Author's response to reviews

Title: Significance of fractional exhaled nitric oxide in chronic eosinophilic pneumonia: a retrospective cohort study

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Version: 2
Date: 16 April 2014

Author's response to reviews: see over
April 16, 2014

Catia Cornacchia, M.D.
Executive Editor
BMC Pulmonary Medicine

Dear Dr. Cornacchia:

Please find herewith our manuscript, now entitled “Significance of fractional exhaled nitric oxide in chronic eosinophilic pneumonia: a retrospective cohort study,” for resubmission as a research article. The previous manuscript ID was 1977228442116594.

We have made several corrections in addition to the revisions suggested by the reviewers. Our point-by-point responses to the reviewers’ comments follow this letter. The revised version has been proofread by Editage, a professional English editing service.

We made additional revision according to Editor’s recommendation on April 11th.
We thank you and the reviewers for your constructive comments and the opportunity to resubmit our article. We hope that the revised manuscript is now suitable for publication in your journal.

Sincerely,

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Reviewer: Mona Bafadhel

Major revisions

Methods

1. What do the authors mean by a change in FeNO or eosinophil count? Could the authors comment when this change was calculated; is this a reflection of treatment response or is it for the exacerbation cohort. There are a lot of figures and correlations that relate to this but I don’t understand what these data points will show

[Page 5]

We have revised the Methods section to clarify the studied parameters (page 5, lines 98–101):

Change in FeNO levels between visits was calculated at every assessment point, as follows: $\Delta$FeNO = FeNO$_n$ – FeNO$_{n-1}$, where $n$ and $n-1$ represent the $n$-th and preceding visits, respectively. Changes in peripheral eosinophil count ($\Delta$eosinophil count) and percentage ($\Delta$eosinophil percentage) were similarly calculated.

Therefore, the calculated values ($\Delta$FeNO) cover both the uncontrolled (including exacerbation events) and the controlled (including the therapeutic phase) states. For example,

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
<th>Visit #....</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient #1</td>
<td>6 ppb</td>
<td>12 ppb</td>
<td>9 ppb</td>
<td>70 ppb</td>
<td>8 ppb</td>
<td></td>
</tr>
<tr>
<td>$\Delta$FeNO</td>
<td>12 – 6 = 6</td>
<td>9 – 12 = –3</td>
<td>70 – 9 = 61</td>
<td>8 – 70 = –62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results:

2. What is the definition of controlled status?

[Page 4]

Controlled CEP was defined as absence of symptoms regardless of corticosteroid dose, so it included the therapeutic phase as well. Please check lines 91 and 92 on page 4 of the revised
3. There is no mention of treatment regimens in those in the controlled status or uncontrolled status; this information is important.

[Page 5]

We have explained the therapeutic regimens in the “Treatment” subsection of the revised manuscript (page 5, lines 108–116).

"The initial regimen for patients with newly diagnosed or uncontrolled CEP was 0.5 mg/kg/day of prednisolone. The dose was gradually tapered according to the clinical state. Patients with controlled CEP generally received a maintenance dose of 2.5- to 5-mg prednisolone daily. If no exacerbation event occurred during 3 months of maintenance treatment, the medication was discontinued. If symptom aggravation, reappearance of radiopacities, and peripheral eosinophilia were noted, suggestive of uncontrolled CEP, the dosage was increased up to 0.5 mg/kg/day."

4. The time course of sampling needs to be explicitly stated.

[Page 4]

Unfortunately, the patients were recalled and examined at various times over 1 year, so the time course of sampling cannot be specified. We have clarified the assessment points as follows (page 4, line 95 to page 5, line 98):

"The recall interval was individualized according to the clinical state: most patients were reexamined every 2–3 months, but some patients with uncontrolled CEP were recalled before the scheduled appointment."

5. Correlation analysis: (results; 3rd paragraph) parameters were measured on the same day… which day? In whom? Is this baseline or study entry? Why are
the measurements here for FeNO and eosinophils different to those presented in table 1 and 2? Why is the range of eosinophil count as low as 0? Does this not infer that CEP was not the correct diagnosis? Or a reflection of treatment – which is again missing from the analysis.

[Page 4]

We performed the measurements at every assessment point throughout the study. To avoid ambiguity, we have deleted this repetition from the Results section.

We have merged Tables 1 and 2 as revised Table 1. Fifteen patients were diagnosed with CEP before entry into the study, so their FeNO levels at diagnosis are not available.

The range of peripheral eosinophil count is as low as 0 because of the corticosteroid treatment, which is now explained in the revised manuscript.

6. The changes in FeNO or eosinophil correlations are not clearly explained. Is this a change between visits? Or treatments?

[Page 5]

Changes in FeNO level and peripheral eosinophil count or percentage were measured between visits at every assessment point. We have clarified the calculations on lines 98–101 of page 5 in the revised manuscript.

Table 1 & 2

7. I would combine both tables together as a baseline characteristic table.

[Page 7, Table 1,2]

Thank you for your suggestion. We have combined the tables and presented the baseline data in revised Table 1.

8. Duration of disease, treatment and smoked pack years need to be added

[Table 1]
We agree with your suggestion. We have added the relevant information in revised Table 1.

Figure 2

9. Are these points only at stable state?

[Figure 2]
The assessment points include all the measurements. Therefore, they represent not only the controlled state (including the therapeutic phase) but also the uncontrolled state (including exacerbation events).

10. Does the correlation take into account repeated measures?

[Figure 2]
Unfortunately, the correlation analysis does not account for repeated measurements. Because of the few cases, we combined the data.

11. Could OCS be a covariate in the correlation?

[Figure 2]
Yes, oral corticosteroid could be a confounding variable. However, its therapeutic effect varies among individuals, so the mean distorting effect of the confounder cannot be easily assumed.

12. Describe the legend more clearly for the change in eos/change in FeNO

[Figure 2, Page 15]
We have improved the description of Figure 2 (page 14, lines 328–331).

"Figure 2 - Relationship of FeNO level and peripheral eosinophilia in CEP. Scattergrams of FeNO level against peripheral eosinophil percentage (A) and count (B) as well as ΔFeNO against Δeosinophil percentage (C) and Δeosinophil count (D) at every
13. Y-axis for absolute number of eosinophils, please put units

[Figure 2]

We apologize for the missing unit in Figure 2. We have added “(cells/µL)” in the y-axis.

Minor revisions

Introduction

14. The concept of an exacerbation is introduced. Is this widely known/established? I would enhance the definition statement here

[Page 4]

The concept of exacerbation is not established, but some authors use this term. Considering previous studies of the long-term prognosis of CEP and our experience, CEP seems to show a waxing-and-waning pattern frequently. No clear remission criteria are available, so we used the term “exacerbation” instead of “relapse.” The term is defined on lines 88 and 89 of page 4 in the Methods section.

Methods

15. Is a new diagnosis the same as an exacerbation? I think this should be classified as untreated CEP; the study does not look at using FeNO for diagnosis so this distinction should be clearer

[Page 4]

New diagnosis is not the same as exacerbation. However, we combined cases of new diagnosis and exacerbation under “uncontrolled CEP” instead of “untreated CEP” because our aim was to determine whether FeNO is a potential marker of eosinophilic parenchymal inflammation in CEP rather than to diagnose CEP. Please see the “Study design and case definition” subsection on page 4.
16. Describe the study design clearly

[Page 4]

We have revised the “Study design” subsection to include all relevant information.

17. There are numerous visits but there is no information as to when these visits occurred; what treatment phase patients were on or whether these visits were scheduled or ad-hoc. Please clarify

[Page 5]

We have clarified the assessment points as follows:

“The recall interval was individualized according to the clinical state: most patients were reexamined every 2–3 months, but some patients with uncontrolled CEP were recalled before the scheduled appointment.”

18. Statistical analysis – please re-phrase the first line; most results implies the description of other results is withheld

[Page 5]

Thank you for the suggestion. We have corrected this sentence.

Results

19. Please change wording to read eighteen patients (10 males) and remove 2nd part of the 2nd sentence

[Page 6]

We have revised the sentence to “Eighteen patients (10 men) were enrolled in the study; fifteen patients had been diagnosed before the study began.”
20. A diagnostic BAL is introduced here. When was this performed? The authors state that 15 patients were already diagnosed. Does this information add anything to the whole dataset? If not remove from the text but leave in the table

[Page 7]

Among the 15 patients who were diagnosed before the study began, only seven underwent BAL. The others did not need BAL fluid analysis for diagnosis of CEP. We have clarified our intended meaning as “Seven patients underwent BAL for diagnosis of CEP.”

21. Clinical course – 1st sentence this has already been referred to. Please remove duplication

[Page 7]

We have deleted the sentence from the revised manuscript.

22. Please describe the ATS guideline of significant change in the methods Section

[Page 5]

Thank you for the suggestion. We have explained the ATS guidelines for significant change in FeNO in the revised manuscript (page 5, lines 106 and 107).

23. The ROC for sensitivity and specificity analysis to diagnose an exacerbation seem quite good – however caution must be drawn here as the number analysed should be 7 (not inclusive of new diagnosis) and at n=10 still very small

[Page 6]

As already mentioned in the “Clinical course” subsection and depicted in Figure 1, 74 FeNO measurements were obtained from the 18 patients, including 10 measurements during exacerbation events and three at diagnosis. Therefore, 13 FeNO measurements were obtained during uncontrolled CEP.
Discussion

24. The authors describe the waxing/waning of the disease process – likely diagnosed because of requirement of corticosteroids. Is this the same as what happens to FeNO? A figure for each of the subjects recruited and their disease time course would be valuable with treatment, FeNO and inflammation for each.

Discretionary revisions

We agree with the reviewer. Unfortunately, we think that the manuscript already contains too many figures. Although Figure 4 is not perfect, it might represent the course of exacerbation, treatment, and FeNO measurement.

25. Can a baseline FeNO predict those that would exacerbate

We think that extensive prospective studies are needed to evaluate the validity of baseline FeNO level to predict exacerbation of CEP.
Reviewer; JAE YEOL KIM

26. In this study, there were only 3 patients who checked the initial level of FeNO. More accumulation of data would be necessary for the determination of cut-off value for the initial diagnosis of CEP. This must be added to the limitation of the present study.

[Page 11]

We agree with your comment. We have explained this limitation as follows: “Fourth, FeNO levels of only three patients were measured at diagnosis of CEP. Additional FeNO data are needed to determine the cutoff value for diagnosis of CEP.” (page 10, lines 220–222)

27. In more than half of CEP patients, BAL fluid eosinophilia were not confirmed, which does not make the diagnosis CEP strong. The reasons for skipping bronchoscopy need to be described in the Discussion.

[Page 9]

BAL fluid analysis would strengthen the diagnosis of CEP, but the patients who did not undergo BAL met the diagnostic criteria on the basis of the typical radiographic findings and clinical course. Furthermore, many authors consider that BAL fluid analysis is not a prerequisite for accurate diagnosis. We have explained our viewpoint on lines 185–187 of page 8 in the revised manuscript.

28. One of the most difficult decision making in the follow-up of CEP patient is the timing of withdrawal of medication. It would be very valuable if authors continue to evaluate FeNO level in CEP patients and to present safe period of medication before discontinuation.

We agree with your viewpoint. Prospective clinical trials based on FeNO levels would be invaluable.
Additional Comments from Editors (April 11 2014)

1. Please provide the average time between patient visits and the average number of visits that were attended by the patients. We added the details of number of visit and interval expressed as median and IQR in accordance to other values in this manuscript. “Median time interval between patient visits was 56 days (IQR 28-77). Median number of visits that were attended by the patients was 4 (IQR 4-5).” (page 8)

2. There is 1 current smoker - were their FeNO levels significantly lower than the other patients and did this impact on the analysis?

There is one current smoker. His FeNO was measured after one hour cessation of smoking according to previous recommendation because smoking may decrease FeNO level[18, 19]. His FeNOs were measured two times in stable state (29 and 33 ppb) which were within controlled state IQR (26-49). I don’t think this finding impacted on the analysis. (page 9)

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

I tried to check all of my revised manuscript confirms the journal style.