Author’s response to reviews

Title: Lung function and systemic inflammation associated with short-term air pollution exposure in chronic obstructive pulmonary disease patients in Beijing, China

Authors:

Nannan Gao (gaonan0570@163.com)
Wenshuai Xu (xuwenshuai100@sina.com)
Jiadong Ji (jiadong@sdufe.edu.cn)
Yanli Yang (iamyyl@126.com)
Shao-Ting Wang (bird0162@163.com)
Jun Wang (kobezijin24@163.com)
Xiang Chen (chenxiang_pumc@foxmail.com)
Shuzhen Meng (1105501363@qq.com)
Xinlun Tian (xinlun_t@sina.com)
Kai-Feng Xu (xukf@pumch.cn)

Version: 1 Date: 13 Nov 2019

Author’s response to reviews:

In our responses, there are many tables and figures which cannot be presented here. So, we also placed the "response to the reviewers" at the end of cover letter for better revision.

Reviewer #1: Summary

Using repeated data over a one-year period, the authors found that exposure to several air pollutants was associated with reduced FVC% predicted, but not with reduced FVC when measured in absolute levels, or any measurement of FEV1, in 84 COPD patients. No consistent associations were apparent in 64 healthy volunteers. Using a subset of this population (30 COPD patients), the authors also found several associations between markers of air pollution and numerous biomarkers of systemic inflammation. Again, no consistent associations appeared among healthy participants. The analysis presented is interesting, but in its current form, is rather confusing due to unclear aspects of the methods, and the huge number of tests conducted and reported.

Response: We thank the reviewer’s comments and carefully revised our manuscript. We wish the clarity of the revised version has substantially improved.
Major points

1. There is a very large number of tests conducted and no consideration of the issue of multiple testing. Do these authors anticipate that all reported associations are "real" and independent of one another? Reporting correlations between the air pollutants, lung function measures and biomarkers might provide some insight here.

Furthermore, given the large number of tests done, I would recommend not strictly looking at p-values < 0.05 to decide on which results to report - many associations for PM10 are right on the border but not mentioned.

Response: We sincerely thank for your constructive suggestions. The p values presented in our study are not corrected. As shown in Additional file 2: Table S4-S6, there are close correlations between air pollutants, lung function and biomarker levels. And, concentrations of air pollutants at different lag days are not independent. Given the high level of correlations between tests, it is difficult to correct the p value or control the false discovery rate. Actually, for one pollutant, Bonferroni correction is the strict method to correct p value. For example, in one pollutant analysis, analyses for lag 07d have 7 hypothesis testing, the corrected p value should be min{7*p, 1} in Bonferroni correction, which may result in the false negative results. Therefore, we presented the unadjusted p value in our results. We had discussed the issue in the limitation (see page 15, line 2-6).

Table S4 Correlation coefficients between different air pollutants.

<table>
<thead>
<tr>
<th>PM10</th>
<th>PM2.5</th>
<th>CO</th>
<th>NO2</th>
<th>O3</th>
<th>SO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM10</td>
<td>1</td>
<td>0.82**</td>
<td>0.66**</td>
<td>0.66**</td>
<td>-0.12**</td>
</tr>
<tr>
<td>PM2.5</td>
<td>1</td>
<td>0.87**</td>
<td>0.79**</td>
<td>-0.20**</td>
<td>0.56**</td>
</tr>
<tr>
<td>CO</td>
<td>1</td>
<td>0.83**</td>
<td>-0.38**</td>
<td>0.61**</td>
<td></td>
</tr>
<tr>
<td>NO2</td>
<td>1</td>
<td>-0.49**</td>
<td>0.64**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O3</td>
<td>1</td>
<td>-0.35**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SO2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** corrected p<0.01

Table S5 Correlation coefficients between FEV1%pred and FVC%pred.

<table>
<thead>
<tr>
<th>FEV1</th>
<th>FEV1%pred</th>
<th>FVC</th>
<th>FVC%pred</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1</td>
<td>1</td>
<td>0.83**</td>
<td>0.76**</td>
</tr>
<tr>
<td>FEV1%pred</td>
<td>1</td>
<td>0.43**</td>
<td>0.73**</td>
</tr>
<tr>
<td>FVC</td>
<td>1</td>
<td>0.51**</td>
<td></td>
</tr>
<tr>
<td>FVC%pred</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** corrected p<0.01; * p<0.05

2. Although I understand that at some point an analysis must be done on the available data, according to the text, the full study sample should have been available as of September 2018. This leaves the reader wondering, why did the authors only use one of the available two years of data in their analysis. Do the results replicate when two years of data is considered?

Response: Thanks for the comment. Participants in our study were enrolled on a rolling basis between
December 2015 and September 2017. The recruitment and follow-ups were processed simultaneously. For example, the first subject was enrolled at December 2015, then his 5th visit was arranged at December 2016. While, the last subject was enrolled at September 2017, then the 5th visit completed at September 2018. Therefore, in our study, only 6 COPD patients and 6 health volunteers completed the 2-year follow-up up to September 2018. So, we only analyzed the first-year data. The replication of results will be tested when all participants completed the 2 years follow-up. We had added the details about the enrollment and follow-up in the methods (Page 6, line 9-14).

3. The models were not adjusted for height and weight. Have the authors tested these as potential confounders? Further, as BMI might lie in the causal pathway, an analysis removing this covariate might be of interest.
Response: Thank you for pointing this out. We had taken height and weight as the potential confounders. Considering that BMI was calculated by height and weight, and most studies adjusted the BMI, therefore we adjusted the BMI index in our statistical analyses. As shown in the followed figures (figure 3.1-3.4), results were barely changed when adjusted for height and weight. If BMI lies in the causal pathway (see figure A below), adjusting the BMI would underestimate the effects of air pollution. While, if associations between BMI, air pollution and lung function/cytokine like figure B, then BMI must be adjusted as a confounder. So, it is prudent to adjust the BMI in this study. Actually, to explore the role of targeted factor as a confounder or lie in the causal pathway is an interesting and hot topic in causal inference. We had added the topic in the discussion (see page 15, line 6-11).

Figure 3.1 Changes in FVC % pred among COPD patients with 1 standard deviation increase in air pollutant levels adjusted for height and weight.

Figure 3.2 Changes in eotaxin, IL-4 and IL-13 levels among COPD patients with a 1 standard deviation increase in air pollutant levels adjusted for height and weight.

Figure 3.3 Changes in IL-2, IL-12 and IFNγ levels among COPD patients with a 1 standard deviation increase in air pollutant levels adjusted for height and weight.

Figure 3.4 Changes in IL-17, sCD40L and MCP-1 levels among COPD patients with a 1 standard deviation increase in air pollutant levels adjusted for height and weight.

Minor points

1. When the authors report that no associations were found for the other cytokines in the results section, could they provide a list for the reader. I would also encourage the authors to provide justification for why they selected to test these particular biomarkers, and if this is hypothesis based, providing the reader in the introduction with a clear expected direction of effect would greatly facilitate the interpretation of the results.
Response: Thank you for your suggestions. We had summarized the associations between air pollution exposure and cytokines levels in the revised Table S3. We selected the measured cytokines from the following aspects. Firstly, the options were based on the MILLIPLEX® MAP human cytokine/chemokine panel. Secondly, we pay attention to the common cytokines expressed by or
targeted at macrophage, T helper cell, neutrophil and eosinophils, which play roles in biological inflammatory and immune response (see page 5, line 16-19). In addition, these cytokines may have effects in lung tissue or airway by searching PUBMED.

Table S3 Summary of the correlations between air pollution exposure and serum cytokine levels.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>COPD population effect exposure</th>
<th>Health population effect exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>↑ SO2, O3</td>
<td>—</td>
</tr>
<tr>
<td>IFNγ</td>
<td>↑ PM2.5, NO2, CO</td>
<td>—</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>↑ NO2</td>
<td>—</td>
</tr>
<tr>
<td>IL-1β</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>IL-2</td>
<td>↑ PM2.5, PM10, NO2, CO</td>
<td>—</td>
</tr>
<tr>
<td>IL-4</td>
<td>↓ PM2.5, PM10, NO2, SO2, CO</td>
<td>—</td>
</tr>
<tr>
<td>IL-5</td>
<td>↑ NO2</td>
<td>—</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>IL-12P70</td>
<td>↑ PM2.5, NO2, SO2, CO</td>
<td>—</td>
</tr>
<tr>
<td>IL-13</td>
<td>↓ CO</td>
<td>—</td>
</tr>
<tr>
<td>IL-17A</td>
<td>↑ PM2.5, NO2</td>
<td>↑ NO2</td>
</tr>
<tr>
<td>IP-10</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>MCP-1</td>
<td>↑ PM10, NO2</td>
<td>—</td>
</tr>
<tr>
<td>MIP-1α</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>sCD40L</td>
<td>↑ PM2.5, PM10, NO2</td>
<td>—</td>
</tr>
<tr>
<td>TNFα</td>
<td></td>
<td>↓ O3</td>
</tr>
<tr>
<td>MIP-1β</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>eotaxin</td>
<td>↓ PM2.5, PM10, SO2, CO</td>
<td>—</td>
</tr>
</tbody>
</table>

↑ The cytokine level increased with air pollutant exposures.
↓ The cytokine level decreased with air pollutant exposures.
— The cytokine level had no association with air pollution levels.

2. More information on how the air pollutants were assigned is required. In the abstract, this is simply described as "achieved". For example, how far were the participants on average from the monitoring data? Were there issues with missing data?
Response: Thank you for the comments. We had added the description of air pollution data in the abstract (see page 2, line 10-12). The average distance of participants to the nearest station in our study was 3.9 km. The hourly data of monitoring stations have missing. In our analyses, the miss rate of daily pollutant levels was 0.45% for COPD cohorts and 0.19% for healthy group. For the missing data, the pollutant levels were calculated as the city daily estimates. We had added the details in the revised manuscript (see page 8, line 20-22; page 9, line 1).

3. There is no discussion as to why %predicted FVC should show the strongest associations, especially as results were reported as null for FVC. Could the authors please expand as to why this may be?
Response: Thank you for the comments. The FVC was the absolute values of the participant’s measurements, associated with age and height of the participant. While, FVC%pred controlled the heterogeneity between participants. So, we speculate that the FVC%pred may have the opportunity to show the strongest associations.
4. Were there significant differences between the air pollution concentrations between the healthy and COPD groups?
Response: Thanks a lot. We compared the pollution differences between healthy and COPD groups, which was presented in revised Table S1. No significant difference was found for PM2.5, PM10, NO2, CO and SO2.

Table S1 Distribution characteristics of air pollutants levels in the study.

<table>
<thead>
<tr>
<th>Overall†</th>
<th>COPD*</th>
<th>Health*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mean±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM10 (μg/m3)</td>
<td>88.62±74.48</td>
<td>94.8±86.05</td>
<td>92.40±71.45</td>
</tr>
<tr>
<td>PM2.5 (μg/m3)</td>
<td>62.74±60.44</td>
<td>64.04±66.13</td>
<td>61.58±51.18</td>
</tr>
<tr>
<td>CO (mg/m3)</td>
<td>1.03±0.89</td>
<td>1.04±0.99</td>
<td>1.03±0.67</td>
</tr>
<tr>
<td>NO2(μg/m3)</td>
<td>42.81±22.4</td>
<td>55.95±25.17</td>
<td>53.37±23.65</td>
</tr>
<tr>
<td>O3 (μg/m3)</td>
<td>61.76±38.69</td>
<td>55.27±44.12</td>
<td>65.42±52.28</td>
</tr>
<tr>
<td>SO2 (μg/m3)</td>
<td>7.55±8.49</td>
<td>8.59±7.40</td>
<td>8.29±6.74</td>
</tr>
</tbody>
</table>

Notes: *mean exposure levels for COPD and healthy group: firstly, calculated each participant exposure, then averaged all COPD or healthy participant exposures. †mean daily air pollutant levels in Beijing city over the study period.

5. In the introduction, the authors state that a limitation of previous studies is that they have only used "short follow-up periods". Could this be quantified? Providing a range of how many months/years were used in previous work would help justify the current manuscript even more.
Response: Thanks for your reminder. We added the follow-up periods in the introduction (see page 4, line 22).

6. The description of the selection and derivation of the study sample needs to be clarified. At the top of page 6, the authors state that all subjects 18-75 years residing in Beijing for at least 1 year were invited. Do the authors mean "eligible" here, instead of "invited"? Also, based on what criteria were 30 COPD and 30 healthy participants selected for serum cytokine detection? After applying the exclusion criteria listed, was a random selection made?
Response: Thanks for your reminder. At the top of page 6, we mean that subjects 18-75 years residing in Beijing for at least 1 year has the eligibility to participate. The selection criteria for serum cytokine detection were shown in the manuscript (page 7, line 22; page 8, line 1-4). The selection was random.

7. Table 1 should present the characteristics of the 75 COPD patients who were analyzed in this study, and the not 84 who were originally recruited.
Response: Thank you for pointing this out. Our study was based on the repeated measurements. In the cohorts, each participant had at least 2 measurements of pulmonary function. There are 9 COPD patients dropped out, however, these patients’ data before drop-out were used in our study. So, Table 1 presented the characteristics of the 84 COPD patients and 64 healthy participants at the baseline.

8. It is unclear how often each sampling took place. For example, "baseline and 1-year" used for blood sampling, "each visit" used for lung function, and "baseline, half-year and 1-year" used for serum cytokine detection all in the Methods, yet "every three months" used in the abstract for serum and spirometry. The number of visits and available samples for each outcome should be made clear.
Response: Thank you for the comments. The description in our manuscript may be confusing. We had summarized and simplified the methods in the revised manuscript. Overall, each participant was
scheduled to follow-up every 3 months. As shown in revised Figure S1, lung function was measured at each visit, while cytokines tests were detected at 1st visit (baseline), 3rd visit (half-year), 5th visit (1-year). However, not all participants in the cohorts completed the pulmonary function tests during the follow-up. For example, at 2nd visit, 84 COPD patients were in the cohorts, 4 of those missed the pulmonary function tests. Therefore, 389 spirometry tests were available totally. A total of 180 samples \((30*3+30*3)\) were detected for cytokines levels (see page 7, line 17-19).

Figure S1 Study flow chart.
Notes: N: number of participants at the corresponding visits for COPD and heath cohort; n: number of pulmonary function tests (PFT) obtained at the corresponding visits.

9. Was an analysis conducted looking at the impact of potential outliers in the biomarkers, as some of these have very high (and very influential) values.
Response: Thanks very much for your valuable suggestion. In our analysis, we took this issue into consideration. There are several outliers for the biomarkers. We try to remove the outliers or replace them by mean value and find that the regression coefficients changed slightly. No influential points were identified. For example, we removed the top four extremes of the cytokine, the results changed slightly and the effect trends remained (see figure 9.1-9.3). Therefore, we applied the raw data in the final analysis.

Figure 9.1 Changes in eotaxin, IL-4 and IL-13 levels among COPD patients with a 1 standard deviation increase in air pollutant levels after excluding the outliers.

Figure 9.2 Changes in IL-2, IL-12 and IFN\(\gamma\) levels among COPD patients with a 1 standard deviation increase in air pollutant levels after excluding the outliers.

Figure 9.3 Changes in IL-17, sCD40L and MCP-1 levels among COPD patients with a 1 standard deviation increase in air pollutant levels after excluding the outliers.

10. Can the authors clarify how those with asthma-COPD were identified in the text?
Response: Thanks a lot. Asthma-COPD overlap (ACO) is characterized by persistent airflow limitation with several features usually associated with asthma and several features usually associated with COPD. ACO is therefore identified by the features that it shares with both asthma and COPD. And in our study, the identification of ACO patients from COPD patients were in accordance with the features in Global Initiative for Asthma guideline. We had added the reference (26) in the revised manuscript.

11. When the authors state that the single lag models for the biomarkers are in the Supplement, could they also briefly describe for the reader if the results were similar.
Response: Thanks for the comment. On the whole, the effects of air pollution exposure on cytokine levels were similar in single-day lag model and multiday lag model. We had integrated the results of single-day lag model into the revised manuscript (see page 11, line 2-21).

12. Very minor, but the results for IL2 are not described in the text, although this is done for all other biomarkers which show associations. Also, CO should likely also be included in the description of the results for MCP-1.
Response: Thank you a lot. We had added the description on IL-2 and MCP-1 in the results (see page 11, line 10-12, 19-21).
13. The statement that the effect estimates for IL2, IL17, sCD40L and MCP-1 in COPD patients were
greater as the lags increased does not appear to be supported by the results, especially not for IL2 and
MCP-1. Please revise, and consider whether this statement is justified in general, given the fact that the
confidence intervals very largely overlap in all cases.
Response: Thanks for your suggestion. We had deleted the statement in the results.

14. Why focus on the PM results in the conclusion, with "especially PM"?
Response: Thanks a lot. As shown in our study, PM is the main air pollutant in Beijing city. Daily
concentrations of PM2.5 (62.74 μg/m3) exceeded the Chinese ambient air quality standards (35 μg/m3)
and were far higher than those reported in the US and Europe. So, we emphasized the PM in the
conclusion. However, as you suggested, this may be not appropriate here. We had removed the term.

15. Why do the authors include the description of CRp, ESR, IgE, for example, when these factors are
never used in the analysis?
Response: Thanks a lot for pointing this out. CRP, ESR and IgE were only detected at the baseline and
5th follow-up. These factors were not included in the final analysis. We deleted the description in the
revised manuscript and Table 1.

16. 18 smokers are listed among the COPD patients in the Results text, but 22 is given in Table 1.
Response: Thank you for pointing this out. We checked our raw data, as shown in Table 1, there are 22
current smokers in our study. And, we have corrected the data in the results.

Grammar/presentation

17. COPD should be defined in first sentence of abstract, not in second.
Response: Thanks for your reminder. We had defined the COPD in the first sentence in the abstract.

18. Some minor grammatical issues remain in the text and should be corrected. For example, "in
association with air pollution" needs to be added to the sentence ...."The researchers found that RH1,
TH17, INFN, Il17 were increased in COPD mice...." on page 13, or else the reader does not know how
this relates to the current work that looks at air pollution effects. There are a few other points in the text
when the grammar/sentences are unclear.
Response: Thanks for pointing this out. We had revised the sentence in the discussion. And, we had
tried to minimize the grammar error in the revised manuscript.

19. The authors could reconsider the use of "change" in the y-axis of the Figures. Is this really what is
modelled, or rather a comparison of mean differences?
Response: Thanks for the comments. Actually, both “change” and “mean difference” terms represent
the regression coefficient of linear mixed linear model. When the independent variable is categorical,
the coefficient means the mean difference between two comparison levels. For the continuous
independent variables, the coefficient means the changed values of dependent variable with each 1 unit
increase in independent variables. In our study, the y-axis means the increased or decreased values of
pulmonary function and cytokine levels with each 1 SD increase in air pollutants. Therefore, we think
the use of “change” term is appropriate in this study.
Reviewer #2: Gao N and co-authors investigated the acute effects of air pollution exposure in lung function parameters and serum cytokines in a group of COPD patients (n=75) and a group of healthy individuals (n=64) in China. The analysis is based on lung function and cytokines measured each 3 months in the first-year follow-up. Results indicate that 4 pollutants (NO2, PM2.5, SO2 and CO) were statistically significantly associated with a lower FVC in COPD patients although only SO2 was inversely associated with lower FVC and FEV1 in healthy subjects. The study further show that short-term air pollutants enhances systemic inflammation in COPD patients, by aggravating the Th1/Th2 and Th17 cytokines imbalance. Overall the manuscript is well written and provide interesting results in the field. Nevertheless, I have some concerns detailed below.

Response: We sincerely appreciate reviewer’s effort in reviewing our manuscript and constructive comments.

1) Overall strategy: This paper is aimed to address effects of air pollution on lung function and systemic inflammation among COPD patients. The reason for including a healthy group is not clearly stated (neither in the introduction, nor in the methods) and by providing results from a large set of associations independently in each group makes the interpretation uneasy. If the authors wish to show that COPD patients are more vulnerable to air pollution then it needs to be explicitly stated (including in the aims) and statistical tests of interaction (disease*air pollutant exposures) are warranted.
Response: Thank you for the insightful suggestion. We want to observe the effects of air pollution on both COPD and healthy participants because scarce studies explored the effects of air pollution on circulating cytokines both in COPD and healthy participants. However, many covariables did not matched between two groups in the study, direct comparison is not appropriate. We took the results of healthy population as indirect reference to COPD patients. We added the description in the revised manuscript (see page 4, line 19; page 5, line 13-21).

2) Population: It is unclear why the study is based only on the first-year follow-up (p.6 line 15-16) although data up-to the 2-year visit have not been recorded and are available according the flow-chart. This needs to be explained and scientifically supported.
Response: Thank you for the reminder. Participants in our study were enrolled on a rolling basis between December 2015 and September 2017. The recruitment and follow-ups were processed simultaneously. Follow-ups of the participants were not performed at the same date. For example, the first subject was enrolled at December 2015, then his 5th visit was arranged at December 2016. While, the last subject was enrolled at September 2017, then the 5th visit completed at September 2018. Therefore, in our study, only 6 COPD patients and 6 health volunteers completed the 2-year follow-up up to September 2018. So, we only analyzed the first-year data. We added the details about the enrollment and follow-up in the methods (Page 6, line 9-14) and revised the flow chart (Figure S1).

Figure S1 Study flow chart.
Notes: N: number of participants at the corresponding visits for COPD and heath cohort; n: number of pulmonary function tests (PFT) obtained at the corresponding visits.

3) Population: The number of subjects retained for the analysis is unclear and inconsistent through the manuscript: from Table 1 (which should be presented for the population on which the analysis is based), 84 COPD vs. 64 healthy individuals; from the flow chart, 69 COPD and 60 healthy individuals; From the Results first paragraph 135 participants, from the Abstract, 75 COPD and 64 healthy individuals. Similarly, the number of spirometry test does not fully converge with the number of subjects (shouldn't it be 135*5-16= 659, rather than 691?).
Response: Thank you for the comments. The description in our original manuscript may be confusing.
We had summarized and simplified the methods in the revised manuscript. Our study was based on the repeated measurements. In the cohorts, each participant had at least 2 measurements of pulmonary function. There are 9 COPD patients dropped out, however, these patients’ data before drop-out were used in our study. So, Table 1 presented the characteristics of the 84 COPD patients and 64 healthy participants at the baseline. In the study, there are 75 COPD patients and 60 healthy individuals completing the 1-year follow-up until September 2018. In addition, as shown in Figure S1, some participants in the cohorts came to our hospital for visits, but he or she didn’t perform the pulmonary function tests due to certain reasons. For example, at 2th visit, 84 COPD patients were in the cohorts, 4 of those missed the pulmonary function tests. Therefore, 389 spirometry tests achieved totally in COPD cohorts. In the original flow chart, we mean that there were 69 COPD and 60 healthy individuals continuing to follow up on September 2018. Avoiding to confuse the readers, we deleted this data and only presented the follow-up details which we analyzed in this article in the revised Figure S1.

4) Population: most of the COPD patients were former or current smokers (almost 90%) although inversely most of the healthy individuals were never smokers (92%). Therefore, differences in the associations observed between COPD and healthy individuals could be attributed to smoking status (that could enhance the harmful effects of air pollutants exposure) and not to COPD disease. This is a major weakness in the interpretation of the results in this study. Ideally, a healthy group mainly composed of smokers would have been appropriate. A sensitivity analysis investigating the robustness of the association in COPD patients after excluding current smokers (if former smokers is defined by no smoking for at least several months) can help to address this issue. This limitation requires discussion.

Response: Thanks very much for the insightful comment. The imbalanced distribution of smoking status in our cohorts may contribute to the different effects of air pollution on pulmonary function not on the cytokine changes. Because, subjects selected for cytokine detection had no current smoking (as shown in the methods: page 8, line 1). We analyzed the effects of air pollution on pulmonary function after excluding the 22 current smokers. As shown in the figure below, PM2.5 and CO remained the adverse association with FVC%pred. Though the nonsignificance for SO2 and NO2 were observed, the p value was marginally significant. The nonsignificance for SO2 and NO2 may be attributed to the decreased sample size after excluding the 22 smokers. We had mentioned the issue in the revised manuscript (see page 13, line 6-8).

Changes in FVC % pred among COPD patients with 1 standard deviation increase in air pollutant levels among non-current smokers.

5) Statistical analysis: multiple comparisons have been tested in this study (&gt;250 tests without considering the different lags for exposure assessment). Although correcting for multiple comparison is not straightforward given the high level of correlation between tests (Bonferroni correction would not be appropriate here), not taking it into account in the analysis makes the interpretation of the results quite difficult. Methods relying on the effective number of independent tests as proposed in the genomic field (ex: Li et al. Hum Genet 2011) could be used here. Also, this multiple comparison issue needs to be addressed in the discussion.

Response: Thank you for the comments. Indeed, multiple testing should be considered in this study. Generally, adjusting the p value or controlling the false discovery rate is the way to address this issue. The method proposed in the reference of Li et al. Hum Genet 2011 was not appropriate in our study. Because, that method need to calculate the correlation matrix of p values from multiple testing. The study described a polynomial approximation that allows the correlation matrix of association test p values to be calculated from the correlation matrix of allele counts. But, the polynomial approximation
depends on the used method to calculate p value and the distribution of independent variable. Variables
and statistical model in our study were different from this reference. This reference mentioned another
method, which calculated effective number of independent tests by correlation matrix of variables, may
be useful for us. According to the above method, the corrected p value of single air pollutant was less
than 0.05/5.5 instead of 0.05/7. So, most results with p&lt;0.01 remained significant for single air
pollutant analyses. We had discussed the issue in the limitation (see page 15, line 2-6).

6) Statistical analysis: Wouldn't it be possible and relevant to address the mediation effect of cytokines
in the association between air pollutants exposures and lung function parameters in COPD patients?
Response: Thank you for the valuable suggestion. Mediation effect analysis is an important research
filed. Our results indicated that some cytokines may have the mediation effect. But, analysis on
mediation effect of cytokines exceeded the research focus in our study. Thanks very much for
providing this good and interesting question. We will explore the mediation effect in further analysis.

7) Results Table S1: Value of 0 for the min PM10 seems incorrect. How many daily PM10
concentration was observed at 0? If it is related to measurement errors, shouldn't it be corrected to the
expected minimum value? In addition, did the authors checked that the distribution of air pollutant
concentrations was similar between COPD patients and Healthy individuals? This information could be
added to the manuscript (Tables S1 presented for each group separately).
Response: Thanks for your kind reminder. Three daily PM10 levels were observed at 0 in the raw data,
which was not related the measurement errors. In addition, we had analyzed the distribution of air
pollutant concentrations between COPD patients and healthy participants in the revised Table S1. No
significant difference was found for PM2.5, PM10, NO2, CO and SO2.

Table S1 Distribution characteristics of air pollutants levels in the study.

<table>
<thead>
<tr>
<th></th>
<th>overall†</th>
<th>COPD*</th>
<th>Health*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mean±SD)</td>
<td>(mean±SD)</td>
<td>(mean±SD)</td>
<td>(mean±SD)</td>
<td></td>
</tr>
<tr>
<td>PM10 (μg/m3)</td>
<td>88.62±74.48</td>
<td>94.8±86.05</td>
<td>92.40±71.45</td>
<td>0.689</td>
</tr>
<tr>
<td>PM2.5 (μg/m3)</td>
<td>62.74±60.44</td>
<td>64.04±66.13</td>
<td>61.58±51.18</td>
<td>0.580</td>
</tr>
<tr>
<td>CO (mg/m3)</td>
<td>1.03±0.89</td>
<td>1.04±0.99</td>
<td>1.03±0.67</td>
<td>0.892</td>
</tr>
<tr>
<td>NO2(μg/m3)</td>
<td>42.81±22.4</td>
<td>55.95±25.17</td>
<td>53.37±23.65</td>
<td>0.165</td>
</tr>
<tr>
<td>O3 (μg/m3)</td>
<td>61.76±38.69</td>
<td>55.27±44.12</td>
<td>65.42±52.28</td>
<td>0.007</td>
</tr>
<tr>
<td>SO2 (μg/m3)</td>
<td>7.55±8.49</td>
<td>8.59±7.40</td>
<td>8.29±6.74</td>
<td>0.578</td>
</tr>
</tbody>
</table>

Notes: *mean exposure levels for COPD and healthy group: firstly, calculated each participant
exposure, then averaged all COPD or healthy participant exposures. †mean daily air pollutant levels in
Beijing city over the study period.

8) Conclusion: The authors concluded on the role of air pollution on aggravating the Th1/Th2 cytokine
imbalance. I agree that results observed on individual cytokines indicate this, but I think it could be
relevant to further investigate this by analyzing ratio between Th1/Th2 cytokines to further document
the Th1/Th2 imbalance.
Response: Thank you for the comment. Indeed, further analysis or experiments on Th1/Th2 ratio may
be needed to illustrated the Th1/Th2 imbalance. We had altered the statement in the revised conclusion
(see page 3, line 9; page 15, line 19).