Author's response to reviews

Title: Occupational exposures, smoking and airway inflammation in refractory asthma

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Author's response to reviews: see over
Dear Professor Mattes,

We thank you for your encouragement and advice, and we would like to resubmit the manuscript entitled ‘Occupational exposures, smoking and airway inflammation in refractory asthma’ (MS: 2146363650141234) for your further consideration as an original research article for publication in BMC Pulmonary Medicine.

We have revised the manuscript to address the concerns raised by the reviewers. The changes in the revision have been marked in red colour, and please see our point-by-point responses below. We will upload the revision with track changes as the supplementary file for your evaluation.

If we can be of any assistance regarding the process of this manuscript, please feel free to contact me. We look forward to hearing from you soon.

Editor’s comment:

Additional comment: Can the authors please provide a reference for the parsimonious model and a further short explanation of this method (line 146: "Backwards stepwise methods were employed to determine the parsimonious model.")

Response: We have clarified our methods as follows “insignificant variables were removed from the full model to obtain the simplest model with greatest explanatory power”

Reviewer: Rakesh Kumar  
Reviewer’s report:

This paper emphasizes the important point that taking an occupational history may be critical in planning the management of an asthmatic patient whose clinical status has deteriorated. It also addresses some other interesting issues, although aspects of the study are somewhat unclear.

- Major Compulsory Revisions

1. The hypothesis being tested in relation to smoke exposure is not articulated explicitly. While it is certainly true that whatever the hypothesis might have been, it was not supported by the data, it would be appropriate to spell this out.

R1 We have amended the hypothesis statement as follows

“We tested the hypothesis that patients with asthma exposed to occupational asthmagens would be more likely to have neutrophilic bronchitis than those without exposure and that exposure to passive cigarette smoke would result in a worsening of neutrophilic bronchitis.”

2. A key limitation of the study, in relation to assessment of inflammation largely in terms of percentage of neutrophils in sputum, is not adequately discussed. Because all of the patients were on high doses of inhaled corticosteroids, and because corticosteroids prolong the survival of neutrophils (Cox G. J Immunol. 1995;154:4719-25) this needs to be considered.
authors do mention the work by Cowan and others (cited ref #39) which points out that inhaled corticosteroids cause phenotype misclassification, but do not discuss this. The point was again emphasised recently by Arron and others (Eur Respir J. 2014;43:627-9) who noted the poor correlation between sputum neutrophil percentages and other measures of airway inflammation in asthmatics.

R2 We agree that corticosteroids are associated with increased neutrophil proportions. However, thanks to your suggestions, we have now tested the dose in the neutrophil model to confirm that there was an effect of corticosteroids on neutrophil proportion. The manuscript and figure 1 have been updated to reflect our improved model. We have also updated the limitation paragraph in the discussion as follows:

“A further limitation is that all participants were taking high doses of inhaled corticosteroids and further studies are needed to determine the effect of occupational exposures in participants with milder disease who do not require treatment with inhaled corticosteroids. Inhaled corticosteroids are known to enhance the survival of airway neutrophils [49] and increase following introduction of inhaled corticosteroids[40].”

The paper by Arron et al. was interesting and certainly suggests caution when applying inflammatory cut points, however in this study we have examined neutrophils as a continuous variable and have not examined the participants in regard to inflammatory subtype. With respect to the question concerning the choice of modeling with the number of neutrophil or the percentage of neutrophils, we performed our analysis on both of these. We found that the results were similar, but the significance of all effects was stronger when using the percentage. For example, the p-values for the effect of age in the number of neutrophils model is p=0.016 and in the percentage of neutrophils model it is p<0.001.

- Minor Essential Revisions

1. What was the randomisation procedure for the selection of macrophages for photography?

The ambiguity of this sentence has clarified it as follows

“Photographs of 50 macrophages were then taken from each slide and used for analysis using ImageJ software [27]. Macrophages were not selected based on the presence of carbon particles, rather, once a macrophage was identified then a further 50 macrophages were assessed as they were identified in each field of view”

2. In the Discussion, please comment on the extent to which exposure to motor exhaust fumes may induce a neutrophilic bronchitis independent of whether or not the person has asthma.

We have amended the first sentences as follows

“Motor exhaust fumes were the most common exposure identified from the participants’ occupation analysis. This may represent a common exposure in
adults with neutrophilic bronchitis as exposure to diesel exhaust can result in a neutrophilic infiltrate even in healthy individuals [35].”

3. Please amend the sentence in the conclusions that reads “Exposure to both environmental and occupational particulate matter may be a key contributor to the presence of neutrophilic asthma.” by deleting the word “key”, as this appears to be an overstatement.

This has been deleted and the sentence now reads “Exposure to both environmental and occupational particulate matter may contribute to the presence of neutrophilic asthma. This may help explain asthma heterogeneity and geographical variations in airway inflammatory phenotypes in asthma.

4. Please correct minor typographical errors (e.g. "verses" should be "versus").

We have corrected these errors.
Reviewer: Sebastian Johnston

Reviewer's report:

Minor Essential Revisions
This is an interesting manuscript describing a study of 66 participants with refractory asthma and the relationships between occupational exposures, smoking and airway inflammation. The authors conclude that sputum neutrophils are elevated in refractory asthma when subjects are exposed to occupational asthmagens. The work is interesting. I have the following comments for revision:

1) Abstract line 21: I suggest “ex/passive smoking” rather than just passive smoking here.

This change has been made at line 21 of the abstract.

2) Abstract line 28: you state “exposure to active and passive cigarette smoke was determined by questionnaire” however I believe active smoking was an exclusion criterion; please clarify.

Thank you for finding this error, we have now rewritten the sentence as “exposure to passive cigarette smoke was determined by questionnaire”

3) Abstract line 37 to 38: the phrase “who were with asthma younger than 30 years of age” does not make sense to me; please clarify.

Thank you for this suggestion, we have now amended the sentence to read as follows

“Sputum neutrophils were elevated in participants with asthma who had occupational exposures, particularly those who were diagnosed with asthma at a more than 30 years of age.”

4) Abstract line 41: I suggest delete “be a key” and just say “exposure may contribute to the presence of neutrophilic asthma.” And then in the next sentence: “This may in part explain” or “may help explain” as it is unlikely to be a complete explanation.

Thank you we have made the suggested changes.

5) Page 5 line 103: please explain how “50 macrophages were randomly selected”.

This sentence has been clarified it as follows

“Photographs of 50 macrophages were then taken from each slide and used for analysis using ImageJ software [27]. Macrophages were not selected based on the presence of carbon particles, rather, once a macrophage was identified then a further 50 macrophages were assessed as they were identified in each field of view”

6) Page 7 line 152: poorly controlled asthma is normally defined as an ACQ
score of #1.5, please clarify whether this was an ACQ or a different asthma controlled questionnaire. If it is a different one please provide a reference justifying the classification of poor asthma control as having a score of >1

We have clarified this statement as follows

“Participants were middle aged (median 60 years), atopic (76%) adults with moderate-severe airflow obstruction without well-controlled asthma (asthma control score >0.75) [31], despite being prescribed a high dose of inhaled corticosteroids (ICS) (median 2000µg daily) consistent with a diagnosis of refractory asthma.”

7) Page 7 line 158 to 160: Please indicate were this data is to be found.

This data is found in Table 2 which has been added to the manuscript

8) Page 7 line 170: 45% of what?

We have clarified this as follows

“The single category with the highest number of participants was those exposed to motor exhaust fumes with nine (45%) of the 20 participants identified as having exposure to asthmagens.”

9) Page 8 line 188 to 193: in this paragraph you state “data not shown” twice, please do show the data as this is an important analysis since both age and occupational exposure were related to neutrophil presence.

After some reconsideration, the phrase “data not shown” was not a good way to explain. There are several exposure variables, several biomarker variables and several more possible confounder variables. Thus the number of possible models to run to seek our relationships between exposures and biomarkers is large, remembering that we must consider all possible interactions between all the exposure and confounder variables. Even to list the p-values for each model would be impossibly voluminous. So we have added the following sentence to be more clear how the reported model was found

“Multivariable linear regression models were fit to assess the effect of possible confounders, such as age, gender and smoking characteristics, on the relationships between exposures and sputum inflammatory cells. More precisely, there are three main cell types examined neutrophils, eosinophils and lymphocytes, and for each of these a model was fit including one of four exposure variables (high weight, low weight, mixed and all), the possible confounders and their interactions as explanatory variables. The aim of was to find any significant association between an exposure variable and a specific inflammatory cell. Of the many models fitted, only exposure, diagnosis age and age had a significant effect on an inflammatory cell type. Of the different types of exposures, only combined exposure, that is, exposure to any agent, reached statistical significance in any model. Consequently, in the results detailed below, “exposure” means “exposure to any agent”.

10) Please justify in greater detail your conclusion that it is occupational
exposure rather than age that is the important relationship. Does your multiple logistic regression analysis have sufficient power/robustness to conclude as boldly as you do that it is exposures rather than age that is important?

Age is important and we have previously reported on the relationship between sputum neutrophils and age (Respirology. 2013 Jul;18(5):857-65). We have used age as the horizontal axis in Figure 1 to show this relationship where neutrophils increase with increasing age, indicating its importance in the model. The p-value has been added into the text at line 203. So we would say that both age and occupational exposure are important. We have revised our conclusions in line with the previous suggestions to tone down the statements as follows:

“Sputum neutrophils are elevated in refractory asthma with exposure to occupational asthmagens. In addition to older age, exposure to both environmental and occupational particulate matter may contribute to the presence of neutrophilic asthma. This may help explain asthma heterogeneity and geographical variations in airway inflammatory phenotypes in asthma.”

11) Page 12 line 292 to 294: Please provide a reference for the recommendation by Papadopoulos and please rephrase “stands out as widely required” as this is unclear to me.