Peer Review File
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Reviewer A

In their narrative review, the authors have reviewed the literature to determine if FeNO is a treatable trait in chronic cough, in comparison to other T2 biomarkers, and summarized major knowledge gaps in clinical application.
The topic is relevant and the paper is really interesting. However, I have some concerns regarding the article.
1. The authors describe in the text the meaning of the concept of "treatable traits" (clinical relevance, trait identifiability/measurability, and treatability). Therefore the Authors should better describe why NO should be considered a treatable traits. In particular they should underline the clinical relevance of NO in the paragraph of NO.

Reply 1: Thank you for your review and valuable feedback. We have modified conclusion as advised to better provide the rationale for FeNO as a treatable trait in chronic cough.

Changes to the text: T2 inflammation has emerged as a treatable trait an important inflammatory phenotype in chronic airway diseases and chronic cough. FeNO is clinically relevant as it has diagnostic utility and treatment response predictability in identifying FeNO measurement may help to identify patients with chronic cough patients with CVA or eosinophilic bronchitis that will benefit from ICS corticosteroid treatment. Therefore, FeNO should be considered a treatable trait in chronic cough. However, strong mechanistic and clinical evidence for the diagnostic roles of FeNO are still lacking. Further studies are warranted to determine the diagnostic roles of FeNO in the management of patients with chronic cough (see Page 16, line 358-365).

We described the clinical relevance of FeNO through evidence of diagnostic utility and treatment response predictability in a section of "Clinical evidence for FeNO in chronic cough". In the end of the paragraph, we highlighted and suggested the ICS treatment in patients with high FeNO: "Considering the overall results through indirect evidence, high FeNO is closely associated with the ICS response. Accordingly, patients with chronic cough and high FeNO could be initially or sequentially treated with ICS." (see Page 15, line 332-334).

2. Furthermore, there are some original articles which have studied the role of exhaled nitric oxide in the diagnosis of chronic cough which have not included in the review (Respirology. 2008 May;13(3):359-64 and Allergy Asthma Immunol Res. 2019 Nov;11(6):830-845)

Reply 2: Suggested references are now included.
Changes to the text: Studies have consistently reported a significantly higher median

FeNO level in asthma and eosinophilic bronchitis compared with other groups, including postinfectious cough, atopic cough, upper airway cough syndrome (UACS), and GERD (56-59) (see Page 12, line 259-262).

58. Liu X, Wang X, Yao X, et al. Value of ehaled ntric oide and FEF(25-75) in ientifying fctors asociated wth cronic cugh in alergic rinitis. Allergy Asthma Immunol Res. 2019;11:830-45.
59. Fujimura M, Ohkura N, Abo M, et al. Exhaled nitric oxide levels in patients with atopic cough and cough variant asthma. Respirology. 2008;13:359-64.

Reviewer B

In this narrative review, the author was aimed to determine if FeNO could be used as a treatable trait in the management of chronic cough by conducting a literature review. It does have great clinical significance, but some concerns existed.

Major comments：

1. In this narrative review, the author conducted a detailed literature review on the role of FeNO in guiding diagnosis and treatment of chronic cough, meanwhile, pointed out the current knowledge gaps and directions of future study. However, the mainly what I concerned was that the focus of this article is not prominent. According to the title and the objective, this review should focus on the role of FeNO as a treatable trait in chronic cough. But the authors took too much space to discuss the mechanism of FeNO and cough reflex, sputum eosinophils and cough reflex, the utility of FeNO in other diseases, etc.

Reply 1: Thank you for your review and valuable feedback.

As described in introduction, this article aims to discuss whether FeNO should be considered as a treatable trait in chronic cough in comparison to other T2 inflammation biomarkers. While the use of FeNO test is increasing, the utility has yet to be determined. Furthermore, the mechanistic relevance of FeNO in the cough reflex has not been comprehensively reviewed. Therefore, this article hopefully helps to identify the current knowledge and gaps regarding the mechanistic and clinical relevance of FeNO in chronic cough.

2. On the whole, the authors just describe different results in different studies, without any specific analysis or comparison in this review. I can’t get a definite answer to the question of “Is FeNO a Treatable Trait in Chronic Cough?”. If the authors wanted to answer the question of “Is FeNO a Treatable Trait in Chronic Cough?” or compare the utility of different biomarkers as a treatable trait, it will be better to conduct a systematic review.

Reply 2: We completely agree with you that systematic review should be conducted to better answer this question. However, definition and assessment of treatable trait in chronic cough were not fully defined. Further, clinical study based on treatable trait and comparative study of different biomarkers in chronic cough is still not published, which might act as limitation in conducting systematic review now.

We have modified conclusion to highlight the importance of FeNO as a treatable trait in chronic cough.

Changes to the text: T2 inflammation has emerged as a treatable trait an important inflammatory phenotype in chronic airway diseases and chronic cough. FeNO is clinically relevant as it has diagnostic utility and treatment response predictability in identifying FeNO measurement may help to identify patients with chronic cough patients with CVA or eosinophilic bronchitis that will benefit from ICS corticosteroid treatment. Therefore, FeNO should be considered a treatable trait in chronic cough. However, strong mechanistic and clinical evidence for the diagnostic roles of FeNO are still lacking. Further studies are warranted to determine the diagnostic roles of FeNO in the management of patients with chronic cough
(see Page 16, line 358-365).

3. Since the objective of this study was to "analyze the literature to determine if FeNO is a treatable trait in chronic cough, in comparison to other T2 biomarkers, and summarized major knowledge gaps in clinical", airway hyperresponsiveness was discussed in this review, however, there is no search term on airway hyperresponsiveness. Adding the content of blood eosinophils and airway hyperresponsiveness make the current review lack of focus. Furthermore, the narrative review did not show a comparison result in the utility of different T2 biomarkers. I preferred deleting content of other biomarkers, including blood eosinophils, sputum eosinophils and airway hyperresponsiveness.

Reply 3: Our primary objective was to compare T2 inflammation biomarkers in terms of treatable traits. We hope the comparison helps to understand the roles of FeNO in relation to eosinophils. However, we removed the section on AHR.

Changes to the text:
Airway hyperresponsiveness
AHR is usually accepted as an independent treatable trait in chronic airway disease, for which the histamine challenge test may predict inhaled corticosteroid (ICS) treatment response. A study of AHR assessed by inhaled mannitol in asthma showed a significant correlation between the degree of AHR and eosinophils in the submucosa. Therefore, AHR could also be a potential biomarker for T2 inflammation in chronic cough. However, the diagnostic utility and therapeutic implication of AHR in patients with isolated chronic cough and normal lung function (without breathlessness or wheezing) remain controversial.

4. I think the Table 3 can be improved further. Firstly, the predictability of FeNO as the key point, would be clearer and comparable if the cut-off value, sensitivity and specificity could be listed separately. Secondly, indirect evidence can be deleted.

Reply 4: We have modified Table 3 as advised, in which indirect evidence (RCT in chronic respiratory symptoms) were deleted and cut-off value, sensitivity and specificity were listed separately.

Changes to the text: See Table 3

5. I suggest the authors add a table to summarize the diagnostic utility of FeNO in chronic cough in different reports, presenting the cut-off point, sensitivity, specificity, etc.

Reply 5: Thank you for the suggestion. However, we respectfully decided not to make additional table because previous meta-analyses already presented the table and figure summarizing diagnostic utility of FeNO with cut-off point, sensitivity, and specificity (see reference 60, 61).

60. Song WJ, Kim HJ, Shim JS, et al. Diagnostic accuracy of fractional exhaled nitric oxide measurement in predicting cough-variant asthma and eosinophilic bronchitis in adults with chronic cough: A systematic review and meta-analysis. J Allergy Clin Immunol. 2017;140:701-9.
61. Zhang L, Liu S, Li M, et al. Diagnostic value of fractional exhaled nitric oxide in cough-variant asthma: an updated meta-analysis. J Asthma. 2020;57:335-42.

Changes to the text: reference 61 was added (See references)

6. What is new when compared this review with the meta-analysis reported by Dr. Song (PMID: 27707661, 28088474)?
Reply 6: Previous papers did not discuss the findings of FeNO with the criteria of treatable traits.

Other comments:
7. A specific objective should be presented in the “Abstract”.

Reply 7: Thank you. The abstract has been updated.

Changes to the text:
Background and Objective: Current management of chronic cough is largely based on sequential therapeutic trials. The concept of treatable traits was first introduced for individualized treatment of chronic airway diseases; however, it has emerged as a potentially useful strategy in revising the management of chronic cough. This narrative review aimed to analyze the literature to discuss if fractional exhaled nitric oxide (FeNO)

is a treatable trait in chronic cough, compared to other T2 biomarkers, and to summarize current knowledge and gaps in the clinical application (see Page 2, line 27-30).

8. Some references should be added in the main text: Line 49-51 "Practical biomarkers…guiding a diagnosis or corticosteroid treatment decision."; Line 150-151 "Respiratory epithelium…dependent on the activity of interleukin-4 (IL-4) and IL-13."; line 111-112 "The degree of improvement in cough sensitivity positively correlated with the change in sputum eosinophil count.", etc.

Reply 8: Related references have been added as follows.

Changes to the text:
Practical biomarkers, such as the fractional exhaled nitric oxide (FeNO) test or blood eosinophil counts, have emerged as alternatives to sputum eosinophil counts in guiding a diagnosis or corticosteroid treatment decision (11,12) (see Page 3, line 69-71).
Respiratory epithelium is the primary source of exhaled nitric oxide (NO) in the airways, and FeNO levels are largely dependent on the activity of interleukin-4 (IL-4) and IL-13 (36) (see Page 8, line 169-170).
The degree of improvement in cough sensitivity positively correlated with the change in sputum eosinophil count (23) (see Page 6, line 130-131).

9. In Line 78-79, the significance of specificity and sensitivity might be mixed.
Reply 9: Our intent was to broadly discuss the utility of treatable trait-measurement tools or biomarkers.

10. For Table 2, what's the standard of the strength of effect rating?

Reply 10: We briefly described the meaning of each criterion of clinical relevance, trait identifiability/measurability, and treatability in "Concept of treatable trait". Unfortunately, no standard to assess the degree of each criterion exists. Therefore, we tried to grade the degree of each criterion based on the strength of clinical effects or evidence levels through literature review and finally author's discretion.
For example: "These findings suggest that sputum eosinophilia determined by induced sputum is a direct noninvasive method with high clinical relevance (Table 2: + + +) and treatability (+ + +) to define causal inflammatory phenotype in chronic cough."

Changes to the text: in footnote of Table 2 we added the explanation of rating.
Strength of effect Grading was based on the strength of clinical effects or evidence level identified through literature review and but was decided by author's discretion (See Table 2).

11. Line 105, "was devised by" should be changed to "was detected by".

Reply 11: We modified the sentence as advised.

Changes to the text: Eosinophilic bronchitis was devised detected by applying induced sputum analysis (see Page 6, line 124).

12. The English written should be improved and carefully revised.

Reply 12: Our manuscript underwent professional English proofreading.

Reviewer C

The primary objective of this study is to investigate the diagnostic utility and treatment response predictability of FeNO in chronic cough management. The article is clearly written. The methods and results are clear and discussion is measured. This paper warrants publication with some minor points to address below.

1. Firstly, the analysis and discussion of the predictive effect of FeNO on the treatment of chronic cough without differentiating between subgroups are not going deep enough since there are many possible causes of chronic cough, and the heterogeneity between different etiologies could be enormous. Therefore, it is recommended to conduct separate subgroup analyses according to the common etiologies of chronic cough.

Reply 1: Thank you very much for your review and valuable feedback.

First, as you mentioned, chronic cough is heterogenous in terms of endotypes and phenotypes. However, it is hard to differentiate them, thus, specific disease can be confirmed only after treatment response. We described the limitation of anatomical approach and suggested the treatable trait approach rather than diagnostic labeling in introduction. Therefore, separate analyses for common etiologies of chronic cough do not match our perspective of treatable trait in this review article.
Second, FeNO levels are higher in CVA or eosinophilic bronchitis compared to other chronic cough phenotypes, as we described in the section of diagnostic utility. However, evidence of FeNO in common etiologies of chronic cough such as UACS and GERD is scarce so that we cannot go further into separate analyses of them regarding FeNO.

2. The specific metrics or parameters used to assess the therapeutic effects of chronic cough were not illustrated in this manuscript. These contents need further elucidation.

Reply 2: We did not fully describe parameters to assess the therapeutic effects of ICS in each study to avoid redundancy in the manuscript. Instead, parameters and criteria for ICS response were described in “Measurement of ICS response” of Table 3.

3. Please explain the specific evaluation and the analysis method of "+", "++", and "+++" in Table 2.

Reply 3: We briefly described the meaning of each criterion of clinical relevance, trait identifiability/measurability, and treatability in "Concept of treatable trait". Unfortunately, no standard to assess the degree of each criterion exists. Therefore, we tried to grade the degree of each criterion based on the strength of clinical effects or evidence levels through literature review and finally author's discretion.
For example: "These findings suggest that sputum eosinophilia determined by induced sputum is a direct noninvasive method with high clinical relevance (Table 2: + + +) and treatability (+ + +) to define causal inflammatory phenotype in chronic cough."

Changes to the text: in footnote of Table 2 we added the explanation of rating.
Strength of effect Grading was based on the strength of clinical effects or evidence level identified through literature review and but was decided by author's discretion (See Table 2).

4. It is recommended to list the specific etiologies of chronic cough and whether there are multiple comorbid conditions, such as CVA with UACS or CVA with GERC in Table 3. The presence of comorbidities may have great influence on the responsiveness of FeNO to predict ICS treatment of chronic cough.

Reply 4: We searched for specific etiologies of chronic cough in each study. Some study did not present them.

Changes in the text: We added the specific etiologies in Table 3 (See Table 3).

5. Line 255-256. "This moderate correlation between sputum eosinophils and FeNO could be explained by the regulation of different cytokines." Please discuss the possible mechanisms of "the regulation of different cytokines".

Reply 5: We explained different mechanisms by which eosinophils and FeNO are regulated.

Endogenous mediators, such as IL-4 and IL-13, can upregulate iNOS expression or activity. In addition, exogenous stimuli, such as bacterial toxins, viruses, and allergens, may induce iNOS. In such pathological conditions, NO production increases, and NO has cytotoxic or pro-inflammatory effects, such as AHR, vasodilation, free radical production, mucus hypersecretion, and ciliary motility inhibition at high concentrations (see Page 8, line 184-189).

Mechanistically, IL-5 is directly related to the increase in eosinophils and is not usually involved in the production of FeNO (see Page 10, line 222-224).