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Effects of small airway dysfunction on the clinical expression of asthma:

A focus on asthma symptoms and bronchial hyperresponsiveness

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Abstract

Background: The small airways are an important site of inflammation in asthma. However, the relation between small airway dysfunction and clinical expression of asthma have hardly been studied.

Aim: To investigate the association of small and large airway dysfunction with asthma symptoms and bronchial hyperresponsiveness (BHR).

Methods: Fifty-eight asthma patients were characterized with spirometry, body plethysmography, impulse oscillometry (IOS), alveolar and bronchial exhaled nitric oxide, and a methacholine provocation. Symptoms of nocturnal asthma, exercise-related symptoms, BHR symptoms and respiratory symptoms were assessed with the Asthma Control Questionnaire (ACQ) and Bronchial Hyperresponsiveness Questionnaire (BHQ). Perception of dyspnea was rated with the Borg score during the provocation test.

Results: Small and large airway dysfunction did not associate with higher scores for nocturnal, exercise-related or BHR symptoms. Only higher scores on *wheezing* were significantly associated with higher values of R5-R20 ($r=0.367$, $p<0.01$) and AX ($r=0.354$, $p<0.01$). Lower $FEF_{25-75\%}$ ($p=0.024$) and higher R5-R20 ($p=0.003$) values, were independently

associated with more severe BHR to methacholine, but not FEV₁ or R20 values. The increase in dyspnea during the methacholine provocation was strongly and independently correlated with the decrease in FEV₁ and X5.

Conclusion: Small and large airway dysfunction poorly associate with asthma symptoms in our patients. However, deteriorations in small airway dysfunction are strongly related to an increase in dyspnea during bronchial provocation with methacholine. Small airway dysfunction contributes also independently to the clinical expression of asthma, as reflected by the severity of BHR.

Author contributions

EvdW, DSP, LSG and TvdM contributed to data collection. EvdW performed the data analyses and EvdW, DSP, NtH and MvdB were involved in interpretation of the data. EvdW wrote the manuscript supervised by MvdB. All authors critically reviewed and approved the final version.

Conflict of interest

DSP, NtH and MvdB received a grant to the University of Groningen from Chiesi during the conduct of the study. DSP reports other to the University of Groningen from Astra Zeneca, Boehringer Ingelheim, Chiesi, Takeda, GSK and TEVA, outside the submitted work. MvdB received grants to the University of Groningen from AstraZeneca and TEVA, outside the submitted work. EvdW, TvdM, LSG have nothing to disclose.

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Key words:

Asthma

Small airways

Signs and Symptoms, Respiratory

Bronchial hyperreactivity, Bronchial hyperresponsiveness

Abbreviations

ACQ: Asthma Control Questionnaire

AX: Reactance area

BDP: Beclomethasone dipropionate

BHQ: Bronchial Hyperresponsiveness Questionnaire

BHR: Bronchial hyperresponsiveness

Calv: Alveolar concentration of eNO

CFC: Chlorofluorocarbon

FEF_{25-75%}: Forced expiratory flow at 25% to 75% of the FVC

FEF_{50%}: Forced expiratory flow at 50% of the FVC

FEV₁: Forced expiratory volume in one second

FVC: Forced vital capacity

HFA: Hydrofluoroalkane

ICS: Inhaled corticosteroids

IOS: Impulse oscillometry

JNO: bronchial flux of eNO

MBNW: Multiple breath nitrogen washout test

eNO: Exhaled nitric oxide

PC₂₀: Provocative concentration causing a 20% fall in FEV₁

PD₂₀: Provocative dose causing a 20% fall in FEV₁

R20: Resistance of the respiratory system at 20 Hertz

R5-R20: Difference between R5 and R20

RV: Residual volume

Sacin: Ventilation heterogeneity generated in the acinar lung zone

Scond: Ventilation heterogeneity generated in the conductive lung zone

TLC: Total lung capacity

X5: Reactance of the respiratory system at 5 Hertz

Introduction

Traditionally, studies investigating the clinical expression of asthma have focused on the role of the large airways and disregarded a contribution of the small airways. Nowadays, it is accepted that the small airways are an important site of airway inflammation and remodeling in asthma(1,2). In addition, some studies suggest that dysfunction of the small airways is related to more severe asthma symptoms, nocturnal asthma and exercise-induced asthma(3-6). Although the small airways have been subject of investigation during the last years, the relation between small airway dysfunction and asthma symptoms has hardly been studied. A better

understanding of how small and large airway dysfunction contribute to asthma symptoms may help to improve asthma management by more targeted therapy.

A few studies have investigated the relation between small airway dysfunction and presence of bronchial hyperresponsiveness (BHR), which is a core clinical feature of asthma(7-9). Wagner *et al* were the first to demonstrate that the small airways are sensitive to provocation with non-specific stimuli leading to an increased small airway resistance(7). Additionally, Downie *et al* showed that ventilation heterogeneity of the small airways also relates to more severe BHR(9). Together these findings indicate that the small airways are involved in BHR.

Small and large airway function can be assessed with several tests, e.g. spirometry, obtaining the $FEF_{25-75\%}$ and FEV_1 reflecting small and large airway function respectively, body plethysmography or exhaled nitric oxide (eNO). Since there is no gold standard to specify small or large airway dysfunction, all variables are assumed to reflect either one or the other, and the sensitivity and specificity of the tests concerning small or large airway function are not known(10,11). A recently rediscovered method that is used to assess small and large airway dysfunction is impulse oscillometry (IOS), a simple technique measuring resistance and reactance of the airways(12). Resistance at 20 Hz is considered to reflect the large airways (R20), and resistance at 5 Hz the total airways. Small airway resistance can be calculated with the difference between the resistance at 5 Hz and 20 Hz (R5-R20). Reactance at 5 Hz (X5) and total reactance area (AX) are also assumed to reflect small airway function(12,13). Yamaguchi *et al* found significantly higher improvements in IOS parameters (R5-R20 and AX) after treatment with small particle hydrofluoroalkane-134a beclomethasone dipropionate (HFA-BDP) than after large particle chlorofluorocarbon (CFC)-

BDP(14). In contrast, the responses of spirometric values were comparable between the two treatments, supporting the notion that IOS is a sensitive measurement to assess small airway function.

The aim of this study was to investigate the association of small and large airway dysfunction with asthma symptoms as well as BHR in 58 asthma patients extensively characterized with respect to small and large airway function.

Methods

Study design

This cross-sectional study was part of a research project developing a questionnaire assessing small airway dysfunction (NCT01360294). Patients between 18-75 years were recruited via general practitioners. Inclusion criteria were: a physician's diagnosis of asthma and either a positive response to the methacholine provocation test ($PC_{20} < 39.3$ mg/mL) or maintenance therapy with inhaled corticosteroids and presence of asthma symptoms in the last three months (BHQ symptom score > 0). Patients attended the pulmonary outpatient department once and filled in questionnaires and performed lung function measurements. All patients gave written informed consent. The study was approved by the local Medical Ethics Committee.

Symptom assessment

Nocturnal symptoms, exercise-related symptoms, BHR symptoms and respiratory symptoms were assessed using the Dutch version of the Asthma Control Questionnaire (ACQ) and the Bronchial Hyperresponsiveness Questionnaire (BHQ)(6,15-17). The ACQ assesses symptoms of last week and the BHQ of the last 3 months. Nocturnal symptoms were measured with the ACQ-1 "*how often were you*

woken by your asthma during the night”, ACQ-2 “how bad were your asthma symptoms when you woke up” and BHQ-2 “waking up at night due to chest tightness”. Symptoms related to exercise were measured with the ACQ-3 “how limited were you in your activities”, BHQ-4 “trouble walking uphill”. The total BHQ-score was used to analyze symptoms of BHR. Respiratory symptoms were investigated with the ACQ-4 “how much shortness of breath did you experience”, ACQ-5 “how much of the time did you wheeze”, BHQ-1 “breathlessness”, BHQ-3 “shortness of breath”, BHQ-6 “chest tightness”, BHQ-10 “acute breathlessness” and BHQ-11 “wheezing”.

Assessment of alveolar and bronchial eNO, IOS, spirometry and body plethysmography

Exhaled Nitric Oxide (eNO) was measured at multiple flow rates (20, 50, 100 and 200 ml/s) with the NIOX (Aerocrine, Stockholm, Sweden) according to current recommendations(18). The modified mathematical model of Tsoukias and George was used to differentiate between the bronchial and alveolar compartment, respectively JNO and Calv, and subsequently corrected for axial back-diffusion(19,20,21). Due to technical problems eNO measurements were performed in 39 patients. The resistance of the respiratory system was analyzed using impulse oscillometry (IOS masterscreen, E. Jaeger, Wurzburg, Germany) according to standard recommendations(22). Patients performed spirometry and body plethysmography tests (Masterlab, Viasys Healthcare) according to international guidelines(23,24)-(25).

Large and small airway function

Selected parameters to reflect large airway dysfunction were FEV₁, FEV₁/FVC and R20. Parameters for small airway dysfunction were FEF_{25-75%}, RV, RV/TLC and IOS parameters R5-R20, X5 and AX(10,11). Bronchial and alveolar eNO were thought to reflect an inflammatory signal from the large and small airways respectively.

Methacholine provocation test including IOS and Borg scores

A 2-minute methacholine provocation test was performed with the Jaeger APS Pro system using a Medic-Aid sidestream nebulizer (Viasys Healthcare, Höchberg, Germany) and doubling concentrations of methacholine-bromide (0.038-39.3 mg/mL)(26). IOS was measured 30s after every provocation step followed by an FEV₁ after 90s. Borg scores, measuring dyspnea, were obtained at baseline and after every step during the provocation test in 37 patients. The provocative concentration causing a 20% fall in FEV₁ (PC₂₀) was determined by linear interpolation using log-transformed concentrations. Patients not reaching a 20% fall in FEV₁ were assigned twice the highest concentration (78.6 mg/mL).

Statistical analysis

Analyses were performed with SPSS version 20. Correlations between large and small airway parameters and asthma symptoms were calculated with the Spearman's correlation coefficient. To correct for multiple comparisons between large and small airway parameters and questionnaire scores, we applied the Bonferroni method. Severity of BHR was expressed by the slope of the FEV₁, calculated as the percentage change between the FEV₁ at the last provocation step compared to the FEV₁ at baseline divided by the last given methacholine

concentration. The slopes of the IOS parameters (R20, R5-R20, X5) and Borg scores of the provocation test were calculated as the absolute change between the values at the last provocation step minus the values at baseline divided by the last methacholine concentration. Univariate regression of severity of BHR and the slopes were analyzed with the Spearman's rank correlation test.

The slope of the FEV₁ was log-transformed to obtain normal distribution. We performed multivariate linear regression to assess contributing factors to severity of BHR. We included variables with a p-value <0.1 in the univariate regression in our multivariate regression model, with a maximum of one small and one large airway parameter assessed by spirometry and one small and one large airway parameter assessed by IOS. Multivariate regression analysis on BHR was adjusted for age and gender.

Results

A total of 58 patients with mild to severe asthma was included in this study. Their clinical characteristics are presented in **Table I**.

Asthma symptoms

Higher scores on nocturnal symptoms and exercise-related symptoms were not significantly correlated with more dysfunction of the large or small airways (**Table II**). Total BHQ score, representing symptoms of BHR, was related to a lower FEV₁ percentage predicted, with a p-value approaching statistical significance ($r=-0.257$, $p=0.052$). Higher scores on BHQ-11 "*wheezing*", representing respiratory symptoms, were significantly associated with higher R5-R20 and AX values (**Table III**). Also an

unexpected correlations was observed, i.e. higher scores on ACQ-3 correlated with a higher FEV₁/FVC value, reflecting less obstruction.

BHR severity measured with a methacholine provocation test

More severe BHR correlated with several small and large airway parameters (**Table IV**). Figure 1 shows the correlation between severity of BHR and FEV₁, and R20 (not significant), reflecting the large airways, and FEF_{25-75%} and R5-R20, reflecting the small airways (**Figure 1**). Multivariate regression analysis of severity of BHR included the large airway parameters, FEV₁ %predicted and R20, and the small airway parameters, FEF_{25-75%} %predicted and R5-R20, with additional adjustment of age, gender and ICS dose. Since severity of BHR was not associated with smoking habits, smoking was not included in the model. Lower age, lower FEF_{25-75%} %predicted and higher R5-R20 values were independent predictors of more severe BHR (**Table V**). FEV₁ and FEV₁/FVC had a comparable correlation coefficient, and when exchanging FEV₁ %predicted by FEV₁/FVC only younger age ($\beta=-0.278$, $p=0.02$) and higher R5-R20 ($\beta=0.299$, $p=0.02$) were independently associated with more severe BHR.

Changes in small and large airway parameters, and dyspnea during methacholine provocation

The slope of the FEV₁ during the methacholine provocation test correlated with the slope of R20, R5-R20 and X5 (**Table VI**). The increase in dyspnea, as assessed with the Borg score, was associated with a deterioration in FEV₁ and R5-R20 and X5 but not with the change in R20. Multivariate regression analysis showed that the slope of X5 was significantly associated with the slope of the Borg score independently of the

slope of the FEV₁ (X5; $\beta=0.431$, $p<0.01$ and FEV₁; $\beta=0.569$, $p<0.01$; R square 0.997).

Discussion

This study provides new insights in the role of small airway dysfunction in relation to asthma symptoms and BHR. Small airway dysfunction associates poorly with patient-perceived symptoms, as only higher small airway resistance and reactance were related to symptoms wheezing. However, when measuring BHR to methacholine, we found small airway dysfunction, i.e. a lower FEF_{25-75%} and a higher R5-R20, to be independently associated with more severe BHR to methacholine, whereas large airway dysfunction as reflected by lower FEV₁ or higher R20, was not.

In the present study, large airway dysfunction was not significantly related to patient-perceived nocturnal symptoms, exercise-related symptoms, BHR symptoms and respiratory symptoms. This is in line with previous studies showing that asthma symptoms poorly correlate with the FEV₁(27,28). In contrast to our expectations, small airway dysfunction also poorly correlated with asthma symptoms. We found that small airway dysfunction was only associated with wheezing, when small airway dysfunction was measured with IOS. These results contrast to the findings of our systematic review where we found suggestive evidence for a relation between small airways dysfunction and respiratory symptoms(6). Though only a few studies have directly investigated this relation(3,15). Takeda *et al* found that the small airway parameters R5-R20 and X5 were associated independently of the large airway parameters FEV₁ and R20 with either Asthma Quality of Life Questionnaire, St. George's Respiratory Questionnaire, ACQ or Baseline Dyspnea Index(3). Next,

Bourdin *et al* investigated 21 patients with asthma and found that a higher ACQ score was correlated with a steeper slope of the single-breath nitrogen washout test and higher RV/TLC values, while the ACQ score did not correlate with FEV₁ and FEF_{25-75%} values(29). In addition, Mansur *et al* found that the increase in X5 following a methacholine provocation test was correlated with the increase in dyspnea, tightness and wheezing. The latter study investigated the change in symptoms during a provocation test and not the severity of symptoms at baseline as our study did. Analysis of the change in symptoms during provocation in the present study, also showed a strong correlation between methacholine-induced deteriorations in large and small airway dysfunction and the concomitant increase in dyspnea. Taken together, it remains uncertain whether both small and large airway dysfunction associate with asthma symptoms at baseline and, if it is present, the association appears to be weak and more frequently related to IOS than spirometric parameters. Since small airway dysfunction was not taken into account with the development of these questionnaires, it is probable that the ACQ and BHQ do not include symptoms related to small airway dysfunction. For this reason, we think we need a new questionnaire to detect symptoms related to especially small airway dysfunction.

When we tested severity of BHR by a methacholine provocation test instead of by the patient-perceived BHQ score, severity of BHR was associated with small but not large airway dysfunction. This finding is in line with a study of Telenga *et al* showing that the FEF_{50%} is an independent predictor of the provocative dose of histamine causing a 20% fall in FEV₁ (PD₂₀)(30). We extended these findings by including IOS, thereby showing that small airway resistance, i.e. R5-R20, is an additional independent predictor of more severe BHR. We did not find any relationship of

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severity of BHR with bronchial and alveolar eNO, which are supposed to reflect central and peripheral airway inflammation respectively. However this can be due to the lower number of patients that performed this eNO measurement.

Besides measuring the fall in FEV₁ during the methacholine provocation test, the airway response was also measured with IOS parameters and dyspnea Borg scores. The change in FEV₁ was closely related to the changes in R5-R20 and X5, however it correlated weakly with the change in R20. This was an unexpected finding since FEV₁ and R20 are both considered to reflect large airway function. We suggest that the FEV₁ not only reflects changes in the large airways, but is also affected by changes in small airway function. Although, it is not exactly known which compartment of the bronchial tree is measured with the R20, we speculate that it mainly reflects the resistance of the trachea and cartilage-containing large central airways which hardly narrow in response to provocation.

A strength of our study is the use of a heterogeneous study population, containing current, ex- and never smokers, obese as well as non-obese patients, steroid-naïve patients and steroid users with asthma. This reflects the real-life situation and our conclusions may be translated to the broad spectrum of asthma patients. A limitation of our study was the relatively small sample size of 58 patients. Future studies with a larger sample size are needed to confirm the poor relationship between asthma symptoms and small airway dysfunction as found in our asthma population.

In conclusion, our results suggest that small airway dysfunction poorly associates with asthma symptoms. However, small airway dysfunction contributes importantly to the clinical expression of asthma as reflected by the severity of BHR when measured with a

methacholine provocation test and by the increase in dyspnea during bronchial constriction. For this reason small airway dysfunction should not be disregarded in asthma management. Moreover, treatment of the small airways may improve patients' well-being with respect to BHR and accompanying dyspnea.

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Table I. Characteristics of the study population

(n=58)		
Gender (m/f)	16/42	
Age (years)	54	(20-75)
BMI (kg/m ²)	29	(21-52)
ICS use (n, %yes)	50	(86)
ICS dose (µg)*	625	(0-3000)
Smoker (%current/ex/never)	7/53/40	
Pack-years (years)	2.5	(0-51)
Asthma control [#] (%well/partly/uncontrolled)	48/28/24	
Treatment step ^{\$} (%step 1-5)	12/14/31/43/0	
FEV ₁ (%pred)	109	(66-140)
FEV ₁ /FVC (%)	78	(52-89)
FEF _{25-75%} (%pred)	77	(23-154)
RV (%pred)	99	(53-160)
RV/TLC (%)	33	(19-45)
R20 (kPa·s·L ⁻¹)	0.36	(0.22;0.67)
R5-R20 (kPa·s·L ⁻¹)	0.09	(-0.03;0.44)
X5 (kPa·s·L ⁻¹)	-0.12	(-0.49;-0.03)
AX (kPa·s·L ⁻¹)	0.61	(0.02;4.20)
Alveolar eNO (Calv, ppb) (n=39)	2.9	(-0.3;67.9)
Bronchial eNO (JNo, Pl/s) (n=39)	0.94	(0.24;12.4)
PC ₂₀ methacholine ≤39.3mg/ml (% yes)	59	
PC ₂₀ methacholine (mg/ml)	11.6	(0.05-78.6)

Data are presented as median (range) or percentage

*beclomethasone equivalent ; [#] ACQ score <0.75; ≥0.75 to <1.5; ≥1.5(31); ^{\$} asthma severity according to GINA guidelines(32)

M: male, f: female, BMI: body mass index, ICS: inhaled corticosteroids, FEV₁: Forced expiratory volume in one second, %pred: percentage of the predicted value, FVC: forced vital capacity, FEF_{25-75%}: forced expiratory flow between 25% and 75% of the FVC, RV: residual volume, TLC: total lung capacity, R20: Resistance of the respiratory system at 20 Hertz, R5-R20: Difference between R5 and R20, X5: Reactance of the respiratory system at 5 Hertz, Calv: Alveolar concentration of eNO, JNO: bronchial flux of eNO, PC₂₀: provocative concentration causing a 20% fall in FEV₁, ACQ: Asthma Control Questionnaire, BHQ: Bronchial Hyperresponsiveness Questionnaire

Table II. Univariate correlations between symptoms of nocturnal asthma and large and small airway parameters (n=58)

	Nocturnal symptoms			Exercise-related symptoms		BHR Symptoms
	<i>ACQ 1 Nocturnal awakening</i>	<i>ACQ 2 Symptom severity upon awakening</i>	<i>BHQ 2 Waking up at night due to chest tightness</i>	<i>ACQ 3 Activity limitation</i>	<i>BHQ 4 Trouble walking uphill</i>	<i>BHQ Total score</i>
FEV ₁ (%pred)	-0.032 p=0.81	-0.023 p=0.87	-0.095 p=0.48	0.035 p=0.79	-0.183 p=0.17	-0.257 p=0.05[‡]
FEV ₁ /FVC (%)	0.239 p=0.07	0.303 p=0.02	0.218 p=0.10	0.354 p=0.01*	0.185 p=0.17	0.183 p=0.17
FEF _{25-75%} (%pred)	0.133 p=0.32	0.178 p=0.18	0.115 p=0.39	0.252 p=0.056	0.063 p=0.16	0.046 p=0.73
RV/TLC (%)	-0.148 p=0.27	-0.104 p=0.44	-0.014 p=0.91	-0.223 p=0.09	-0.032 p=0.81	-0.078 p=0.56
R20 (kPa·s·L ⁻¹)	-0.086 p=0.52	-0.078 p=0.56	0.063 p=0.64	-0.009 p=0.95	0.140 p=0.30	0.085 p=0.53
R5-R20 (kPa·s·L ⁻¹)	0.069 p=0.61	-0.062 p=0.65	0.181 p=0.18	-0.014 p=0.92	0.214 p=0.11	0.102 p=0.45
X5 (kPa·s·L ⁻¹)	0.042 p=0.76	0.192 p=0.15	-0.078 p=0.57	0.080 p=0.56	-0.184 p=0.18	-0.102 p=0.45
AX (kPa·s·L ⁻¹)	0.065 p=0.63	-0.109 p=0.42	-0.153 p=0.26	-0.030 p=0.83	0.200 p=0.14	0.094 p=0.49
Alveolar eNO (Calv, ppb) (n=39)	-0.055 p=0.74	-0.113 p=0.49	-0.267 p=0.10	-0.042 p=0.80	-0.198 p=0.23	-0.062 p=0.71
Bronchial eNO (JNo, Pl/s) (n=39)	-0.062 p=0.71	-0.370 p=0.02	-0.167 p=0.31	0.096 p=0.56	0.124 p=0.45	-0.163 p=0.32

p-values considered significant: $p < 0.017$ for nocturnal symptoms, $p < 0.025$ for exercise-related symptoms, $p < 0.05$ for BHR symptoms. *significant after Bonferroni correction, [‡] p-value = 0.052. (Spearman's correlation coefficient)

ACQ: asthma control questionnaire, BHQ: bronchial hyperresponsiveness questionnaire, FEV₁: forced expiratory volume in one second, %pred: percentage of the predicted value, FVC: forced vital capacity, FEF_{25-75%}: forced expiratory flow between 25% and 75% of the FVC, RV: residual volume, TLC: total lung capacity, R20: Resistance of the respiratory system at 20 Hertz, R5-R20: Difference between R5 and R20, X5: Reactance of the respiratory system at 5 Hertz, Calv: Alveolar concentration of eNO, JNO: bronchial flux of eNO

Table III. Univariate correlations between respiratory symptoms and large and small airway parameters (n=58)

	Respiratory symptoms						
	ACQ 4 Shortness of breath	ACQ 5 Wheezing	BHQ 1 breathlessness	BHQ 3 Shortness of breath	BHQ 6 Chest tightness	BHQ 10 Acute breathlessness	BHQ 11 Wheezing
FEV ₁ (%pred)	-0.157 p=0.24	-0.073 p=0.59	-0.169 p=0.21	-0.295 p=0.03	-0.019 p=0.89	-0.071 p=0.60	-0.184 p=0.17
FEV ₁ /FVC (%)	0.269 p=0.04	0.191 p=0.15	0.300 p=0.02	0.217 p=0.10	0.181 p=0.17	0.277 p=0.04	0.134 p=0.32
FEF _{25-75%} (%pred)	0.10 p=0.44	0.086 p=0.52	0.180 p=0.18	0.074 p=0.58	0.134 p=0.31	0.137 p=0.30	0.039 p=0.77
RV/TLC (%)	-0.106 p=0.43	-0.038 p=0.78	-0.177 p=0.18	-0.107 p=0.43	-0.068 p=0.61	0.015 p=0.91	0.052 p=0.70
R20 (kPa·s·L ⁻¹)	0.059 p=0.66	0.121 p=0.37	0.064 p=0.64	0.105 p=0.44	0.004 p=0.98	0.022 p=0.87	0.017 p=0.90
R5-R20	0.054	0.135	0.157	0.277	0.007	0.097	0.367

(kPa·s·L ⁻¹)	p=0.69	p=0.32	p=0.24	p=0.04	p=0.96	p=0.47	p<0.01* ‡
X5 (kPa·s·L ⁻¹)	0.039	-0.047	-0.092	-0.206	0.027	-0.052	-0.249
	p=0.77	p=0.73	p=0.50	p=0.12	p=0.84	p=0.70	p=0.06
AX (kPa·s·L ⁻¹)	0.041	0.135	0.115	0.281	-0.003	0.095	0.354
	p=0.77	p=0.32	p=0.40	p=0.04	p=0.98	p=0.48	p<0.01* §
Alveolar eNO (Calv, ppb) (n=39)	0.084	0.189	-0.147	-0.115	0.076	-0.296	-0.059
	p=0.61	p=0.25	p=0.37	p=0.49	p=0.65	p=0.07	p=0.72
Bronchial eNO (JNo, Pl/s) (n=39)	0.034	-0.034	-0.121	0.050	0.017	-0.007	-0.02
	p=0.84	p=0.84	p=0.46	p=0.76	p=0.92	p=0.97	p=0.88

p-values considered significant: p≤0.007 for respiratory symptoms

* significant after Bonferroni correction, ‡ p-value= 0.005, § p-value= 0.007 for

multiple comparisons. (Spearman's correlation coefficient)

ACQ: asthma control questionnaire, BHQ: bronchial hyperresponsiveness questionnaire, FEV₁: forced expiratory volume in one second, %pred: percentage of the predicted value, FVC: forced vital capacity, FEF_{25-75%}: forced expiratory flow between 25% and 75% of the FVC, RV: residual volume, TLC: total lung capacity, R20: Resistance of the respiratory system at 20 Hertz, R5-R20: Difference between R5 and R20, X5: Reactance of the respiratory system at 5 Hertz, Calv: Alveolar concentration of eNO, JNo: bronchial flux of eNO

Table IV. Univariate correlations of severity of BHR, reflected by the FEV₁ slope (n=58)

Variable	Correlation coefficient	P value
Age (years)	-0.142	0.29
BMI (kg/m ²)	-0.017	0.90
ICS dose (µg)*	-0.307	0.02
FEV ₁ (%pred)	-0.434	<0.01
FEV ₁ /FVC (%)	-0.436	<0.01
FEF _{25-75%} (%pred)	-0.488	<0.01
RV (%pred)	0.394	<0.01
RV/TLC (%)	0.208	0.12
R20 (kPa·s·L ⁻¹)	0.228	0.09
R5-R20 (kPa·s·L ⁻¹)	0.523	<0.01
X5 (kPa·s·L ⁻¹)	-0.382	<0.01
AX (kPa·s·L ⁻¹)	0.466	<0.01
Alveolar eNO (Calv, ppb) (n=39)	0.122	0.46
Bronchial eNO (JNO, Pl/s) (n=39)	0.183	0.27

*beclomethasone equivalent (Spearman correlation coefficient)

P-value <0.05 was considered significant.

BMI: body mass index, ICS: inhaled corticosteroids, FEV₁: Forced expiratory volume in one second, FVC: forced vital capacity, %pred: percentage of the predicted value, FEF_{25-75%}: forced expiratory flow between 25% and 75% of the FVC, RV: residual volume, TLC: total lung capacity, R20: Resistance of the respiratory system at 20 Hertz, R5-R20: Difference between R5 and R20, X5: Reactance of the respiratory system at 5 Hertz, Calv: Alveolar concentration of eNO (n=40), JNO: bronchial flux of eNO (n=40).

Table V. Multivariate linear regression of predictors of BHR severity, reflected by the FEV₁ slope*

	β	P value
Age (years)	-0.366	0.01
Gender (m/f)	0.043	0.69
ICS dose (μ g)	-1.47	0.20
FEV ₁ (%pred)	0.349	0.12
FEF _{25-75%} (%pred)	-0.484	0.02
RV (%pred)	0.174	0.21
R20 (kPa·s·L ⁻¹)	-0.021	0.85
R5-R20 (kPa·s·L ⁻¹)	0.439	<0.01
R ² = 0.52		

* the FEV₁ slope was log-transformed

P-value <0.05 was considered significant.

M: male, f: female (is coded 1), ICS: inhaled corticosteroids, FEV₁: Forced expiratory volume in one second, %pred: percentage of the predicted value, FEF_{25-75%}: forced expiratory flow between 25% and 75% of the forced vital capacity, RV: residual volume, R5-R20: difference between resistance at 5 Hertz and 20 Hertz, R20: resistance of the respiratory system at 20 Hertz

Table VI. Univariate correlations of the change in FEV₁ and IOS parameters with the change in dyspnea score during a provocation test, reflected by the slopes of the FEV₁, R20, R5-R20, X5 and Borg score.

	Slope of R20	Slope of R5-R20	Slope of X5	Slope of Borg
Slope of FEV ₁	0.315, (p=0.02)	0.846, (p<0.01)	0.763, (p<0.01)	0.902, (p<0.01)
Slope of R20	-	0.311, (p=0.02)	0.183, (p=0.17)	0.146, (p=0.39)
Slope of R5-R20	-	-	0.865, (p<0.01)	0.872, (p<0.01)
Slope of X5	-	-	-	0.730, (p<0.01)

(Spearman's correlation coefficient)

Analysis included data of 58 patients with respect to the slope of the FEV₁, R20, R5-R20, X5, and data of 37 patients with respect to the slope of the Borg score.

P-value <0.05 was considered significant.

FEV₁ slope (% decline in FEV₁/ mg·ml⁻¹); slopes of the R20, R5-R20 and X5 (kPa·s·L⁻¹/mg·ml⁻¹); Borg slope (Borg score/mg·ml⁻¹) FEV₁: Forced expiratory volume in one second, R20: Resistance of the respiratory system at 20 Hertz, R5-R20: Difference between R5 and R20, X5: Reactance of the respiratory system at 5 Hertz

Figure legends

Figure I: Correlation plot of the severity of BHR, i.e. slope of the FEV₁, (%decline in FEV₁/[mg/ml]) with **(A)** the FEV₁ (%pred) and **(B)** the FEF_{25-75%} (%pred) and **(C)** the R20(kPa·s·L⁻¹) and **(D)** the R5-R20 (kPa·s·L⁻¹)



