Peer Review File

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We would like to express our sincere thanks to the reviewers for their constructive and helpful comments, which have certainly helped us improve our manuscript.

Reviewer A:

This is an interesting and topical paper examining the expression of autoantibodies in both lung and systemic compartments in COPD. I am concerned however both with the choice of autoantibodies measured and related to this, the age of many of the papers referenced.

Major revisions:

1. The introduction needs updating with more recent literature in the area of autoimmunity and COPD. There is a recent systematic review on the role of autoantibodies in COPD that would be useful here.

Reply: Thank you for your constructive suggestion. The systematic review you suggested is very useful. We have updated the introduction in the revised manuscript. (Page 5, line 80-99)

2. Information related to inhaled corticosteroids should be included. Was this an exclusion criteria? This is key as samples were taken from the lung.

Reply: Thank you for your constructive comment.

In the current study, we matched the medications used in the participants in two groups of COPD patients but did not exclude patients using inhaled medications. According to your comments, we have added more details regarding inhaled medications to the revised manuscript (Table 1).

3. Why did the authors only measure IgG? There is evidence for the role of IgM autoantibodies in COPD (Shindi et al, 2017) and so the justification for not measuring IgM should be included.

Reply: Thank you for your constructive comment. We fully concur that IgM autoantibodies play a role in COPD.

As stated in the Discussion section where we cover the limitations of this work, we detected IgM autoantibodies using liquid chip, but we did not relay these data in the study because we did not think the IgM data were reliable enough to include: (1) most values were close to the lower detection limit and similar to IgM in normal controls; (2) most of the pathogenic autoantibodies were IgG, and we validated the reliability of IgG autoantibody detection (using liquid chip) by comparing patients with autoimmune diseases to normal controls, so we only showed reliable IgG data.

However, your point is very important for our further research. We have addressed this limitation in Discussion of the revised manuscript. (Page 17, line 331-333)

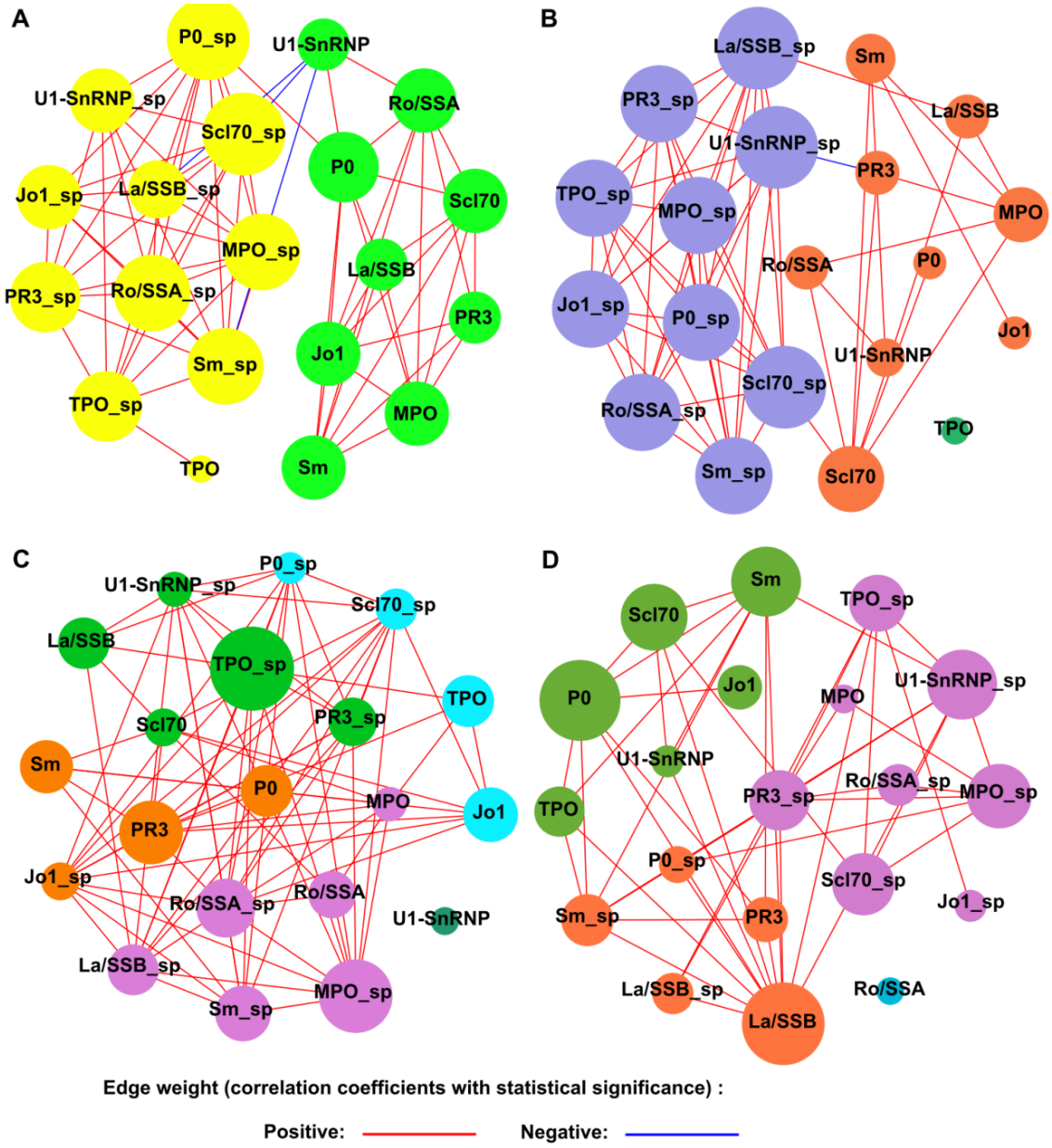
4. The results are not well described. For example, the Spearman's rank correlation matrix for autoAb levels in sputum and serum requires more explanation. Also, looking at the images of this in figure 2, it looks like the non-COPD control group has a very similar pattern to both COPD groups. The hierarchical clustering images are more useful and informative and maybe negate the need for figure 2. The colour scheme for each target autoAb response in Figure 4 is not clear and from my version, I cannot see any correlation coefficients between two nodes for the red autoantibody responses in Figure 4B, again this needs expanding.

Reply: Thank you for your important and helpful comments. We fully concur and are happy to revise and expand the descriptions as you suggest.

(1) As you said, it looks like the non-COPD control group has a very similar pattern to both COPD groups. We used an unsupervised method to classify the autoantibody profiles. We showed only the original matrix (Figure 2) in order to allow clear visual assessment, but as you said, this figure is not necessarily

needed. Per your comment, we have removed figure 2 from the revised manuscript.

(2) We have adjusted the color scheme in Figure 4 (Figure 3 in the revised manuscript) in order to make it clearer to see (see below). We have expanded the explanation in figure legends. (Page 26-27, line 487-490)



Minor revision

1. I am surprised at the 1:180 dilution of serum to measure autoantibody responses. Did the authors test a variety of dilutions to come to this value? Normal dilutions for serum are in the region of 1:1000

Reply: Thank you for your careful comment.

For human autoantibody testing, the recommended dilution ratio for commercially ELISA kits (IBL International, TMB substrate) is 1:101. Because the detection sensitivity of liquid chips is higher than that of ELISA (TMB substrate), we chose a dilution ratio of 1:180. We tested a variety of dilutions before we arrived at this value.

10.2. Dilution of Samples

Sample	to be diluted	With	Relation	Remarks
Serum	generally	SAMPLEDIL	1:101	e.g. 5 µL + 500 µL

Samples containing concentrations higher than the highest Calibrator have to be diluted further.

2. The COPD groups contain mainly male participants, why is this? This is not the case for the non-COPD controls and the CTD-ILD patients.

Reply: Thank you for your constructive comment. This is indeed a limitation of the current study.

The COPD participants in the current study were predominantly male, and the skew toward more male participants may be attributable to the uneven sex distribution of the most important COPD risk factor: According to the Global Adults Tobacco Survey of 2018, 50.5% of males and 2.1% of females in China smoked (1). Cigarette smoking is the main risk factor for COPD in China overall, although in rural southern China the main COPD risk factor is exposure to biomass fuel (2). Participants in the present study were recruited from a medical center in Guangzhou (the largest city in southern China). Thus, cigarette smoking would have been a major risk factor for COPD in this population. Because many more males than females are smokers, there was a corresponding sexual bias among our study participants.

We have addressed this limitation in the Discussion section of the revised manuscript (see Page 17, line 334-338).

References:

1. World Health Organization. Global Adult Tobacco Survey (GATS) (China 2018), 2019.

2. Liu S, Zhou Y, Wang X, et al. Biomass fuels are the probable risk factor for chronic obstructive pulmonary disease in rural South China. Thorax 2007; 62:889-97.

Reviewer B:

There is growing evidence that autoimmunity has a role in the pathogenesis of the stable chronic obstructive pulmonary disease (COPD). Although direct, indirect, and circumstantial evidence of a role for autoimmunity in stable COPD has been identified, no cause-and-effect relationship between autoimmunity and COPD mechanisms has been established and represents an area of intense active research. In this paper, the authors try to investigate the relationship between airway and systemic autoantibody by findings dissociation between airway and systemic autoantibody responses in chronic obstructive pulmonary disease. This result was feasible because the bloodstream is like a sink that receives much information from all the cells and organs of our body. So it is very important to investigate the site of the diseases of interest and so sample from small airways is the key target in COPD. Unfortunately, small airways are a difficult area for sampling to obtain reproducible results. This study is very interesting because try to investigate the role of autoantibody production at the site of the disease. Despite the relevance of the topic, I have several comments that are summarized below.

Major comments

- Because smoking exposure is correlated with the presence of autoantibodies It is very important to best characterize the amount of smoking exposure in terms of pack-years in all the cohort of patients and controls included and reported in table n.1.

Reply: Thank you for your constructive comments. Per your suggestions, we have added smoking intensity (pack-years) for all cohorts of patients and controls (Table 1).

- The real motivation to include two cohorts of patients with stable COPD must be specified and discussed.

Reply: Thank you for your comments. We are happy to expand upon this point and added more details on it.

In the beginning of the current work, we included only one cohort for analysis and found the interesting phenomenon of dissociation between airway and circulating autoantibodies in COPD. Then, we added the second cohort, which has a larger sample size, to validate the results.

We have added more details to the Discussion section. (Page 17, line 321-323)

- In the exclusion criteria, the authors stated that an exclusion criterion was a history of significant inflammatory disease other than COPD. This very important point must be specified. Was the presence of any possible comorbidity leading to the presence of systemic inflammation considered a criterion for exclusion? Please clarify. Considering the mean absolute value of FEV1 reported in table 1 concerning cohorts of COPD patients was around 50% of the predicted value so the patient with COPD recruited was in the moderate/severe stage of classification of airflow limitation severity of COPD based on GOLD classification 2020. It is very unlikely that many of these patients do not have comorbidities that could affect the state of systemic inflammation. This is a potential bias of the study that must be discussed.

Reply: Thank you for your important comments. We are happy to expand upon this point.

In COPD groups, we excluded significant inflammatory disease such as inflammatory bowel disease (Crohn's disease and ulcerative colitis), systematic lupus erythematosus, rheumatoid arthritis, and autoimmune thyroiditis, but did not exclude chronic comorbidities such as hypertension, diabetes, coronary heart disease, hypercholesterolemia, and benign prostatic hyperplasia. We acknowledge that we could not exclude all of the comorbidities which may bring bias to the study.

We have added more details to the Methods and Discussion sections of the revised manuscript. (Page 7, line 147-149; Page 17, line 338-341)

- In the discussion authors comment that sputum but not serum autoantibody levels were associated with the risk of disease exacerbation. This is an important point that needs to be mentioned in the result first.

Reply: Thank you for your constructive suggestion. We have reorganized the results in the revised manuscript (Page 12, line 219-227).

- From table 1 is evident that Patients in the single cohorts were not matched for age and sex ($p<0.001$). This could be a bias of the study that must be mentioned.

Reply: Thank you for your careful review. Due to differences in risk factors and prognoses between groups, we could not match sex or age between groups. However, the goal of the current study was to elucidate the relationship between airway and systemic autoantibody responses in COPD patients. In this way, this limitation may not affect the main conclusions of our study. Avoiding this limitation will be very important to the design of future research.

We have mentioned these limitations and their potential bias in the Discussion section of the revised manuscript. (Page 17-18, line 338-343)

- Pre-and post-bronchodilator variation in absolute value and % was missing and must be reported in table 1. The positivity of the acute bronchodilation test as an exclusion criterion must be added.

Reply: Thank you for your careful review. According to your comments, we have added the data in Table 1. Also, we have more detailed in the exclusion criterion of the revised manuscript.

In the current study, we excluded patients with a current primary diagnosis of asthma, but patients with a primary diagnosis of COPD who also had asthma were included. We also did not exclude COPD patients with positive bronchodilation tests. These exclusion criteria were similar to those of several previous studies (1-4).

We have added more details to the Methods section of the revised manuscript. (Page 7, line 122-124)

References:

1. Lipson DA, Barnhart F, Brealey N, et al. Once-Daily Single-Inhaler Triple versus Dual Therapy in Patients with COPD. N Engl J Med 2018; 378:1671-1680.
2. Kim V, Zhao H, Regan E, et al. The St. George's Respiratory Questionnaire Definition of Chronic Bronchitis May Be a Better Predictor of COPD Exacerbations Compared With the Classic Definition. Chest 2019; 156:685-695.
3. Bhatt SP, Soler X, Wang X, et al. Association between Functional Small Airway Disease and FEV1 Decline in Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 2016; 194:178-84.
4. Regan EA, Hokanson JE, Murphy JR, et al. Genetic epidemiology of COPD (COPDGene) study design. COPD 2010; 7: 32–43.

Minor comments

- The exact time of day when the venous blood sample was taken and the sputum induction should be specified.

Reply: Thank you for your careful review. Pulmonary function test (before sputum induction), venous blood sample collection, and sputum induction were performed from 8:30 a.m. – 10:00 a.m.

Reason: Because the sputum lab in our institute performs disinfection at noon every day, we have to finish sputum processing (which takes about 1.5 h per one sample) before 12:00 p.m. This means that we have to complete sample collection (venous blood and sputum) before 10:00 a.m.

We have added these details to the methods section of the revised manuscript. (Page 9, line 170-172)

- The value of FVC predicted was missing in table 1

Reply: Thank you for your careful review. We have added these data to Table 1 of the revised manuscript.

- In table 1 authors reported the percentage of respiratory medication utilization. What was the percentage of patients using multi-drug combinations?

Reply: Thank you for your careful review. Per your comments, we have added the data regarding multi-drug combinations in Table 1 of the revised manuscript.

Reviewer C:

In this study, the authors use a sensitive detection method to investigate autoantibody levels in airway and circulation in COPD patients. The main finding of this study was the dissociation between airway and circulating autoantibody levels in patients with stable COPD. They also found that sputum autoantibodies are more clinically relevant than serum autoantibodies. However, only 47 patients were included, Is the different autoantibodies between the two samples (sputum and serum) due to the small sample size? The specificity of the autoantibody is not strong, the authors should describe in the discussion section

Reply: Thank you for your comments. We are happy to expand the descriptions.

(1) Sample size

We agree with you and acknowledge that the sample size of the current was relatively small. For this reason, we added a second cohort with a larger sample size to validate the results. Several convincing studies with similar analysis had a similar or smaller sample size (1-3), so we think that this sample size may be acceptable. (Page 17, line 321-323)

(2) Specificity of the autoantibody

We agree with you that the autoantibodies in the current study are not specific to COPD, which could be a potential limiting factor to identify pathological autoantibodies involved in COPD mechanisms.

We have described this limitation in the discussion section of the revised manuscript. (Page 17, line 328-331)

References:

1. Hurst JR, Perera WR, Wilkinson TM, Donaldson GC, Wedzicha JA. Systemic and upper and lower airway inflammation at exacerbation of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006; 173:71-8.
2. Vernooy JH, Kucukaycan M, Jacobs JA, et al. Local and systemic inflammation in patients with chronic obstructive pulmonary disease: soluble tumor necrosis factor receptors are increased in sputum. Am J Respir Crit Care Med 2002; 166:1218-24.

3. Roland M, Bhowmik A, Sapsford RJ, et al. Sputum and plasma endothelin-1 levels in exacerbations of chronic obstructive pulmonary disease. Thorax 2001; 56:30-5.