-1057). The authors did not mention the possibility of downregulation in their discussion part (page 11). Are there other mechanisms/bacteria known to downregulate SLPI? If so its seems possible and therefore might be an interesting point to discuss that M. catarrhalis could also be directly involved in downregulation of SLPI since it is not related to the extent of airway inflammation (colonization vs exacerbation).
Response: This point has been added to the Discussion.

Comment: 4.) Page 12 Line 14: A further limitation is and could be stated is that only one protease and antiprotease was investigated despite the fact that more proteases and antiproteases are involved in the imbalance hypothesis.
Response: Done (Page 14).

Minor comments:
Comment: The authors should use the abbreviation M. catarrhalis instead of M catarrhalis
Response: Done.

Comment: Page 4 line 10: in the lower airways
Page 4 line 20: bacteria-associated
Page 12: line 8: NF-#B
Response: Text changed as suggested.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
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'
Reviewer's report
Title: Moraxella catarrhalis acquisition, airway inflammation and protease-antiprotease balance in chronic obstructive pulmonary disease
Version: 1 Date: 30 June 2009
Reviewer: Nestor Soler
Reviewer's report:
In the present study, Parameswaran and colleagues have performed a prospective study to determine the effects of acquisition of a new strain of pathogenic bacteria -M. catarrhalis- in COPD patients on the airway inflammatory pattern. During 1994-2000 period, 50 patients acquired a new strain of M. catarrhalis (120 acquisitions). The authors analyze changes in protease-antiprotease balance in bacterial colonization as well as exacerbation. Seventy-six acquisition samples (34 associated with exacerbation, 42 with colonization), with available pre-acquisition samples, were included and analyzed for IL-8, TNF-alpha, alastase and secretory leukoprotease inhibitor (SLPI). All changes were compared in paired samples from each patient. Specifically, they reported that IL-8, TNF-alpha and elastase were significantly increased after acquisition of M. catarrhalis in colonization and exacerbation. In contrast, SLPI was significantly lower after acquisition. They suggest that acquisition of M. catarrhalis in COPD increased airway inflammation. The worsening of the protease-antiprotease imbalance could contribute to progression of airway disease. The study is scientifically accurate, show original findings and its contents are complete and appropriate. However, there are concerns that require further clarification.

Comment: 1. Discretionary revisions
The major concern about this manuscript is the relatability of sputum cultures in clinically stable stable. The authors have to provide data about the quality of the samples and the number of them that could not be obtained. I understand, that only 76 pre-acquisition samples were used for a total of 120 new acquisitions. With respect of bacteriological data, how authors can explain the differences between acquisition samples in episodes of exacerbation versus colonization during clinical stable period?. Would have been convenient that the authors clarify this point and the results should be better structured.
Response: Of the 120 new strain acquisitions, samples were available for analysis in 76 episodes (the rest of the samples- 44- had been used up in prior studies exploring other areas of M. catarrhalis). The text has been altered to clarify this (Page 9).

In summary, I have 3 minor problems:
Comment: 1-Is sputum culture a reliable, reproducible, and comparable respiratory sample for this type of study? Authors have to give convincing reasons about this point.

Response: Sputum culture is the standard test used in multiple prior papers examining the role of bacteria in COPD. Moreover, sputum is available for repeated, longitudinal testing. Bronchoscopy and BAL would be too invasive to perform repeatedly. A prior paper has shown that expectorated sputum, as obtained in our study clinic, approximates closely to induced sputum and has the molecular markers of lower airway secretions (cited). This point has been added to Discussion (15).

Comment: 2-The study have a limitations to reach his major conclusion:
- The absence of cellular inflammation (sputum neutrophilia and eosinophilia) in respiratory samples.
- The lack of information of inflammation charge and severity of airway obstruction.
- The investigation of a single pathogen species clearly limit to generalize the results.

Response: These points are discussed in Discussion as limitations (Page 14).

Comment: 3. The information reported by the authors does not permit conclude that the protease-antiprotease imbalance could contribute to progression of airway disease in COPD.

Response: We have not claimed this. This paper investigates changes in airways of COPD patients when they acquire a new strain of M. catarrhalis. Whether such changes contribute to progression of COPD will have to be investigated in a separate longitudinal study.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests in relation to the paper.

Reviewer’s report
Title: Moraxella catarrhalis acquisition, airway inflammation and protease-antiprotease balance in chronic obstructive pulmonary disease
Version: 1 Date: 9 July 2009
Reviewer: Terence AR Seemungal
Reviewer’s report:
Major Comments
Comment: 1. Abstract is weak. It does not reflect the contents of the paper.
Response: Abstract has been changed to better reflect the main text as suggested.

Comment: 2. Background of abstract, the phrase: 'are not well studied'. This phrase in the background is argumentative. Please rephrase and be factual. If something is not well studies please say exactly what it is. The study does not appear justified from the background as stated here. Why have you chosen to look at SLPI, IL6 and IL8 amongst the many inflammatory markers found in the lower airway?

Response: Abstract re-phrased as suggested. IL-8, TNF-α and NE are established markers of airway inflammation and have been used as such by prior authors (References cited). Similarly SLPI has been used by prior authors as an indicator of anti-protease levels in airways (Reference cited). With limited resources, it is not possible to study all described markers of airway inflammation.

Comment: 3. What is the hypothesis to be tested? Please state concisely in abstract.
Response: The hypothesis has been added to the Abstract.

Comment: 4. Results of abstract: need to show the data.
Response: Abstract revised as suggested.

Comment: 5. Introduction: The hypothesis needs to be more clearly focused. Are you testing effects of M Cat on SLPI?
Response: We are testing the effects of M. catarrhalis acquisition on the balance between NE and SLPI. Hypothesis explained more in Introduction, as suggested.

Comment: 6. Methods: you state that between 1994 to 2000, there were 120 acquisitions of M Cat. Kindly define what you mean by an acquisition

Response: Text altered to clarify “Acquisition” (Page 6).

Comment: 7. Secondly, you refer to your 2005 paper in AJRCCM (ref 12), are you therefore reanalysing data that was already published? If this is a new analysis of data that is already published then it may well be extremely important but you do need to say so and can you state how this study differs from the 2005 data so far published? Was the data in this study used in some form in the 2005 paper which you quoted?
Response: No, this paper is not a re-analysis of the 2005 study quoted. The 2005 study reported on patterns of Moraxella catarrhalis carriage in COPD from the same cohort of patients, but did not measure airway inflammation or protease-antiprotease balance. We have quoted the study as the samples used were from the same cohort, but the measurements were all done specifically for this paper in 2008-2009.
Comment: 8. In the first paragraph of the methods you state or give the impression that all patients had COPD, is this correct ie was the FEV1/FVC less than 70% for all patients?
Response: These patients all had tobacco smoke-induced chronic bronchitis. Text amended to clarify this. The majority has FEV1/FVC of <70. Patients and samples from this study clinic have been used in multiple prior peer-reviewed publications (References 5, 11 and 12 cited in Methods).

Comment: 9. The paper seems to be about 74 ‘acquisitions of M Cat’ but in the methods you refer to 120 acquisitions? Which is it?
Response: We have altered the text to explain this better (Page 9). Of 120 acquisitions, paired samples (pre-acquisition and acquisition) for 76 acquisitions were available for study. Samples from the rest of the 120 acquisitions had been used up in previous studies, examining other aspects such as immune responses.

Comment: 10. Results: the statement of the results is sometimes confusing: Eg: ‘We analyzed the data further to determine whether airway inflammation increases with colonization by M. catarrhalis, and to compare these changes with those seen with exacerbation.’ But in the paragraph above you already looked at changes with M Cat.
Response: The first paragraph looked at changes with acquisition, compared to pre-acquisition samples. The acquisition samples contained colonization and exacerbation samples. In the second paragraph, we separately analyze changes in colonization and exacerbation.

Comment: 11. The phrase: ‘SLPI is a major anti-protease in the airway lumen, while NE reflects uninhibited protease activity’ appears for the first time in the results. This should form part of your background. You are suggesting that there is a reciprocal relationship between these two parameters but further on the discussion you admit that this relationship is not well understood.
Response: The reciprocal relationship between NE and SLPI has been described by prior authors during exacerbations of COPD. However, the mechanism underlying this change, whether it is decreased production of SLPI or extracellular degradation of SLPI, is not fully understood. We have discussed possible mechanisms of this relationship (Page 13).

Comment: 12. Figure 5 shows trend line for the relation between SLPI and NE. You state that this is a linear regression. However you do not show the data for the regression calculations anywhere nor is the model explained. This is confusing. Further, is the line drawn in Fig 5 actually the regression line, if so what software was used?
**Response**: Linear regression figures (5,6 and 7) have been changed to better reflect these points. More data included. We have indicated the software used in the Methods section (Page 9).

**Comment**: 13. ‘Our clinic recruits patients with smoking-induced’ is stated in the discussion of limitations. Thus have you included COPD or Chronic bronchitics in the study or did you recruits have both conditions? Please state clearly in our inclusion criteria.

**Response**: Please see response to Comment 8. Our clinic recruits patients with smoke-induced chronic bronchitis. The majority has FEV1/FVC <70. The clinic, patients and methods have been described in many prior papers (cited).

**Comment**: 14. You state in your discussion that : ‘Colonization episodes by definition were not associated with symptoms of respiratory infection, making a concomitant viral infection when these samples were collected unlikely’. Surely you are assuming an acute viral infection? What about latent viral infection?

**Response**: We intend to say that there were no symptoms suggestive of an acute viral infection, and have altered the text accordingly (Page 14-15).

**Level of interest:** An article of limited interest
**Quality of written English:** Acceptable
**Statistical review:** No, the manuscript does not need to be seen by a statistician.
**Declaration of competing interests:**
None to declare