Peer Review File

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Reviewer A

The authors described an association between wheezing and exacerbations of COPD with or without ACO.

Comments;
1. Table 1. Eos count - can the authors show the % of patients with Eos > 300 in the 4 groups of patients? Please add the units for Eos counts (cells/ microL).

Response: We appreciate your thoughtful comment. The following table displays the proportion of patients with eosinophils ≥300 cells/μL, 100-300 cells/μL, <100 cells/μL in the four groups. Since eosinophils ≥300 cells/μL were used as criteria for defining ACO in this study, there were no patients with eosinophils ≥300 cells/μL in the non-ACO group.

	Total (n=1,121)	ACO (n=273)		Non-ACO (n=848)	
		With wheezing (n=155)	Without wheezing (n=118)	With wheezing (n=475)	Without wheezing (n=373)
Eosinophil ≥300cells/μL	259 (23.1)	147 (94.8)	112 (94.9)	0 (0)	0 (0)
100≤ Eosinophil <300cells/μL	566 (50.5)	7 (4.5)	5 (4.2)	318 (66.9)	236 (63.3)
Eosinophil <100cells/μL	296 (26.4)	1 (0.6)	1 (0.8)	157 (33.1)	98.5 (36.7)

We have included the relevant findings in Table 1 and provided units for blood eosinophil count (cells/μL) in Table 1 and Table S1.

2. Exacerbations - what was the % of patients who required systemic steroids or antibiotics for exacerbations in the 4 groups? It may be reasonable to guess that patients in the ACO group are more likely to required steroid while patients in the non-ACO more likely to require antibiotics.

Response: We appreciate your insightful feedback. This study involved an analysis of data from the KOCOSS (Korean COPD Subgroup Study) cohort. The KOCOSS cohort captures information about the frequency of moderate or severe exacerbations during the follow-up period, yet it does not gather data on whether patients received antibiotics or steroids as part of their treatment. Consequently, we were unable to assess the percentage of patients who were administered antibiotics or steroids during their exacerbation. We acknowledge the significance of this aspect and regret our inability to provide this information.

3. The presence of wheezing was apparently not associated with the Eos count - patients without wheezing in the ACO group had high Eos count, while patients non-ACO group had low Eos count whether they have wheezing or not (Table 1). Can the authors explain or postulate what are the possible pathophysiological causes of wheezing in patients in COPD and ACO vs non-ACO?

Response: Thank you for bringing attention to this aspect. Wheezing in COPD patients can emerge as a result of the intricate interplay among various factors, encompassing airway inflammation, bronchoconstriction, and mucus production. While eosinophilic inflammation is a characteristic feature of asthma, it can also manifest in patients with ACO. Nonetheless, the absence of a direct correlation between eosinophil count and wheezing in our study may be attributed to the predominant influence of non-

eosinophilic mechanisms in instigating wheezing episodes within the ACO or non-ACO group.
Based on your valuable feedback, we have incorporated additional findings related to the association between blood eosinophil count and wheezing into the abstract and results sections. Furthermore, we have expanded our discussion concerning the potential pathophysiological origins of wheezing in COPD patients, as outlined below:

"There was no association between blood eosinophil count and wheezing in both the ACO and non-ACO groups." [Abstract, page 3, lines 55-56].

"There was no difference in blood eosinophil count between patients with wheezing and those without wheezing in both ACO and non-ACO groups." [Results section, page 9, lines 179-181]

"The blood eosinophil count showed no statistically significant difference between the groups with and without wheezing." [Results section, page 10, lines 197-198]

"Moreover, our study revealed that the presence of wheezing did not correlate with eosinophil count, regardless of patients being classified into either the ACO or non-ACO group. While eosinophilic inflammation can also manifest in ACO patients, it is plausible that chronic non-eosinophilic inflammation or the decline in lung elasticity like emphysema exert a more predominant impact on driving wheezing episodes within the ACO or non-ACO patient population." [Discussion section, page 15, lines 299-304]

4. Only a relatively low % of patients were treated with ICS - lowest 28.2% in the non-ACO group without wheezing, highest 40.6% in the ACO group with wheezing. The % of patients with moderate to severe exacerbations in the 4 groups ranged from 14.4% in ACO without wheezing to 25.2% in ACO with wheezing. Were ICS given to the patients with exacerbations or not? Can the authors clarify the usefulness of ICS in preventing exacerbations in patients with ACO and non-ACO, with or without wheezing?

Response: As previously mentioned, we analyzed data from the national COPD cohort. COPD medications, including ICS, were prescribed at the discretion of the physician although the standard practice for using ICS is applied to patients with a history of a significant acute exacerbation in the previous year or a peripheral blood eosinophil count > 300 cells/μL, following the GOLD guidelines.
Consistent with this, the proportion of patients treated with ICS in the group that experienced exacerbation in the previous year was significantly higher than in the group that did not within our study (54% vs. 32%, p<0.001).

	Exacerbation group (Patients with exacerbation in the previous year) (n=221)	Control group (Patients without exacerbation in the previous year) (n=900)	p-value
ICS use			
Total	120 (54.3)	289 (32.1)	<0.001
ACO with wheezing (n=155)	14/39 (35.9)	49/116 (42.2)	0.485
ACO without wheezing (n=118)	8/17 (47.1)	32/101 (31.7)	0.215
Non-ACO with wheezing (n=475)	68/108 (63.0)	133/367 (36.2)	<0.001
Non-ACO without wheezing (n=373)	30/57 (52.6)	75/316 (23.7)	<0.001

We added this finding in the results section as follows:

"An ICS-containing regimen was more commonly utilized in the group that had experienced moderate to severe exacerbations in the previous year compared to the group that had not (54% vs. 32%, p<0.001)."

[Results section, page 10, line 187-189]

Additionally, we investigated the relationship between ICS usage and exacerbation risk among our patients. The use of ICS did not demonstrate an association with a reduced risk of exacerbation in the entire population, although there is a subpopulation that is likely to benefit from ICS treatment. However, it's important to note that this study, conducted as a non-interventional observational study using a national cohort, might not be suitable for evaluating the preventive effects of specific medications. For instance, ICS were probably more commonly prescribed to individuals with more severe COPD. Adjusting for variables, including disease severity, cannot completely mitigate this limitation. Furthermore, the one-year follow-up period may be too short to accurately assess the risk of exacerbations and may have underestimated the role of ICS, as discussed in the discussion section.
Thus, we mentioned this point in the discussion section as follows:

"Finally, assessing the effectiveness of ICS in preventing exacerbations based on the presence of wheezing was hindered by the inherent bias associated with an observational study." [Discussion section, page 15, line 317-319]

5. It has been found ICS was more effective in preventing exacerbations in COPD patients with Eos >300. In this study, non-ACO patients have apparently low Eos level in both patients with or without wheezing. The patients with wheezing in the non-ACO group had higher exacerbation rate despite higher % was prescribed ICS (42.3% vs 28.2%). On the other hand, ACO patients have higher Eos whether they have wheezing or not. The ACO patients with wheezing have higher exacerbation rate than those without wheezing despite slightly more common prescription of ICS and similar Eos level. Overall, it appears that wheezing is a more important determinant of exacerbation than Eos level or treatment of ICS. Please clarify.

Response: Your observations regarding the relationship between wheezing, eosinophil levels, ICS treatment, and exacerbations in the ACO patient group are indeed relevant. Our study's findings present the elevated exacerbation rate in the ACO group with wheezing, despite the slightly higher prescription rate of ICS. As stated in the previous comment 4, this observational study is not suitable to assess the effectiveness of certain medication.
Nevertheless, our finding suggests that exacerbation rates in COPD patients with wheezing, whether non-ACO or ACO, may be influenced by factors extending beyond eosinophil count. These factors could potentially encompass non-eosinophilic inflammation, as indicated by the lack of association between eosinophil count and wheezing in our study. We believe that this comment falls within the continuum of the previous comment 3 & 4. Additionally, we revised the conclusion section as follows:

"Wheezing, as an indicator of more pronounced airflow restriction and a predictor of exacerbation development, could be considered a severe phenotype of COPD rather than a characteristic of an ACO phenotype." [Conclusion section, page 16, lines 326-328]

6. Overall, it appears that patients with wheezing have poorer lung function in the ACO and non-ACO group, irrespective of Eos level and treatment with ICS. Is it possible that the presence of wheezing just reflected more severe airflow obstruction and hence higher CAT score, mMRC grade, SGRQ-C and more frequent exacerbation? Eos level and treatment with ICS apparently had relatively minor effect on the generation of wheezing in COPD patients, whether they have ACO or non-ACO. Please clarify.

Response: Thank you for your thoughtful comments. You've rightly pointed out an important aspect of our findings. The presence of wheezing indeed seems to be associated with poorer lung function across both the ACO and non-ACO groups, regardless of Eos levels and ICS treatment. It's worth noting that eosinophil levels and ICS treatment appeared to have a relatively minor effect on the occurrence of wheezing in both

ACO and non-ACO patients. This suggests that wheezing might be influenced more strongly by factors such as the underlying persistent airflow obstruction severity rather than hyperresponsiveness or specific eosinophil levels or ICS therapy. In light of your valuable feedback, we have elaborated on our discussion, as outlined below:

"These results are consistent with our findings, as our study revealed that patients with wheezing had lower lung function in both the ACO and non-ACO group. This implies that the presence of wheezing is not indicative of an ACO phenotype, but rather signifies more prominent airflow obstruction. This correlation could potentially be associated with heightened symptoms and an increased risk of exacerbations." [Discussion section, page 13, lines 256-260]

7. If the proposition in 6 is correct, the presence of wheezing may be an indicator of more severe airflow obstruction in patients who are too weak or refused to perform a lung function test.

Response: Thank you for your insightful comment. While we did not explicitly explore the concept you've raised, we recognize that wheezing could potentially function as a valuable indicator of more severe airflow obstruction, especially in particular patient populations where conducting lung function tests might be impeded by factors such as frailty or patient preferences. We've included this perspective in the "Discussion" section as follows:

"For individuals facing challenges in performing lung function tests, the presence of wheezing could potentially signal a higher degree of airflow obstruction." [Discussion section, page 13, lines 260-261]

We believe that we have faithfully addressed all the reviewer's concerns.
Thank you.
Authors

Reviewer B

1. Figure 1
*The number of included patients is not correct after the first exclusion. Please check.

=> Some patients who met the criteria for the first exclusion in Figure 1 exhibit overlaps.
- 'Smoking history <10 pack-years' and 'missing data for eosinophil count': n=50
- 'Missing data for eosinophil count' and 'missing data for presence of wheezing': n=5
- 'Smoking history <10 pack-years' and 'missing data for eosinophil count' and 'missing data for presence of wheezing': n=2
Consequently, 633 patients were included in the initial exclusion.
For better understanding, we have incorporated the expression 'some exhibit overlapping criteria' into Figure 1.
Please confirm whether the equation based on the flowchart is correct:
2202-(286+392+14)+(50+5+2)=1567
Total patients enrolled-(excluded)+(overlap)=included patients

=> We apologize for any confusion this may have caused. Please see the following Venn diagram. Two patients with 'Smoking history <10 pack-years' and 'missing data for eosinophil count' and 'missing data for presence of wheezing' are overlapping twice with cases having 'Smoking history <10 pack-years' and 'missing data for eosinophil count,' and cases with 'missing data for eosinophil count' and 'missing data for presence of wheezing'. Thus, the number of included patients is as follows:

2202 - (286 + 392 + 14) + (50 + 5 + 2 + 2) = 1569

2. Figure 2: A label is required for the y axis.

=> The label has been presented.

3. A header is required for the first column of each table.

=> The header has been presented in each table.

4. Table S1: Please add year as the unit of Age.

=> The unit of age has been presented.

5. Table 2: A table should not have multiple column headers.

=> Comorbidity data from Table 2 has been merged into Table 1.

6. 6MWT and 6MWD are used at the same time in the tables and abbreviation lists in both Table 1 and Table S1. Please be consistent.

=> Thank you for pointing out the inconsistency regarding the use of "6MWT" and "6MWD" in both Table 1 and Table S1. We have ensured consistency by using "6MWD" consistently throughout the tables.