Author’s response to reviews

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

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Author’s response to reviews:

To Prof. Peter B Noble
Associate Editor
BMC Pulmonary Medicine
https://bmcpulmmed.biomedcentral.com/
Re: submission to BMC Pulmonary Medicine - PULM-D-17-00203 – second revision

Nerve ablation after bronchial thermoplasty and sustained improvement in severe asthma.

Authors: Nicola Facciolongo (corresponding author); Antonio Di Stefano; Vladimiro Pietrini; Carla Galeone; Maria Federica Bellanova; Francesco Menzella; Nicola Scichilone; Roberto Piro; Gianluigi Bajocchi; Bruno Balbi; Lorenzo Agostini; Pierpaolo Salsi; Debora Formisano; Mirco Lusuardi

Replies to reviewers’ comments.

Dear Prof. Noble,

We wish to thank you and the reviewers for your comments that have been an invaluable help to significantly improve this second revision of the paper.

A point-by-point reply to reviewers’ comments is enclosed.

We are confident that now the manuscript can meet a favourable evaluation from you.

Please don't hesitate to contact us for any further question.

Thank you again and best wishes,

Nicola

[Nicola Facciolongo, corresponding Author]

Editor Comments:

R3 has indicated new literature that should be referenced, and one of these (Salem et al., 2016 in Annals ATS) indicates ASM reduction at 27 months, so please reconsider your response to R1 - comment 9.

Modified, see below among responses to R1 comments

In general, R1 was not convinced by your rebuttal, and you should consider whether additional changes to the MS are required, rather than only providing comment in the author-reply. Some of R1's remaining concerns are highlighted below:

R1 comments:
-A particular example is my original Point #2 regarding the C-fibre data. Apparently the data in Fig 2 plot the maximum scores at each time point? The authors have not provided information on N values, or how they were derived, as requested in my original review. Or at least I can not see any. Further, the data ranges presented are enormous. A clear description how the data were derived is needed, especially in the figure legend and probably also in Statistics.

Response: We understand that our explanation on original point 2 was not particularly clear and it was in part, incomplete. We try again to better explain the procedure adopted: at the end of each bronchoscopic procedure 6-8 biopsies were taken. Two bronchial biopsies per patient per time point (T0, T1, T2, T12) were sent to the laboratory of Dr. Di Stefano in Veruno Hospital for immunohistochemistry and quantitation of inflammatory cells. Six μm thick cryostat sections were cut from the best selected and oriented biopsy; one cryostat section was stained for each inflammatory molecule studied and read at light microscopy. These data were expressed as number of each inflammatory cell per square millimeter of tissue examined, as reported in the section methods and cited literature from the same group. Three to four biopsies per patient per time point (T0, T1, T2, T12) were sent to the laboratory of Dr. Pietrini at the University of Parma for nerve fiber immunohistochemistry and quantitation. Six serial sections were cut from each biopsy, therefore 6 sections x 3-4 biopsies (for a total of 18-24 sections per Time point-T0, T1, T3, T12) were stained and analyzed. On the basis of morphology and overall area disposable 2-3 best sections were selected from each biopsy and each time point. These sections were scored (0-3 values) for nerve fibers and the final score reported was the best (highest) one determined in the semi-quantitative analysis (by two observers). This description is reported in the text in the section methods (see paragraph Immunohistochemistry for nerve fiber evaluation).

Data ranges presented are enormous.

Response: ranges look actually large in figures, but in a semiquantitative score from 0 to 3 range are unavoidably from 0 to 3

-My original Point #1. Page 4 line 18 is still unclear and should add that biopsies were also taken at T12.

Response: The point has been added.

(Salem et al., 2016 in Annals ATS) indicates ASM reduction at 27 months, so please reconsider your response to R1 - comment 9.

Response: Data in the literature confirm persistence of ASM ablation at 27 months (see reference 29). Our paper is the first demonstrating a persistent ablation of nerve fibers at 12 months. Whether reduction of ASM function is due to persistence of ASM ablation or reduction of nerves stimulating ASM are both plausible explanation that need further studies. Pretolani et al (ref. 10) demonstrated a significant correlation between ASM and nerve fiber reduction at 3 months with clinical improvement at 12 months. In the present study we added the new notion that nerve fibers were reduced at 12 months, which could be considered relevant in light of Pretolani’s data. However, a direct cause-effect relationship between biological and clinical data for long periods after BT treatment need to be further studied.
-My original Point #10 was acknowledged in their Response, but the authors chose not to consider the point further in their Discussion. This is not essential, however.

The point has been commented in discussion (see paragraph before “In conclusion”)

-Page 6 line 5, missed a C-fibre.

Response: following comments of reviewer 2 in the first revision the terminology C-fiber proved to be incorrect and has been modified throughout the text to nerve fiber(s) except one point in introduction and one in discussion just referring to C-fibers.

Ynuk Bosse (Reviewer 3): Facciolongo et al. have followed 12 severe asthmatics undergoing bronchial thermoplasty (BT). The symptoms and lung function were monitored before and after BT with validated questionnaires and spirometry, respectively. They have also collected bronchial biopsies before and at different time-points after the procedures to study the structural and inflammatory changes caused by BT. In addition to the expected improvements in symptoms, the authors reported a decreased number of nerve fibers within the airway smooth muscle and the submucosa.

While the concept is intriguing and likely possible, the current prove provided by the authors relies exclusively on a subjective measure. Is it possible to measure the mRNA expression of a nerve-specific gene in some of these bronchial biopsies? A decrease of which pre- versus post-BT would provide a quantitative assessment that is purely objective.

Response: We find very interesting the reviewer’s suggestion, but mRNA expression is often only in part correlated with protein translation, therefore our attention has been focused mainly on nerve fiber proteins. Quantitative biochemical evaluation of PGP9.5 on biopsy homogenates has been planned but a number of technical problems are still to be solved before presentation of these last data. Furthermore, quantitation of nerve fibers by using the mentioned antibody (anti PGP9.5) is anyway considered a reliable method, as reported in the specialized literature (refs below) and allows to evaluate nerve fibers in the different layers of airways mucosa.


4 additional papers have shown a decreased content of airway smooth muscle post-BT. These should be cited.

Chakir et al., 2014 in Annals ATS
Pretolani et al., 2014 in the Blue
Salem et al., 2016 in Annals ATS
Denner et al., 2015 in Annals ATS

Response: Above references have been included.

It is not clear why the authors have not quantified the effect of BT on the airway smooth muscle content. Is it because bronchial biopsies are not adequate specimens to quantify the amount of airway smooth muscle around the airways (Bullone et al. 2014 in JAP)?

Response: We decided not to evaluate effects of BT on airways smooth muscle for 2 main reasons:

1. Limited adequacy of bioptic specimens (P.G. Woodruff and A.L. Innes. Quantitative morphology using bronchial biopsies Eur Respir Rev 2006; 15: 101, 157–161; the paper suggested by the reviewer – Bullone M et al. JAP 1985 - is very interesting but it refers to horses and we do not know whether data can be transferred to humans)

2. Many publications in literature all in agreement with a reduction effect of BT on smooth muscle (see also the previous comment)

Why repeated measures ANOVAs have not been used for symptoms?

Response: First of all we do apologize for the several inaccuracies of statistics, relevant to this comment and the following two.

In this case it was a writing mistake, since the ANOVA reported in table 4 legend as “comparison among group” was actually ANOVAs repeated measures

Page 6, line 13. Student's unpaired t-test is not a posteriori test for ANOVAs.

Response: Repeated measures ANOVA has been followed by post-hoc analysis with Bonferroni correction and not paired-t-test. Data have been checked and corrected where necessary. To allow a better visualization and understanding of data, in table 4 we omitted non-significant p-values.

How a Friedman test can be used when data are missing at T12 (n = 7)?
Response: According to our consultant statistician an exact Friedman test was used, that can be applied to small series and allows correction for missing data.

The quality of the figure 1 and 2 is poor. Neither the title nor the units are displayed on the y-axis for each graph presented in Figure 2.

Response: Title and y-axis units in figure 2 are now reported in legends.

Page 3, line 9. Pretolani is the first author on this paper. It needs to be referred to accordingly.

Response: Corrected

Page 6, lines 14-15. This sentence is unreadable.

Response: a verbe was missing, the sentence has been corrected

Page 17, Line 16. The authors meant over 18 years I suppose (age > 18 years).

Response: Sorry, but the age < 18 years is correct since it is included among exclusion criteria.

Table 4. RV not VR

Response: Corrected