Reviewer’s report

Title: Nerve ablation after bronchial thermoplasty and sustained improvement in severe asthma

Version: 0 Date: 04 Jun 2017

Reviewer: Howard Mitchell

Reviewer’s report:

Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

1. The protocols are a little hard to follow but it appears that there were 3 bronchial thermoplasty (BT) exposures followed by various studies extending 12 months after the last. As well as reviewing the wording of relevant text, the authors should clarify the legends and headings associated with Tables.

2. The statistical treatment is obscure. What are the N values for the histology and C-fibre work? The authors say that 6 serial sections (50 micron thick) were cut from each of 6-8 biopsy specimens. How are these averaged to provide an N value?

3. Table 2 (page 16, lines 43-47) says biopsies were from lower lobes. Yet Methods say they were from the carina (page 4, lines 24-25).

4. Table 2 (page 16, lines 43-47) indicates that the control biopsy (T0) was taken from the left lung while biopsies at the time periods under investigation (T1, 2 and 12) were taken from the other side of the lung. Without supporting data, the two sides cannot be confidently compared.

5. Shouldn't measurement (biopsy, clinical) happen just before the next BT rather than immediately after, as stated in Methods (page 4, lines 24-25)?

6. PGP9.5 was used to determine C-fibres. PGP9.5 is a pan neuronal marker. In that case it will reveal the rich cholinergic innervation of the airway as well as everything else. How then is the C-fibre identified?

7. The heading of Table 5 (page 20) says several structural measures (basement membrane thickness, disepithelization degree, endothelium) are presented yet only the basement membrane thickness is shown. Did BT ablate the epithelium too and what happens to ASM? Which endothelial cells were studied?
8. The Title, Introduction, Methods prepare the reader for a study focusing on C-fibre, particularly the density in the epithelium, glands and importantly ASM (page 4, lines 55-57). Yet by the Results (page 7, lines 9-15) the authors explain that technical or sampling problems prevented the density of nerves to be determined in most areas of the airway, except for the submucosa. Whilst appreciating the technical difficulties raised by the authors, this limitation restricts the weight of the investigation. Of particular importance would be the density of C-fibres in the ASM layer, since this is the way C-fibres could reduce airway narrowing (see comments elsewhere).

9. The authors show that effect of BT on C-fibres persists up to 12 months. It is unclear to me how this observation supports the hypothesis that mechanisms other than ASM ablation (eg. C-fibre ablation) are involved in the clinically beneficial effects of BT? Perhaps the ASM is also ablated up to 12 months?

10. Regarding the rationale for the study and the experimental approach, it is not entirely surprising that BT ablates nerve as well as muscle (as shown by others). Hypothetically, if the muscle is knocked out surely it hardly matters whether the nerves remain intact or not because without some functional muscle to work on the nerves cannot do anything much in terms of airway narrowing. C fibre derived tachykinins cause airway narrowing by activating ASM. The hypothesis that C-fibres and BT are linked only holds water if you can separate the effects of BT on ASM, C-fibres and clinical outcomes.

11. To this reviewer, the paper addresses inflammatory cellular changes in the airway wall as much as C-fibres. Most Methods, Results and Discussion focus on cellular contributions rather than C-fibres, about which few findings are reported due in part to the technical difficulties alluded to before.

12. Is there an error in TLC at T3 (Table 4)? Ranges for TLC go up to 80L?

13. The sequence of endoscopic procedure is shown in Table 2, not Table 3 as stated (page 4, line 26).

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.
I agree to the open peer review policy of the journal