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**Reviewer A**

**This is a retrospective study report on the association between serum Apolipoprotein B and fractional exhaled nitric oxide in bronchial asthma patients. The study deals with a potentially interesting for clinicians working with such a patient population. This reviewer, however, has some concerns that the authors need to address before resubmission. Please find them below.**

**Major concerns**

1. **I have a major concern with the study design. Retrospective studies analyze pre-existing data and are subject to numerous biases. I found myself inquisitive to understand how the authors controlled for the outcome assessment and quality of the data that they collected from the medical records.**

→ Thank you for your comment about the study design. We agree to your opinion that retrospective studies are subject to numerous biases. To control for the outcome assessment and quality of data, we enrolled the asthma patients limited to pulmonology/allergy clinic in one tertiary hospital. The lung function measurement and FeNO was performed by skilled technicians according to international guideline, and retrospective chart review was done by one pulmonology specialist (HY Lee) to maintain consistency of the collected data. Moreover, data quality and statistical analysis was performed by statistician from the department of applied statistics (SJ Han).

2. **This study design needs large sample sizes. I am not sure whether this study powered enough for the measured variables and analysis type?**

→ We agree to your comment about the small sample size. However, statistical significance about the relationship between log(FeNO) and apolipoprotein B level was definite in multivariate analysis after adjustment of other variables (asthma & smoking history) affecting the FeNo level. P value showed statistical significance ($P$ = 0.001) in multivariate analysis with R-squared 0.459 (table 3). We added R-squared value and $P$-value in table 3. Additionally, to respond to comments from another reviewer, the result of multivariate analysis for post BD FVC (%) was added to Table 3-1 and Table 3-2 & 3-3 have been revised. The results of multivariate analysis including VIF or generalized VIF were shown below.

- Table 3-1) Regression analysis for dependent variable post BD FVC (%)

| Variable | Parameter Estimate | Standard Error | P Value | VIF |
|---|---|---|---|---|
| (Intercept) | 95.648 | 4.259 | <0.001 | |
| Age | -0.128 | 0.067 | 0.060 | 1.254 |
| Statin | -1.748 | 3.771 | 0.644 | 1.885 |
| Dyslipidemia | -2.239 | 3.830 | 0.560 | 1.790 |
| Allergic_rhinitis | 2.086 | 2.457 | 0.397 | 1.216 |

R-squared : 0.07162
P-value of F-statistic : 0.03524

- Table 3-2) Regression analysis for dependent variable post BD FEV1 (%)

| Variable | Parameter Estimate | Standard Error | P Value | VIF |
|---|---|---|---|---|
| (Intercept) | 92.898 | 8.010 | <0.001 | |
| Age | -0.179 | 0.080 | 0.027 | 1.089 |
| Sex | -3.567 | 3.312 | 0.284 | 1.118 |
| Allergy | 6.396 | 3.068 | 0.040 | 1.122 |
| ApoB | -0.105 | 0.062 | 0.095 | 1.002 |

R-squared : 0.1112

P-value of F-statistic : 0.01998

- Table 3-3) Regression analysis for dependent variable log(FeNO)

| Variable | Parameter Estimate | Standard Error | P Value | normalized GVIF |
|---|---|---|---|---|
| (Intercept) | 1.848 | 0.413 | <0.001 | |
| Asthma | 0.595 | 0.207 | 0.007 | 1.006 |
| ApoB | 0.016 | 0.005 | 0.001 | 1.018 |
| Smoking_ Never(ref) | 1.000 | | | |
| Smoking_ EX | 0.492 | 0.248 | 0.056 | 1.012 |
| Smoking_ Current | -1.741 | 0.617 | 0.008 | |

R-squared : 0.459

P-value of F-statistic : 0.0002567

**3. Other factors may be present and were not analyzed and may confound the results (i.e., medication). Is it possible to provide more details about medications (i.e., dosages and durations) that the participates have taken?**

→ Thank you for your precious comment. We already analyzed about the medication of the participants. We added this results in Table 2. Differences in budesonide equivalent showed no statistical significance between two groups (*P* = 0.11). We added this result in result section (line 240-241)

| | PFT-diagnosed Asthma (N=93) | Physician-diagnosed Asthma (N=74) | *P* |
|---|---|---|---|
| Treatment agents, n (%) | | | < 0.001 |
| ICS only | 7 (7.5) | 5 (6.8) | |
| Low dose ICS/LABA | 52 (55.9) | 22 (29.7) | |
| Medium dose ICS/LABA | 13 (14.0) | 4 (5.4) | |
| High dose ICS/LABA | 14 (15.1) | 4 (5.4) | |
| LAMA | 8 (8.6) | 3 (4.1) | |
| Systemic steroids | 10 (10.8) | 11 (14.9) | |
| Biologics | 1 (1.1) | 0 (0) | |
| LTRA | 53 (57.0) | 33 (44.6) | |
| None | 5 (5.4) | 35 (47.3) | |
| Budesonide equivalent (mg) | 320 | 180 | 0.11 |

| | | | |
|---|---|---|---|
| (Median, IQR) | (160-640) | (0-730) | |

**Minor comments**

4. **Abstract: the study objective should be stated clearly in the abstract, which is currently lacking.**

-> Thank you for your comment, we had modified abstract more clearly to state the study objective.

5. **I think mentioning statistical tests (in the patients and methods section) in the abstract is not a common practice. Authors can use the space to give more details about the study methodology.**

**->** Thank you for your comment, we totally agree to your comment. We corrected the abstract as your comment (line 58-60).

6. **The study cannot determine causation, only association. This should be mentioned in the study limitation.**

-> Thank you for your comment. We mentioned the study result as limitation of our study in discussion section. (line 373-375)

7. **Given the retrospective nature, it would be interesting if authors can give more details about chart reviews (data collection from the medical records of patients), how the outcome measures were assessed.**

-> We added the data collection and analysis process in method section (line 166-169).

8. **Asthma diagnostic criteria. Were these criteria being the basis for asthma diagnosis in the setting where data were collected? This should be clarified.**

-> Asthma diagnosis criteria was based on international Global INitiative for Asthma (GINA) 2020 guideline. We added this reference in method section (line 175-177)

9. **The basis on which asthma and non-asthma groups were designated is not clear. Is there any supporting reference?**

-> Thank you for your comment. We agree to your opinion. Authors had discussed about the classification of the study groups and decided to change the labeling of the groups. Based on GINA guideline, asthma is diagnosed by history of respiratory symptoms and evidence of variable expiratory airflow limitation. However, trial of asthma treatment can be performed before the diagnostic lung function tests when the patients have history/examination of asthma diagnosis without alternative diagnosis. In clinical process, patients who had positive effects on asthma medications could be diagnosed as asthma by specialists without pulmonary function test results. So we re-named “asthma” patients to “pulmonary function test (PFT)-proven asthma” and “non-asthma” patients to “physician-diagnosed” asthma. We added this point in method section (178-184)

10. **Data normality assessment should be mentioned in the "Statistical analysis" section. To justify the selection of other tests for computing differences between groups, correlation, and regression analysis.**

-> Thank you for your comment. We added about the data normality assessment in method section (line 206-208)

11. **Pg 8; Ln 157-159 "In the asthma group, more than 85 patients were defined as high Apo B and less than 85 were defined as low Apo B based on the median value of Apo B". Please add a reference for cutoff level for the designation of high and low Apo B.**

-> Thank you for your precious comment. As far as we know, there has been no reference for cutoff level of ApoB value in patients with bronchial asthma. However, ApoB predicted risk of ischemic cardiovascular disease and ischemic stroke in women with hazard ratios of 1.9 (1.2-2.9) and 1.6 (1.0-2.8) in large-scale Copenhagen City Heart study. They divided patients into 3 groups according to the ApoB levels as lower tertile (0-74 mg/dL), middle tertile (75-94) and upper tertile (95-242) [1]. Moreover, the American Diabetes Association and the American College of Cardiology Foundation have suggested a treatment target of 80mg/dL ApoB in patients with known cardiovascular disease or with diabetes and another cardiovascular risk factors [2, 3]. Therefore, the cutoff of ApoB level (85 mg/dL) for high/low ApoB assignment could be an appropriate option to assess the association between blood ApoB and lung function parameters. We added the reference in result section (line 264-269)

12. **The discussion is satisfactory and based on the literature on this topic given the limited publication on the topic. However, the authors should discuss their perspectives and interpretation of the findings and the clinical implication.**

-> Thank you for your comment. We added the clinical perspective of our findings in discussion section. (line 343-359)

**Reviewer B**

**In this study, the authors investigated the relationship between lipid profile and FeNO and revealed a significant association between Apo B and FeNO in asthmatic patients. Although the study is interesting, I have several concerns to be addressed as follows;**

1. **The main concern is the definition of a non-asthma group. It is not clear what the diagnosis of the cases in this group is? The authors should precisely define this group as I think the nomination of this group is not accurate.**

-> Thank you for your comment. We agree to your opinion. Authors had discussed about the classification of the study groups and decided to change the labeling of the groups. Based on GINA guideline, asthma is diagnosed by history of respiratory symptoms and evidence of variable expiratory airflow limitation. However, trial of asthma treatment can be performed before the diagnostic lung function tests when the patients have history/examination of asthma diagnosis without alternative diagnosis. In clinical process, patients who had positive

effects on asthma medications could be diagnosed as asthma by specialists without pulmonary function test results. So we re-named “asthma” patients to “pulmonary function test (PFT)-proven asthma” and “non-asthma” patients to “physician-diagnosed” asthma. We added this point in method section (178-184)

2. **Methods: “Lung function tests were performed according to the American Thoracic Society / European Respiratory Society standardization guidelines.” Line 108. A reference for this guideline should be added.**

-> Thank you for your comment. We added the reference for this guideline. (line 157, reference 19)

3. **Table 3-1: Multivariable analysis should be performed for the significant marker in the univariate for dependent variable post-BD FVC (%) as age, statin, dyslipidemia, allergic rhinitis.**

-> Thank you for your comment. We added the multivariable analysis result in table 3-1.

4. **I noticed that many variables that were not significant in the univariate as ApoA (table 3-2), age, sex, current smoker (table3-3) were included in the multivariate analysis which is not appropriate.**

-> Thank you for your comment and we are very sorry for the confusing result. We added age and gender because of the clinical significance of FVC and FEV1, however, we agree with your opinion that it would be appropriate to include the parameters based on the p value. Exceptionally, current smoking was included in multivariate analysis because the effect of current smoking is important in analysis of FeNO result [4]. We re-analyzed multivariate analysis and corrected the table 3-2 and 3-3.

5. **Discussion: this section is too long and redundant with much-repeated information in the introduction section. Please rewrite this section. Also, other pathways and markers associated with bronchial asthma and obesity and dyslipidemia should be illustrated following this recent reference http://sci-hub.tw/10.1007/s11033-020-05531-2**

-> Thank you for your comment. We agree to your comment and re-wrote discussion eliminating the repeated information in introduction section. Morever, clinical implication of this study result was added (line 343-359).
In addition, we are very sorry to say that internet link of your reference is not currently active, we could not find the reference journal. Instead, we found other recent review about the metabolic dysfunction as asthma [5], added in discussion section (283-291).

**References**

[1] M. Benn, B.G. Nordestgaard, G.B. Jensen, A. Tybjaerg-Hansen, Improving prediction of ischemic cardiovascular disease in the general population using apolipoprotein B: the Copenhagen City Heart Study, Arterioscler Thromb Vasc Biol 27(3) (2007) 661-70.
[2] J.D. Brunzell, M. Davidson, C.D. Furberg, R.B. Goldberg, B.V. Howard, J.H. Stein, J.L.

Witztum, Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation, J Am Coll Cardiol 51(15) (2008) 1512-24.
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[4] A. Malinovschi, C. Janson, T. Holmkvist, D. Norback, P. Merilainen, M. Hogman, Effect of smoking on exhaled nitric oxide and flow-independent nitric oxide exchange parameters., European Respiratory Journal 28(339-345) (2006) 339-45.
[5] H. Pite, L. Aguiar, J. Morello, E.C. Monteiro, A.C. Alves, M. Bourbon, M. Morais-Almeida, Metabolic Dysfunction and Asthma: Current Perspectives, J Asthma Allergy 13 (2020) 237-247.