PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effects of short-term smoking on lung function and airway hyperresponsiveness in young patients with untreated intermittent adult-onset asthma: a retrospective cross-sectional study at a primary–tertiary care hospital in Japan
AUTHORS	Watai, Kentaro; Sekiya, Kiyoshi; Hayashi, Hiroaki; Fukutomi, Yuma; Taniguchi, Masami

VERSION 1 - REVIEW

REVIEWER	Hannu Kankaanranta Seinäjoki Central Hospital and University of Tampere Finland
REVIEW RETURNED	17-Apr-2018

GENERAL COMMENTS	This study of Watai and co-workers evaluates a clinically highly important aspect, namely the effect of smoking on lung function in patients with adult-onset asthma who are smoking. In general the study is interesting, well conducted and the results are important. There is two major strengths in this study, namely the selection of adult-onset asthma patients and not excluding smokers and secondly, the diagnosis of asthma is based on objective lung function test results. However, there remains some room for improvement. Especially referral and discussion with current relevant literature needs to be improved. Major: 1. Introduction, first lines. This referee does not completely agree with first sentence. There is surprisingly small number of studies demonstrating these. Usually smoking patients with asthma have been excluded and there is only limited number of papers out in smoking asthmatics. In fact, the first clinical follow-up study to show the effect of smoking on lung function decline in asthmatic patients was only recently published (Tommola M, et al. Eur Respir J 2016). Before that, landmark population-based studies have been published by Prof. Lange and his group (a very relevant one in AJRCCM 2015 by Colak Y et al.). Furthermore, ref 4, even though a landmark study in its field, describes increased asthma risk in smokers and not exactly those described in first sentence. Of relevance to the current study (smoking and adult-onset asthma), Tuomisto L, et al. (Respir Med 2016) recently reported that smoking is associated with uncontrolled asthma in patients with adult-onset asthma. Recently, the effects of smoking in asthma have been reviewed by Thomson and Polosa in Eur

	Respir J and I recommend that as a reading and reference. It can possibly replace some of the old references. 2. Even though I highly agree with the authors that patients with adult-onset asthma (younger and older) are a highly important target group to study the effects of smoking, it is a common (mis)belief that asthma generally starts in childhood. In fact, recently, evidence has been published to state that adult-onset asthma is very common (Sood A, et al. Ann Am Thorac Soc 2013 and Kankaanranta H, et al. JACI Practice 2017) and these studies form the logical basis for the selection of a group of patients with adult-onset asthma. In addition, the recent division of asthma to different phenotypes with childhood and adult-onset (e.g. the smoking associated neutrophilc asthma) deserves to be described in the introduction. 3. Table 1. BMI needs to be reported in these groups. 4. Table 2. Are the reported values per 1 cigarette or 1 year or 1 pack-year? Probably a cutpoint was used and it should be reported i.e. the methodology should be explained better. 5. The authors adjusted their models with age and sex usually, but not with BMI. As BMI affects especially FVC, I would consider that significant thing to add to the analysis. In addition, data on FVC should be reported. 6. Discussion and results on AHR and pack-years. The OR for AHR with >4 pack-years is 2.02 (p=0.080). Given that there is only 38 patients in that group, I would consider the small n-value as the reason why pack-years do not appear significant. Thus, my conclusion is that, rather than being opposite to what Juusela and co-workers reported, the current study supports their findings (with even lower pack-year history). This could be more clearly discussed. 7. Discussion on smoking and eosinophilia. Refs 26-28 are not exactly studies in clinical asthma (as the current study is). More relevant to the current study (smoking, eosinophils and adult-onset asthma) are those e.g. by Tommola M, et al. Eur Respir J 2017 and Ilmarinen P, et al. JACI Practice 2017). 8. Discussion (general). The findings of this study enlarge our current knowledge by suggesting that even lower number smoked cigarettes /pack-years is associated with lower lung function in adult asthma. Even though this is not a follow-up study, it can be seen as a continuum in the lower end of smoking with that data obtained from clinical cohorts or population based studies reporting lung function decline in adults patients with asthma with relevant smoking history (Tommola M, et al. ERJ 2016; 10 pack-year and above and Colak Y, et al. AJRCCM 2015; 13 and 26 mean pack-year history). This could be more clearly discussed. 9. Major weaknesses: The authors used bronchodilatation response and/or hyperreactivity as inclusion criteria. However, the same variables are also outcome measures. Clinically this makes sense, but creates a possible bias where use of the outcome variable as inclusion criteria may limit the generalizability of the findings. This possible bias should be discussed. 10. Fig 2 A-C and Tables. The authors report FEV1/FVC ratio (%). As it is somewhat unclear whether this means FEV1/FVC x 100 or FEV1/FVC ratio (% predicted) I would suggest using crude FEV1/FVC ratio given as 0.xx Minor: 1. Discussion: Juusela, not Jussella (ref 25).

REVIEWER	Dr. Malcolm Sears McMaster University Ontario,Canada
REVIEW RETURNED	24-Apr-2018

GENERAL COMMENTS	The authors have retrospectively reviewed pulmonary function records from 2004-2011, identifying patients aged 20-34 years with intermittent asthma of adult onset, with no treatment, and assessed lung function and airway responsiveness among those who were never smokers and current smokers. They report that even in smokers with a relatively short history, and total consumption less than 10 pack years, there is abnormal lung function not fully responsive to bronchodilator and increased airway responsiveness. I have a number of questions 1. Adult onset asthma, especially in the early adult years, is uncommon, as the majority of young adults with asthma have a childhood history. The investigators stated that they excluded those with childhood asthma, and the mean age of onset of asthma is stated to be 25 years, only 3 years before the average age at the time of study of 28 years. Do the authors have any way of being absolutely certain that none of these individuals had childhood asthma, which, as they note, may well lead to impaired lung function as young adults and impaired bronchodilator reversibility? 2. It would be of interest to know how many individuals (of the 7291 screened) were in the age group 20-34 years and of those, how many had a childhood history and were therefore excluded from this analysis. If that number is substantial this would provide more reassurance that childhood asthma was indeed excluded. 3. Page 9, line 54, the authors refer to “increased” AHR, defined as a PC20 less than 2 mg/mL, In fact all individuals of adult age with PC20 < 8 mg/mL have increased airway responsiveness, and it would be better to substitute “marked AHR” as they use in table 3. All subjects with PC20 < 8 have AHR, but PC20 < 2 is a useful cut point for more severe or marked AHR. 4. One of the most interesting findings to me is that the number of cigarettes smoked per day is the strongest predictor of marked airway hyper responsiveness, as shown in table 3. Looking at the results overall it would seem that the cumulative exposure to cigarette smoke and duration of smoking have an impact on lung function as measured by standard spirometric measures, but it is the current exposure in terms of number of cigarettes per day which impacts airway responsiveness. This suggests that airway responsiveness is impacted by acute inflammatory stimuli in cigarette smoke, whereas the persistent abnormality of lung function is more a feature of chronic exposure with a degree of airway remodeling. This point could be stated more explicitly. 5. Page 18, line 32, please clarify that you are referring to your present study when you say “patients with a history of childhood asthma were excluded” as otherwise a reader may infer that the statement refers to the Hancox paper referenced in the first half of the sentence. It would be better to state “patients with a history of childhood asthma were excluded from our study”

	6. Page 19, line 26, I would rephrase this sentence “This finding might be explained….” as this is somewhat speculative. It is certainly a possibility but there are no data in this paper to substantiate that. 7. Page 21, line 12, replace “it” with “we”. 8. The figures in figure 2 are quite small, although readable. Perhaps the journal can enhance the size of these figures by splitting this into 3 separate somewhat larger figures.

REVIEWER	Constantinos Koshiaris University of Oxford, United Kingdom
REVIEW RETURNED	02-Jul-2018

GENERAL COMMENTS	Thanks for giving me the opportunity to review this study. My review will give emphasis to the statistical methods and analyses used. The study examines how short term smoking can affect lung function in young adults (<35) with intermittent asthma where the authors demonstrate that even shot term smoking can be associated with decreased lung function. The study is of clinical importance but the methods of analysis and the reporting of results for some of the outcomes should be revisited. Specific comments: 1. Methods and results need to be made clearer with respect to which outcome variables were used for each analysis. For example table 2 shows the association between lung function and smoking history but it is not very clear if this analysis was done on the “pre” values or the “post” values (both in text and in table). 2. Page 10 line 15-17: “The statistical significance of differences in continuous variables between the two groups was analysed using the Mann-Whitney U test”. Was the decision to use a non-parametric test and report the median and IQR for some of the continuous variables due to the fact that these variables were not normally distributed? If that is the case state it in the methods section. 3. Page 10 line 24-27: “The association between lung function and smoking history was evaluated using analysis of covariance (ANCOVA) and multiple regression analysis”. Why use both ANCOVA and multiple linear regression? They are essentially the same method and although these methods have separate commands in statistical packages they produce the same results. Regression might be preferable in this case as it produces coefficients similar to table 2 so it can be easier to interpret. 4. Page 10 lines 29-32: Why use Bonferonni adjustment only when using the ANCOVA procedure where the association between lung function and smoking status is examined and not for the rest of the analyses (HisPC20 for example)? 5. Page 10 lines 35-39: Alternative to the paired t-test a more robust way to analyse studies with pre and post design is to use the regression approach described by Vickers and Altman which can be seen at the attached paper. This paper also elaborates on

	my comment above on why the ANCOVA approach is a regression method (https://www.bmj.com/content/323/7321/1123). 6. Page 14. Analysis reported in table 2 a. The sample size used for the analysis is not displayed. b. Confidence intervals should be reported c. It seems that predictors in this case are continuous. if that is the case it should be mentioned in the text. c. Was the analysis restricted only on smokers or the full sample? If the analysis was for the full sample then how were the non-smokers included in the model? d. Are these three separate models or one model containing all three smoking parameters? It is not very clear. Was collinearity checked for this analysis similar to the logistic regression? 7. When using regression methods with continuous predictors have you checked the model assumptions (like linearity of predictors in relation to the outcome)? Given that you report the median and IQR for some of the continuous variables it suggests that these variables are not normally distributed so transformations might be needed if assumptions are violated (might not be the case but it should be checked) 8. Page 15 line 4. I would replace the mutual linearly statistically with collinearity which is the official term for what your describing i.e. “Smoking history parameters were collinear thus they could not be included in the same model” 9. The sample size especially for the logistic regression seems small which is evident from the wide confidence intervals. It is possible that you might not have had enough power to detect some of the associations between smoking parameters and HisPC20 so I would acknowledge this in your limitations section. 10. Throughout the results section the authors report whether there is a statistically significant difference but not the direction of the association making it hard to interpret the findings. 11. The analyses presented were adjusted for age, gender and disease duration. Why the variables total IgE and eosinophil were not included in the adjusted analysis?

VERSION 1 – AUTHOR RESPONSE

RESPONSE TO COMMENTS FROM REVIEWER #1:
Reviewer Name: Hannu Kankaanranta
Institution and Country: Seinäjoki Central Hospital and University of Tampere Finland

MAJOR COMMENTS
COMMENT 1.
Introduction, first lines. This referee does not completely agree with first sentence. There is surprisingly small number of studies demonstrating these. Usually smoking patients with asthma have been excluded and there is only limited number of papers out in smoking asthmatics. In fact, the first clinical follow-up study to show the effect of smoking on lung function decline in asthmatic patients was only recently published (Tommola M, et al. Eur Respir J 2016). Before that, landmark population-based studies have been published by Prof. Lange and his group (a very relevant one in AJRCCM 2015 by Colak Y et al.). Furthermore, ref 4, even though a landmark study in its field, describes increased

asthma risk in smokers and not exactly those described in first sentence. Of relevance to the current study (smoking and adult-onset asthma), Tuomisto L, et al. (Respir Med 2016) recently reported that smoking is associated with uncontrolled asthma in patients with adult-onset asthma. Recently, the effects of smoking in asthma have been reviewed by Thomson and Polosa in Eur Respir J and I recommend that as a reading and reference. It can possibly replace some of the old references.

RESPONSE 1.
Thank you for your valuable comments.
In accordance with your suggestion, we have made the following changes in the clean manuscript (lines 56 to 59).
(Original)
~~'Although many studies have shown that long-term cigarette smoking by patients with asthma is associated with the worsening of symptoms, an accelerated decrease in the lung function, and an increase in small airway remodeling, [1-4] these studies included smokers with a long-term smoking history.'~~
(Revised)
'Very few studies have demonstrated the effects of smoking on lung function in adult patients with asthma. Because smokers with asthma are generally excluded from surveys to avoid affecting results by including chronic obstructive pulmonary disease (COPD), there are limited number of studies including this patient population.[1-4]'

Accordingly, we have replaced references 1–4 (lines 372 to 384) in the clean manuscript.
(Original)
~~1. Lange P, Parner J, Vestbo J, et al. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med 1998;339:1194–200 doi:10.1056/NEJM199810223391703.~~
~~2. Apostol GG, Jacobs DR Jr, Tsai AW, et al. Early life factors contribute to the decrease in lung function between ages 18 and 40: the Coronary Artery Risk Development in Young Adults study. Am J Respir Crit Care Med 2002;166:166–72 doi:10.1164/rccm.2007035.~~
~~3. St-Laurent J, Bergeron C, Page N, et al. Influence of smoking on airway inflammation and remodelling in asthma. Clin Exp Allergy 2008;38:1582–9 doi:10.1111/j.1365-2222.2008.03032.x [published Online First: 3 August 2003].~~
~~4. Polosa R, Knoke JD, Russo C, et al. Cigarette smoking is associated with a greater risk of incident asthma in allergic rhinitis. J Allergy Clin Immunol 2008;121:1428–34 doi:10.1016/j.jaci.2008.02.041 [published Online First: 25 April 2008].~~

(Revised)
1. Polosa R, Thomson NC. Smoking and asthma: dangerous liaisons. Eur Respir J 2013;41:716–26 doi:10.1183/09031936.00073312 [published Online First: 16 August 2012].
2. Çolak Y, Afzal S, Nordestgaard BG, et al. Characteristics and Prognosis of Never-Smokers and Smokers with Asthma in the Copenhagen General Population Study. A Prospective Cohort Study. Am J Respir Crit Care Med 2015;192:172–81 doi:10.1164/rccm.201502-0302OC.
3. Tommola M, Ilmarinen P, Tuomisto LE, et al. The effect of smoking on lung function: a clinical study of adult-onset asthma. Eur Respir J 2016;48:1298–306 doi:10.1183/13993003.00850-2016 [published Online First: 22 September 2016].
4. Tuomisto LE, Ilmarinen P, Niemelä O, et al. A 12-year prognosis of adult-onset asthma: Seinäjoki Adult Asthma Study. Respir Med 2016;117:223–9 doi:10.1016/j.rmed.2016.06.017 [published Online First: 23 June 2016].

COMMENT 2.
Even though I highly agree with the authors that patients with adult-onset asthma (younger and older) are a highly important target group to study the effects of smoking, it is a common (mis)belief that asthma generally starts in childhood. In fact, recently, evidence has been published to state that adult-onset asthma is very common (Sood A, et al. Ann Am Thorac Soc 2013 and Kankaanranta H, et al. JACI Practice 2017) and these studies form the logical basis for the selection of a group of patients with adult-onset asthma. In addition, the recent division of asthma to different phenotypes with childhood and adult-onset (e.g. the smoking associated neutrophilc asthma) deserves to be described in the introduction.

RESPONSE 2.
Thank you for your valuable comments.

In accordance with your suggestion, we have added the following sentences (lines 61 to 70) in the clean manuscript.
'Generally, asthma is recognised as a disease that develops in childhood, although adult-onset asthma reportedly appears as a dominant phenotype in women by the age of 40 years.[5] Differentiation of severe asthmatics on the basis of the age at onset identifies the phenotypes of asthma.[6] Patients with late- or adult-onset asthma are frequently non-atopic and exhibit lower lung function than do patients with early- or childhood-onset asthma, even though the disease duration is shorter in the former than in the latter.[7] Another study identified five clusters of adult-onset asthma: smoking, obesity-related, female, atopic, and nonrhinitic asthma. The authors found that smoking and obesity-related asthma showed the poorest outcomes and maximum unmet needs in terms of treatment.[8] Thus, investigation of the effects of smoking in patients with adult-onset asthma is clinically meaningful.'

Accordingly, we have added the following references.
5. Sood A, Qualls C, Schuyler M, et al. Adult-onset asthma becomes the dominant phenotype among women by age 40 years. The longitudinal CARDIA study. Ann Am Thorac Soc 2013;10:188–97 doi:10.1513/AnnalsATS.201212-115OC.
6. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;18:716–25 doi:10.1038/nm.2678.
7. Miranda C, Busacker A, Balzar S, et al. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. J Allergy Clin Immunol 2004;113:101–8 doi:10.1016/j.jaci.2003.10.0418.
8. Ilmarinen P, Tuomisto LE, Niemelä O, et al. Cluster Analysis on Longitudinal Data of Patients with Adult-Onset Asthma. J Allergy Clin Immunol Pract 2017;5:967–78.e3 doi:10.1016/j.jaip.2017.01.027 [published Online First: 25 April 2017].

COMMENT 3.
Table 1. BMI needs to be reported in these groups.

RESPONSE 3.
Thank you for your valuable comment.
We have added BMI in Table 1, Table S1, and Table S2. We have also added it as an adjustment factor (covariate) in the subsequent multivariate analyses (Table 2, Table 3).

COMMENT 4
Table 2. Are the reported values per 1 cigarette or 1 year or 1 pack-year? Probably a cutpoint was used and it should be reported i.e. the methodology should be explained better.

RESPONSE 4.
Thank you for your valuable comments.
In the original manuscript, the reported values in Table 2 were per 1 cigarette, 1 year, or 1 pack-year. In accordance with the comments of the other reviewers, the ANCOVA analysis shown in Figure 2 in the original manuscript has been changed to multiple linear regression analysis. Accordingly, we have deleted the original Table 2 and Figure 2 and added a revised Table 2, which is similar to the original Table 2.

COMMENT 5.
The authors adjusted their models with age and sex usually, but not with BMI. As BMI affects especially FVC, I would consider that significant thing to add to the analysis. In addition, data on FVC should be reported.
RESPONSE 5.
Thank you for your valuable comments.
In accordance with your suggestion, we have added BMI as an adjustment factor in our multivariate analyses (Table 2 and Table 3).
With regard to FVC data, we regret to inform you that we do not have post-bronchodilator FVC data recorded in our database. The medical charts of patients are generally kept for 5 years from the time of recording, so there is no way we can access those records again. Therefore, we have only added pre-bronchodilator FVC data in revised Table 1, Table S1, and Table S2.
Moreover, we added the following comment in the footnote of Table 1, Table S1, and Table S2.
'Post-bronchodilator FVC is missing value, therefore, the data is not shown.'

COMMENT 6.
Discussion and results on AHR and pack-years. The OR for AHR with >4 pack-years is 2.02 (p=0.080). Given that there is only 38 patients in that group, I would consider the small n-value as the reason why pack-years do not appear significant. Thus, my conclusion is that, rather than being opposite to what Juusela and co-workers reported, the current study supports their findings (with even lower pack-year history). This could be more clearly discussed.

RESPONSE 6.
Thank you for your valuable comments.
In accordance with your suggestion, we have made the following chnages in the clean manuscript (lines 308 to 312).
(Original)
~~'Compared with the study by Juusela et al., our study included younger patients with intermittent asthma and a short smoking history (median age [IQR], 28.0 [26.0–30.0] years; median pack-years [IQR], 4.0 [2.0–6.3] years). Therefore, the number of pack-years was not significantly associated with AHR below 2 mg/mL of HisPC20 in the present study.'~~

(Revised)
'The >4 pack year group (n = 38) in our study did not show a significant OR for marked AHR (OR, 1.96 [95% CI, 0.90–4.26], p = 0.09); however, we believe the lack of significance was due to the small n-value. Therefore, our finding regarding the association between pack-years and marked AHR is considered to be consistent with the findings of Juusela et al.[26]'

COMMENT 7.
Discussion on smoking and eosinophilia. Refs 26-28 are not exactly studies in clinical asthma (as the current study is). More relevant to the current study (smoking, eosinophils and adult-onset asthma) are those e.g. by Tommola M, et al. Eur Respir J 2017 and Ilmarinen P, et al. JACI Practice 2017).

RESPONSE 7.
Thank you for your valuable comments. We have replaced the original references 26 to 28 with the ones suggested by you (line 393 and 450).
8. Ilmarinen P, Tuomisto LE, Niemelä O, et al. Cluster Analysis on Longitudinal Data of Patients with Adult-Onset Asthma. J Allergy Clin Immunol Pract 2017;5:967–978.e3 doi:10.1016/j.jaip.2017.01.027 [published Online First: 25 April 2017].
24. Tommola M, Ilmarinen P, Tuomisto LE, et al. Differences between asthma-COPD overlap syndrome and adult-onset asthma. Eur Respir J 2017;49. pii: 1602383 doi:10.1183/13993003.02383-2016.

In addition, we have changed the corresponding sentences in the clean manuscript (lines 285 to 290).
'The reason for increased peripheral blood cells in smokers with asthma remains unknown. In a previous cluster analysis,[8] a cluster of patients with adult-onset asthma and the strongest history of smoking (smoking asthma) was identified, and this was the only group that showed no decrease in blood eosinophils from diagnosis to the 12-year follow-up. On the other hand, patients with asthma–COPD overlap in another study showed higher blood neutrophil levels than did patients with adult-onset asthma.[24]'

COMMENT 8.
Discussion (general). The findings of this study enlarge our current knowledge by suggesting that even lower number smoked cigarettes /pack-years is associated with lower lung function in adult asthma. Even though this is not a follow-up study, it can be seen as a continuum in the lower end of smoking with that data obtained from clinical cohorts or population based studies reporting lung function decline in adults patients with asthma with relevant smoking history (Tommola M, et al. ERJ 2016; 10 pack-year and above and Colak Y, et al. AJRCCM 2015; 13 and 26 mean pack-year history). This could be more clearly discussed.

RESPONSE 8.
Thank you for your valuable comments. In accordance with your suggestion, we have made the necessary changes in the clean manuscript (lines 262 to 275).

'Our study showed the effects of short-term smoking (<10 pack-years) in patients with adult-onset asthma and revealed that a cumulative smoking history of <10 pack years was associated with lower

lung function not fully responsive to bronchodilator and marked AHR. Epidemiological evidence suggests that a smoking history of ≥10 pack-years causes an accelerated decline in lung function in patients with adult asthma.[2,3] Elderly current smokers with asthma who have a prolonged high pack-years (mean ± SD, 41 ± 23) comprise a population that is at high risk of severe or life-threatening disease exacerbation, regardless of the relatively small disease duration.[23] Although our study is not a longitudinal study, it can be considered to be at the lower end of a continuum of studies reporting lung function declines in adult patients with asthma and a relevant smoking history. Even after the inhalation of a bronchodilator, there was a significant difference in lung function between the never smokers and current smokers in our study. This finding indicates that even a short smoking duration (<10 pack-years) is associated with a future risk of persistent airflow limitation.'

References

2. Çolak Y, Afzal S, Nordestgaard BG, et al. Characteristics and Prognosis of Never-Smokers and Smokers with Asthma in the Copenhagen General Population Study. A Prospective Cohort Study. Am J Respir Crit Care Med 2015;192:172–81 doi:10.1164/rccm.201502-0302OC.
3. Tommola M, Ilmarinen P, Tuomisto LE, et al. The effect of smoking on lung function: a clinical study of adult-onset asthma. Eur Respir J 2016;48:1298–306 doi:10.1183/13993003.00850-2016 [published Online First: 22 September 2016].

COMMENT 9.
Major weaknesses: The authors used bronchodilatation response and/or hyperreactivity as inclusion criteria. However, the same variables are also outcome measures. Clinically this makes sense, but creates a possible bias where use of the outcome variable as inclusion criteria may limit the generalizability of the findings. This possible bias should be discussed.

RESPONSE 9.
Thank you for your valuable comments.
We added the following sentence in the limitations paragraph (lines 324 to 326 in the clean manuscript. 'Second, bronchodilator response and/or AHR were used as both inclusion criteria and outcome variables, which could have led to bias that may limit the generalisability of the findings.'

COMMENT 10.
Fig 2 A-C and Tables. The authors report FEV1/FVC ratio (%). As it is somewhat unclear whether this means FEV1/FVC x 100 or FEV1/FVC ratio (% predicted) I would suggest using crude FEV1/FVC ratio given as 0.xx
RESPONSE 10.
Thank you for your valuable comments.
In accordance with your suggestion, we have included a crude FEV_1/FVC ratio given as 0.xx in the revised Tables 1, S1, S2, and 2 (The original Figure 2 and Table 2 have been deleted and a revised Table 2 has been added in this version of the manuscript).

MINOR COMMENT:
COMMENT 1.
Discussion: Juusela, not Jussella (ref 25).

RESPONSE 1.
We apologise for the typographical error. We have rectified it at all relevant instances in the revised manuscript.

We appreciate your review and valuable suggestions that have helped us in improving our paper to a considerable extent.

RESPONSE TO COMMENTS FROM REVIEWER #2:
Reviewer Name: Dr. Malcolm Sears
Institution and Country: McMaster University, Ontario,Canada

COMMENT 1.
Adult onset asthma, especially in the early adult years, is uncommon, as the majority of young adults with asthma have a childhood history. The investigators stated that they excluded those with childhood

asthma, and the mean age of onset of asthma is stated to be 25 years, only 3 years before the average age at the time of study of 28 years. Do the authors have any way of being absolutely certain that none of these individuals had childhood asthma, which, as they note, may well lead to impaired lung function as young adults and impaired bronchodilator reversibility?

RESPONSE 1.
Thank you for your valuable comments.
We have added the following sentence in the Methods section (lines 96 to 98).
'The parents of majority of the patients were not present at the time of examination, so we could only record a surveyed self-reported history of childhood asthma.'
We have also added the following sentence in the limitations paragraph (lines 340 to 343).
'Sixth, although we carefully determined the smoking history and history of childhood asthma, both were self-reported by the patients. Therefore, there is a possibility that patients with subclinical childhood asthma were included in this study.'

COMMENT 2.
It would be of interest to know how many individuals (of the 7291 screened) were in the age group 20-34 years and of those, how many had a childhood history and were therefore excluded from this analysis. If that number is substantial this would provide more reassurance that childhood asthma was indeed excluded.

RESPONSE 2.
Thank you for your valuable comments.
We have modified Figure 1 to show the total number of individuals (of the 7291 screened) and those with a history of childhood asthma in the age group of 20–34 years.
Our department deals with adult patients with respiratory and allergic conditions, and according to the medical system in Japan, only patients aged >16 years can visit our department. Therefore, the ratio shown in Figure 1 could be different from that for the general population.
We diagnosed chronic cough using The Japanese Respiratory Society guidelines for the management of cough [1]. Atopic cough is a condition presenting with chronic bronchodilator-resistant non-productive cough, and patients exhibit global atopic tendency and airway cough hypersensitivity without non-specific bronchial hyperresponsiveness [2]. Although we conduct a bronchodilator reversibility test during the course of examinations for chronic cough, we mainly conduct this test when we suspect asthma. Therefore, the rate of asthma in Figure 1 (64%) accounts for a lot compared with other epidemiological data on chronic cough [3, 4]. Moreover, our hospital is a tertiary care hospital and provides examinations for refractory cough. Therefore, 'others' include psychogenic or environmental factor-associated cough [4, 5].

References in RESPONSE 2
1. Kohno S, Ishida T, Uchida Y, et al. The Japanese Respiratory Society guidelines for management of cough. Committee for the Japanese Respiratory Society Guidelines for Management of Cough. Respirology 2006;11:S135–86.
2. Fujimura M, Ogawa H, Yasui M, et al. Eosinophilic tracheobronchitis and airway cough hypersensitivity in chronic non-productive cough. Clin Exp Allergy 2000;30:41–7.
3. Niimi A, Nguyen LT, Usmani O, et al. Reduced pH and chloride levels in exhaled breath condensate of patients with chronic cough. Thorax 2004;59:608–12.
4. Matsumoto H, Niimi A, Takemura M, et al. Prevalence and clinical manifestations of gastro-oesophageal reflux-associated chronic cough in the Japanese population. Cough 2007;3:1–4.
5. Aaron SD, Boulet LP, Reddel HK, Gershon A. Under-diagnosis and Over-diagnosis of Asthma Am J Respir Crit Care Med 2018 doi:10.1164/rccm.201804-0682CI [Epub ahead of print].

COMMENT 3.
Page 9, line 54, the authors refer to 'increased' AHR, defined as a PC20 less than 2 mg/mL, In fact all individuals of adult age with PC20 < 8 mg/mL have increased airway responsiveness, and it would be better to substitute 'marked AHR' as they use in table 3. All subjects with PC20 < 8 have AHR, but PC20 < 2 is a useful cut point for more severe or marked AHR.

RESPONSE 3.
Thank you for your valuable suggestion.
We have replaced 'increased' AHR to 'marked' AHR as the term representing a PC20 value of <2 mg/mL

at all relevant instances in the manuscript.

COMMENT 4.
One of the most interesting findings to me is that the number of cigarettes smoked per day is the strongest predictor of marked airway hyper responsiveness, as shown in table 3. Looking at the results overall it would seem that the cumulative exposure to cigarette smoke and duration of smoking have an impact on lung function as measured by standard spirometric measures, but it is the current exposure in terms of number of cigarettes per day which impacts airway responsiveness. This suggests that airway responsiveness is impacted by acute inflammatory stimuli in cigarette smoke, whereas the persistent abnormality of lung function is more a feature of chronic exposure with a degree of airway remodeling. This point could be stated more explicitly.

RESPONSE 4.
Thank you for your valuable comments.
In accordance with your suggestion, we have added the following sentences in the Results section (lines 236 to 239).
'Among the smoking parameters, only the number of cigarettes per day remained significant. The odds ratio (OR) for marked AHR in the ≥11 cigarettes/day group was 2.23 (95% confidence interval [CI], 1.03–4.80). Moreover, the association between the number of cigarettes per day and marked AHR showed the highest chi-square value.'
We have also added the following sentences in the Discussion section (lines 296 to 303).
'Intriguingly, only the number of cigarettes per day, and not the duration of smoking and cumulative consumption, was a significant predictor of marked AHR (HisPC20 < 2 mg/mL) in our study, even after adjusting for age, sex, disease duration, and BMI. Moreover, the association between the number of cigarettes per day and marked AHR showed the highest chi-square value. These findings suggest that the number of cigarettes smoked per day has a greater effect on AHR compared with the smoking duration. AHR could be influenced by acute inflammatory stimuli with daily cigarette smoke, whereas persistent airflow limitation may be better associated with chronic exposure.'

COMMENT 5
Page 18, line 32, please clarify that you are referring to your present study when you say 'patients with a history of childhood asthma were excluded' as otherwise a reader may infer that the statement refers to the Hancox paper referenced in the first half of the sentence. It would be better to state 'patients with a history of childhood asthma were excluded from our study'

RESPONSE 5.
Thank you for pointing this out. During the process of revision, the original sentence was entirely changed (lines 316 to 320).
'The strength of our study is that we focused on adult-onset asthma with short smoking history (<10 pack-years) and excluded patients with childhood asthma, which is a risk factor for persistent airflow limitation, to evaluate the direct effects of smoking on lung function,[28] even though Hancox et al.[29] found no evidence of additive or multiplicative effects of childhood-persistent asthma on airflow obstruction.'

COMMENT 6.
Page 19, line 26, I would rephrase this sentence 'This finding might be explained….' as this is somewhat speculative. It is certainly a possibility but there are no data in this paper to substantiate that.

RESPONSE 6.
Thank you for your valuable comments.
In accordance with your suggestion, we have modified the phrase (lines 277 to 279) in the clean manuscript.
'This finding suggests that the smoking duration can have a profound effect on lung function when compared with the other smoking parameters (cigarettes per day and pack-years).'

COMMENT 7.
Page 21, line 12, replace 'it' with 'we'.

RESPONSE 7.
Thank you for your suggestion. We have made the necessary replacement in the revised manuscript

and during the process of revision, the original sentence was entirely changed (line 295).
'however, the number of patients with non-atopic asthma was too small for statistical analysis.'

COMMENT 8.
The figures in figure 2 are quite small, although readable. Perhaps the journal can enhance the size of these figures by splitting this into 3 separate somewhat larger figures.

RESPONSE 8.
Thank you for your valuable suggestions.

For better representation of the results and in accordance with comments concerning Figure 2 from the other reviewers, we have deleted Figure 2 and added a revised Table 2.

We appreciate your review and valuable suggestions that have helped us in improving our paper to a considerable extent.

RESPONSE TO COMMENTS FROM REVIEWER #3:
Reviewer Name: Constantinos Koshiaris
Institution and Country: University of Oxford, United Kingdom

COMMENT 1.
Methods and results need to be made clearer with respect to which outcome variables were used for each analysis. For example table 2 shows the association between lung function and smoking history but it is not very clear if this analysis was done on the 'pre' values or the 'post' values (both in text and in table).

RESPONSE 1.
Thank you for your valuable comments. We apologise for the confusion. We have now modified the results of lung function so that the variables, i.e. pre- or post-bronchodilator values, are clear. We have also revised Tables 1, S1, S2, and 2.
In addition, we have changed the outcome to 'post-bronchodilator lung function' at all relevant instances in the revised manuscript.

COMMENT 2.
Page 10 line 15-17: 'The statistical significance of differences in continuous variables between the two groups was analysed using the Mann-Whitney U test'. Was the decision to use a non-parametric test and report the median and IQR for some of the continuous variables due to the fact that these variables were not normally distributed? If that is the case state it in the methods section.

RESPONSE 2.
Thank you for your valuable comments.
In accordance with your suggestions, we have made the necessary changes in the clean manuscript (lines 166 to 169).
'The statistical significance of differences in normally and non-normally distributed continuous variables among the three groups was analysed using analysis of variance (ANOVA) and the Kruskal–Wallis test, respectively.'

COMMENT 3
Page 10 line 24-27: 'The association between lung function and smoking history was evaluated using analysis of covariance (ANCOVA) and multiple regression analysis'. Why use both ANCOVA and multiple linear regression? They are essentially the same method and although these methods have separate commands in statistical packages they produce the same results. Regression might be preferable in this case as it produces coefficients similar to table 2 so it can be easier to interpret.

RESPONSE 3.
Thank you for your valuable comments.
We have deleted Figure 2 and added a revised Table 2 to demonstrate the findings of multiple linear regression analysis.
Only the association between post-bronchodilator lung function and the smoking parameter is shown

in the revised Table 2, because post-bronchodilator lung function is considered more important than pre-bronchodilator lung function in clinical practice.[1] Findings regarding the association between pre-bronchodilator lung function and the smoking parameter were similar to those regarding the association between post-bronchodilator lung function and the smoking status.

Reference in RESPONSE 3

1. Brehm JM, Man Tse S, Croteau-Chonka DC, et al. A Genome-Wide Association Study of Post-bronchodilator Lung Function in Children with Asthma. Am J Respir Crit Care Med 2015;192:634–7 doi:10.1164/rccm.201501-0047LE.

COMMENT 4.

Page 10 lines 29-32: Why use Bonferonni adjustment only when using the ANCOVA procedure where the association between lung function and smoking status is examined and not for the rest of the analyses (HisPC20 for example)?

RESPONSE 4.

Thank you for your valuable comments.

We have now eliminated ANCOVA (original Figure 2) and retained only multiple linear regression analyses without Bonferroni adjustment (revised Table 2).

COMMENT 5.

Page 10 lines 35-39: Alternative to the paired t-test a more robust way to analyse studies with pre and post design is to use the regression approach described by Vickers and Altman which can be seen at the attached paper. This paper also elaborates on my comment above on why the ANCOVA approach is a regression method (https://www.bmj.com/content/323/7321/1123).

RESPONSE 5.

Thank you for your advice regarding an excellent and robust analysis technique.

With reference to your suggested method, we analyzed differences between pre- and post-bronchodilator lung function using post-bronchodilator lung function as the dependent variable, each smoking parameter as an independent variable, and pre-bronchodilator lung function, age, sex, disease duration, and BMI as covariates.

As a result, the partial regression coefficient for each smoking parameter was not significant. That is, no smoking parameter was a significant factor affecting the difference between pre- and post-bronchodilator lung function. In other words, the effect of bronchodilator treatment was not correlated with the smoking history. As shown by ΔFEV1 (%) in the revised Table 1, the change in lung function after bronchodilator use was not significant, consistent with the finding obtained by using your suggested method.

The p-value for ΔFEV1 in Table 1 is approximately 0.1, and there is a possibility of it becoming significant with an increase in the number of subjects. Moreover, we believe that ΔFEV_1 tends to be larger for smokers than for never smokers in a group of subjects with a very short smoking history, such as those in our study. This is because baseline function is lower in current smokers than in never smokers; moreover, current smokers with a short smoking history respond better to bronchodilator treatment.

Our analyses using paired t-tests is misleading. In terms of clinical practice, the intergroup difference (‘lung function of smokers is lower than that of never smokers even after bronchodilator use’) is more important than the amount of change after bronchodilator use. Lower lung function even after bronchodilator treatment is called persistent airflow limitation,[1] which is one of the most serious problems in asthma.

On the basis of these concepts, we have prepared the revised table 2 without pre and post comparisons.

Thank you very much for pointing out the risk of comparing pre and post values by using only paired t-tests.

In order to avoid confusion, and because this study is not focused on the effects of bronchodilators, we have deleted the description of pre and post comparisons. However, we would surely like to implement your suggested method in future studies comparing values before and after treatment.

Reference in RESPONSE 5

1. Lee JH, Haselkorn T, Borish L, et al. Risk factors associated with persistent airflow limitation in severe or difficult-to-treat asthma: insights from the TENOR study. Chest 2007;132:1882–9.

COMMENT 6.
Page 14. Analysis reported in table 2
a. The sample size used for the analysis is not displayed.
b. Confidence intervals should be reported
c. It seems that predictors in this case are continuous. if that is the case it should be mentioned in the text.
d. Was the analysis restricted only on smokers or the full sample? If the analysis was for the full sample then how were the non-smokers included in the model?
e. Are these three separate models or one model containing all three smoking parameters? It is not very clear. Was collinearity checked for this analysis similar to the logistic regression?

Thank you for your valuable comments.
RESPONSE 6-a, b, c
As mentioned in previous responses, the original figure 2 has been deleted and a revised Table 2 showing the findings of multiple linear regression analysis has been added. The revised Table 2 was similar to original Table 2, which has now been deleted.
Your advice was very useful for preparation of the revised Table 2, where we have incorporated your suggestions.

RESPONSE 6-d
As mentioned in previous responses, the original figure 2 has been deleted and a revised Table 2 showing the findings of multiple linear regression analysis has been added. The revised Table 2 was similar to original Table 1, which has now been deleted.
The original Table 2 included full samples (never smokers with asthma and current smokers with asthma). Never smokers were defined as those with 0 cigarettes per day, 0 years of smoking, and 0 pack-years.
We agree that your suggested method of multiple linear regression analysis is appropriate for the analysis of subjects, including never smokers.

RESPONSE 6-e
We apologise for the ambiguity.
In the original Table 2, three separate models were presented. Because smoking parameters have strong collinearity, we used three separate models that included the number of cigarettes per day, duration of smoking, and pack-years, respectively, as independent variables.
Your advice was very useful for preparation of the revised Table 2. We have mentioned the following in the table footnote.
'Each lung function parameter was separately analysed after adjustment for sex, age, disease duration (log transformed), and body mass index.'

COMMENT 7.
When using regression methods with continuous predictors have you checked the model assumptions (like linearity of predictors in relation to the outcome)? Given that you report the median and IQR for some of the continuous variables it suggests that these variables are not normally distributed so transformations might be needed if assumptions are violated (might not be the case but it should be checked)

RESPONSE 7.
Thank you for your valuable comments.
The smoking history parameters were collinear, so they could not be included in the same model.
The variable 'duration of asthma' was not normally distributed; therefore, we used logarithmic transformation for this variable.
With regard to the validity of the model, we checked the normality of the residuals and confirmed a normal distribution.
The variables 'total IgE value' and 'peripheral blood eosinophil count' were not normally distributed; data with logarithmic transformation show more normal distribution. However, they were not included in the regression analysis. The logarithmically converted values made it difficult to determine the actual values. Accordingly, we have shown actual values in the revised Table 1.
HisPC20 was categorised as ≥2 mg/mL and <2 mg/mL for multivariate logistic regression analysis.
Finally, we have added the following sentence in the Methods section (lines 174 to 175).
'Non-normally distributed covariates were log transformed. Moreover, we validated the models by

confirming that the residuals were normally distributed.'

COMMENT 8.
Page 15 line 4. I would replace the mutual linearly statistically with collinearity which is the official term for what your describing i.e. 'Smoking history parameters were collinear thus they could not be included in the same model'

RESPONSE 8.
Thank you for your valuable comments. In accordance with your suggestion, we have made the necessary changes (line 170 to 171) in the clean manuscript.
(Original)
'~~Since each parameter of smoking history (number of cigarettes per day, smoking duration, and pack-year) has mutual linearity statistically, simultaneous input could not be done.~~'
(Revised)
'The smoking parameters were collinear, so they could not be included in the same model.'

COMMENT 9.
The sample size especially for the logistic regression seems small which is evident from the wide confidence intervals. It is possible that you might not have had enough power to detect some of the associations between smoking parameters and HisPC20 so I would acknowledge this in your limitations section.

RESPONSE 9.
Thank you for your valuable comments. In accordance with your suggestion, we have added the following sentences in the limitations paragraph (lines 343 to 345).
'Finally, the sample size was small. Therefore, our study may not have had enough power for the detection of some associations between smoking parameters and lung function and between smoking parameters and marked AHR.'

COMMENT 10.
Throughout the results section the authors report whether there is a statistically significant difference but not the direction of the association making it hard to interpret the findings.

RESPONSE 10.
Thank you for your valuable comments.
In order to clarify the relationship between each smoking parameter and lung function and between each smoking parameter and airway hyperresponsiveness, we have provided clear findings in Tables 2 and 3.
Furthermore, we have only focused on the most important findings and deleted the rest in the Results section.

COMMENT 11.
The analyses presented were adjusted for age, gender and disease duration. Why the variables total IgE and eosinophil were not included in the adjusted analysis?

RESPONSE 11.
Thank you for your valuable comments.
The total IgE level and eosinophil count are intermediate variables for the associations between smoking and lung function and between smoking and airway hyperresponsiveness.[1-6] Therefore, we did not include them as covariates.

References in RESPONSE 11
1. Tommola M, Ilmarinen P, Tuomisto LE, et al. Differences between asthma-COPD overlap syndrome and adult-onset asthma. Eur Respir J 2017;49. pii: 1602383 doi:10.1183/13993003.02383-2016.
2. Ilmarinen P, Tuomisto LE, Niemelä O, et al. Cluster Analysis on Longitudinal Data of Patients with Adult-Onset Asthma. J Allergy Clin Immunol Pract 2017;5:967–78.e3 doi:10.1016/j.jaip.2017.01.027 [published Online First: 25 April 2017].
3. Halonen M, Barbee RA, Lebowitz MD, et al. An epidemiologic study of interrelationships of total serum immunoglobulin E, allergy skin-test reactivity, and eosinophilia. J Allergy Clin Immunol 1982;69:221–8 doi:10.1016/0091-6749(82)90103-8.

4. Taylor RG, Gross E, Joyce H, et al. Smoking, allergy, and the differential white blood cell count. Thorax 1985;40:17–22 doi:10.1136/thx.40.1.17.
5. Jensen EJ, Pedersen B, Narvestadt E, et al. Blood eosinophil and monocyte counts are related to smoking and lung function. Respir Med 1998;92:63–9 doi:10.1016/S0954-6111(98)90034-8.
6. Sunyer J, Springer G, Jamieson B, et al. Effects of asthma on cell components in peripheral blood among smokers and non-smokers. Clin Exp Allergy 2003;33:1500–5 doi:10.1046/j.1365-2222.2003.01730.x.

VERSION 2 – REVIEW

REVIEWER	Constantinos Koshiaris University of Oxford, United Kingdom
REVIEW RETURNED	28-Aug-2018

GENERAL COMMENTS	I would like to complement the authors for the significant rework of the manuscript which has definitely improved. There are still some things that need to be addressed regarding the methods and in particular the analysis, presentation of results and interpretation: 1. The main outcome of the study is post-bronchodilator lung function. I would justify in the methods why you have decided to focus on this outcome and not for example the pre values. In both cases (pre and post) reduced lung function is observed in smokers which makes your argument stronger. 2. What is the unit of measurement for HisPC20 in table 1? It seems to be different than what the methods report. Table 1 reports it in the thousands. 3. In table 1 and in the discussion you mention the pre and post change in FEV1 but you do not mention anything about it in the methods. Since you are including it in those two instances then it should be mentioned in the methods section too. In addition you are saying in the discussion that the ΔFEV1 was higher in smokers compared to never smokers but this can be misleading as the result was not significant. I would rephrase to “The ΔFEV1 before and after bronchodilator inhalation tended to be higher for smokers than for never smokers but this was not statistically significant”. Although an increase in the sample size could make it significant we can’t know that. The best you can do is to calculate a confidence interval and comment on its width which might give you an idea of the precision of the estimated difference. If the CI is very wide then there is the possibility of a small sample size. You should also make it clear in the methods section that the change between pre and post is examining the effectiveness of bronchodilator treatment between smokers and non-smokers even if it is not the primary aim of the study. 4. Page 13. Lines 212 to 215. I would remove this particular sentence regarding the F-values. The F-value shows you whether the fitted model is better than an intercept only model. Usually if you do not have any significant variables in your model then the p.value for the F-test will not be significant. If you want to include a measure of goodness of fit for a linear model I would use the R-squared which is always displayed when doing a linear regression model for all packages.

	5. Page 15. Lines 238-239. I suggest removing this sentence and also the chi squared from table 3. The chi squared test you report is the likelihood ratio chi squared test and it has the same function as the F-test for linear regression. It tells you whether the fitted model is better than an empty model. If you want to include a goodness of fit measure I would suggest the pseudo-R2 for logistic regression. My general advice thought would be to omit measures of goodness of fit unless you want to do model comparison but this seems to be outside the scope of the paper. 6. Page 20. Line 20. Be careful with wording and generalization (comment 3). Although there is the possibility that your sample size is small this does not change the fact that the results are not significant. You could say that your results are consistent with other literature but were not significant which could be due to small number of events. 7. Page 21: Lines 330-332: consider merging these two sentences

VERSION 2 – AUTHOR RESPONSE

RESPONSE TO COMMENTS FROM REVIEWER #3:
Reviewer Name: Constantinos Koshiaris
Institution and Country: University of Oxford, United Kingdom

COMMENT 1.
The main outcome of the study is post-bronchodilator lung function. I would justify in the methods why you have decided to focus on this outcome and not for example the pre values. In both cases (pre and post) reduced lung function is observed in smokers which makes your argument stronger.

RESPONSE 1.
Thank you for your valuable comments.
In accordance with your suggestion, we added the following sentences in the clean manuscript.
(Lines 153 to 157)
'Because irreversible lower lung function is associated with a fixed airflow limitation, "post"-bronchodilator lung function is used as a marker of airway remodeling.[20] Therefore, the primary outcome was the association of "post"-bronchodilator lung function with daily smoking frequency (number of cigarettes per day), smoking duration (years), and cumulative smoking history (pack-years). '

We added the following reference as No. 20:
20.Rasmussen F, Taylor DR, Flannery EM, et al. Risk factors for airway remodeling in asthma manifested by a low postbronchodilator FEV_1/vital capacity ratio: a longitudinal population study from childhood to adulthood. Am J Respir Crit Care Med 2002;165:1480–8. doi:10.1164/rccm.2108009

COMMENT 2.
What is the unit of measurement for HisPC20 in table 1? It seems to be different than what the methods report. Table 1 reports it in the thousands.

RESPONSE 2.
We apologise for the error. We changed the values of HisPC20 to better suit the unit; "mg/mL" in Table 1, Supplement Table 1, and Supplement Table 2.

COMMENT 3.
In table 1 and in the discussion you mention the pre and post change in FEV1 but you do not mention anything about it in the methods. Since you are including it in those two instances then it should be mentioned in the methods section too.

In addition you are saying in the discussion that the ΔFEV1 was higher in smokers compared to never smokers but this can be misleading as the result was not significant. I would rephrase to “The ΔFEV1 before and after bronchodilator inhalation tended to be higher for smokers than for never smokers but this was not statistically significant”. Although an increase in the sample size could make it significant we can’t know that. The best you can do is to calculate a confidence interval and comment on its width which might give you an idea of the precision of the estimated difference. If the CI is very wide then there is the possibility of a small sample size. You should also make it clear in the methods section that the change between pre and post is examining the effectiveness of bronchodilator treatment between smokers and non-smokers even if it is not the primary aim of the study.

RESPONSE 3.
Thank you for your valuable comments.
In accordance with your suggestion, we added the following sentences in the clean manuscript.
(Lines 149 to 153)
‘Bronchial asthma is characterized by airway reversibility following bronchodilator inhalation. Airway reversibility demonstrates the effectiveness of the bronchodilator.[17] Therefore, delta FEV_1 (%) [(Post-bronchodilator FEV_1 – pre-bronchodilator FEV_1)/pre-bronchodilator FEV_1 (L) ×100], was calculated.[19]’

In accordance with your suggestion, we have made the following changes in the clean manuscript. (lines 309 to 312)
(Original)
‘The ΔFEV1 before and after bronchodilator inhalation tended to be higher for smokers than for never smokers; this was because the smoking group exhibited poor lung function at baseline.’
(Revised)
‘The ΔFEV1 before and after bronchodilator inhalation tended to be higher for smokers than for never smokers, but the difference was not statistically significant. This was because the smoking group exhibited poor lung function at baseline, but statistical significance was not shown owing to the small sample size of this study.’

COMMENT 4.
Page 13. Lines 212 to 215. I would remove this particular sentence regarding the F-values. The F-value shows you whether the fitted model is better than an intercept only model. Usually if you do not have any significant variables in your model then the p.value for the F-test will not be significant. If you want to include a measure of goodness of fit for a linear model I would use the R-squared which is always displayed when doing a linear regression model for all packages.

RESPONSE 4.
Thank you for your valuable comments.
In accordance with your suggestion, we removed the following sentences.
‘Although similar results were obtained for all three smoking parameters, we have showed the F-values for each regression equation in Table 2. The duration of smoking showed the highest F-value among the three smoking parameters.’

Instead, the proposed R-squared is shown in Table 2.
As you pointed out, measures of goodness of fit are outside the main scope of this study.
However, we needed above statistical method for responding to the comment of REVIEWER #2.
Below is the comment of REVIEWER #2:
‘One of the most interesting findings to me is that the number of cigarettes smoked per day is the strongest predictor of marked airway hyper responsiveness, as shown in table 3. Looking at the results overall it would seem that the cumulative exposure to cigarette smoke and duration of smoking have an impact on lung function as measured by standard spirometric measures, but it is the current exposure in terms of number of cigarettes per day which impacts airway responsiveness. This suggests that airway responsiveness is impacted by acute inflammatory stimuli in cigarette smoke, whereas the persistent abnormality of lung function is more a feature of chronic exposure with a degree of airway remodeling. This point could be stated more explicitly.’
Therefore, we added the following sentences in the clean manuscript.
(Lines 229 to 231)
‘Although similar results were obtained for all three smoking parameters, we have showed the R-squared for each regression equation in Table 2. The duration of smoking showed the highest R-squared among the three smoking parameters.’

Moreover, the corresponding part in the Discussion section was changed, as shown below (Lines 303 to 308 in the clean manuscript).
(Original)
The smoking duration exhibited the highest F-value in the multiple linear regression analysis adjusted for age, sex, disease duration, and BMI. This finding suggests that the smoking duration can have a profound effect on lung function when compared with the results of other smoking parameters (cigarettes per day and pack-years).
(Revised)
The smoking duration exhibited the highest R-squared in the multiple linear regression analysis adjusted for age, sex, disease duration, and BMI. This finding suggests that the smoking duration can have a profound effect on lung function when compared with the results of other smoking parameters (cigarettes per day and pack-years). However, the prediction accuracy of the models is not very high. This implies that the influence of smoking may vary among individuals.

COMMENT 5.
Page 15. Lines 238-239. I suggest removing this sentence and also the chi squared from table 3. The chi squared test you report is the likelihood ratio chi squared test and it has the same function as the F-test for linear regression. It tells you whether the fitted model is better than an empty model. If you want to include a goodness of fit measure I would suggest the pseudo-R2 for logistic regression. My general advice thought would be to omit measures of goodness of fit unless you want to do model comparison but this seems to be outside the scope of the paper.

RESPONSE 5.
Thank you for your valuable comments.
In accordance with your suggestion, we removed the following sentence:
'Moreover, the association between the number of cigarettes per day and marked AHR showed the highest chi-square value.'

Instead of reporting the chi-squared value, the proposed McFadden pseudo R-squared is shown in Table 3.

Similar to Response 4, we added the following sentences in the clean manuscript. (Lines 260 to 261 in the clean manuscript)
'Moreover, the association between the number of cigarettes per day and marked AHR showed the highest pseudo R-squared result.'

Moreover, the corresponding part in the Discussion section was changed, as shown below (Lines 329 to 333 in the clean manuscript).
(Original)
Moreover, the association between the number of cigarettes per day and marked AHR showed the highest chi-square value. These findings suggest that the number of cigarettes smoked per day has a greater effect on AHR compared with the smoking duration.
(Revised)
Moreover, the association between the number of cigarettes per day and marked AHR showed the highest pseudo R-squared. These findings suggest that the number of cigarettes smoked per day has a greater effect on AHR compared with the smoking duration. As noted earlier, it may be that the influence of smoking may vary among individuals.

COMMENT 6.
Page 20. Line 20. Be careful with wording and generalization (comment 3). Although there is the possibility that your sample size is small this does not change the fact that the results are not significant. You could say that your results are consistent with other literature but were not significant which could be due to small number of events.

RESPONSE 6.
Thank you for your valuable comments.
In accordance with your suggestion, we have made the following changes in the clean manuscript.
(Lines 342 to 344)
(Original)
'however, we believe the lack of significance was due to the small n-value. Therefore, our finding

regarding the association between pack-years and marked AHR is considered to be consistent with the findings of Juusela et al.[26]'
(Revised)
'Our finding regarding the association between pack-years and marked AHR are consistent with the finding of Juusela et al.[27] but was not significant, which could be due to the small number of events.'

COMMENT 7.
Page 21: Lines 330-332: consider merging these two sentences

RESPONSE 7.
Thank you for your valuable comments.
In accordance with your suggestion, we have made the following changes in the clean manuscript (lines 362 to 364).
(Original)
'Fourth, we did not evaluate the effects of passive smoking on the lung function of never smokers. Moreover, the effects of passive smoking on lung function in childhood were not evaluated.' (Revised)
'Fourth, we did not evaluate the effects of childhood or current passive smoking on the lung function of never smokers.'

VERSION 3 - REVIEW

REVIEWER	Constantinos Koshiaris University of Oxford United Kingdom
REVIEW RETURNED	04-Mar-2019

GENERAL COMMENTS	All of my comments have been addressed thus I recommend this paper for publication. Congratulations on the authors for the all the work they have put on the manuscript and the significant rework.