

High fractional exhaled nitric oxide levels may predict short-term worsening of respiratory oscillometry in asthma

To the Editor,

High fractional exhaled nitric oxide (FeNO) levels, reflecting uncontrolled airway inflammation, are regarded as a risk factor for an upcoming asthma exacerbation.¹ A previous study found that persistently high FeNO (>40 ppb) was associated with a rapid decline in FEV1, even in controlled asthma patients, in a 3-year prospective study.² However, little is known about the effect of high FeNO levels on short-term course of lung function. Oscillometry, also known as the forced oscillation technique, is a noninvasive method with which to measure respiratory mechanics and is useful for the evaluation or management of asthma.³ The aim of this study was to assess whether high FeNO levels (>35 ppb) would predict the short-term worsening of lung function, especially oscillometric parameters.

This was a multicenter, prospective observational study. It was approved by the institutional review board of Shizuoka General Hospital and written informed consent was obtained from all subjects prior to the study. The study subjects included adult asthma patients who had routine check-ups at each institution from April 2015 to December 2016. Patients received standard treatment, including inhaled corticosteroid (ICS) and other medications, according to the Global Initiative for Asthma (GINA) report⁴ and the medications were not changed for at least 3 months prior to the study. Patients were excluded from the study if they had an exacerbation upon enrollment. Subjects who could not perform oscillometry and spirometry were also excluded.

On the examination days (baseline and after 3 months), eligible subjects underwent the Asthma Control Test (ACT), FeNO, oscillometry, and spirometry, in that order. The patients were classified into high or low FeNO groups (FeNO ≥ 35 ppb or <35 ppb) based on the baseline FeNO levels according to the previous study.⁵ After a 3-month period of observation without changes in medications, the changes in parameters were compared between the two groups. The primary outcomes were the changes in oscillometric parameters. Secondary outcomes included the changes in ACT scores, FEV1, and FeNO levels. The correlations between baseline FeNO and the other parameters were evaluated.

One hundred and forty-three patients were screened and 46 patients were excluded upon entry because of asthma exacerbation, a change in medications, or difficulty in taking measurements. The clinical characteristics of the patients at baseline are shown in

Table S1. Of the 97 subjects who completed the study, 28 (28.9%) patients had FeNO levels ≥ 35 ppb. There was no difference in the demographic, ACT, and lung function data between high and low FeNO groups at baseline except for FeNO levels (Table S2). Overall, median FeNO levels significantly decreased (29.0 ppb to 24.0 ppb; $P = .008$). However, there was no change in the ACT scores or FEV1. A comparison between the groups revealed that FeNO decreased significantly in the high FeNO group but not in the low FeNO group (Table S2). There was no difference in the ACT score and lung function data between high and low FeNO groups after 3 months except for FeNO levels (Table S2). Respiratory system resistance at 5 Hz (R5), the difference between respiratory system resistance at 5 Hz and 20 Hz (R5-R20), respiratory system reactance at 5 Hz (X5), resonant frequency (Fres), and area under the reactance (AX) significantly worsened in the high FeNO group but not in the low FeNO group (Figure 1 and Table S2). In the high FeNO group, there were no differences in changes in FEV1 and oscillometric parameters between patients who had FeNO <35 ppb after 3 months and those who had ≥ 35 ppb after 3 months. There was no significant change in the ACT scores, FEV1, or R20 in any group. In the high FeNO group, baseline FeNO levels correlated with the changes in R5, R20, and R5-R20, but not with changes in X5, Fres, and AX (Figure 2). Furthermore, a trend toward a negative association between changes in FeNO levels and the changes in R5 and R20 was found (p -value between 0.05 and 0.10) (Figure S1). Univariate and multivariate logistic regression analysis revealed that changes in R5 were associated with age and higher FeNO levels and that changes in R5-R20 were associated with higher FeNO levels (Table S3).

This study found that oscillometric parameters, including R5, R5-R20, X5, Fres, and AX, but not FEV1, significantly worsened after a 3-month period of observation in the high FeNO group but not in the low FeNO group. FeNO decreased significantly in the high FeNO group but not in the low FeNO group. There were correlations between baseline FeNO levels and changes in R5 and R5-R20 in the high FeNO group. Multiple logistic regression analysis revealed that R5 and R5-R20 were associated with higher FeNO levels.

Respiratory resistance, including R5 and R5-R20, is interpreted as a marker of airway caliber.⁶ Oscillometry is measured during tidal breathing while spirometry is performed during forced expiration. In clinical practice, oscillometric parameters sometimes

Abbreviations: AX, area under the reactance; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; Fres, resonant frequency; FVC, forced vital capacity; ICS, inhaled corticosteroids; LABA, long-acting β_2 -agonists; R20, respiratory system resistance at 20 Hz; R5, respiratory system resistance at 5 Hz; R5-R20, the difference between R5 and R20; X5, respiratory system reactance at 5 Hz.

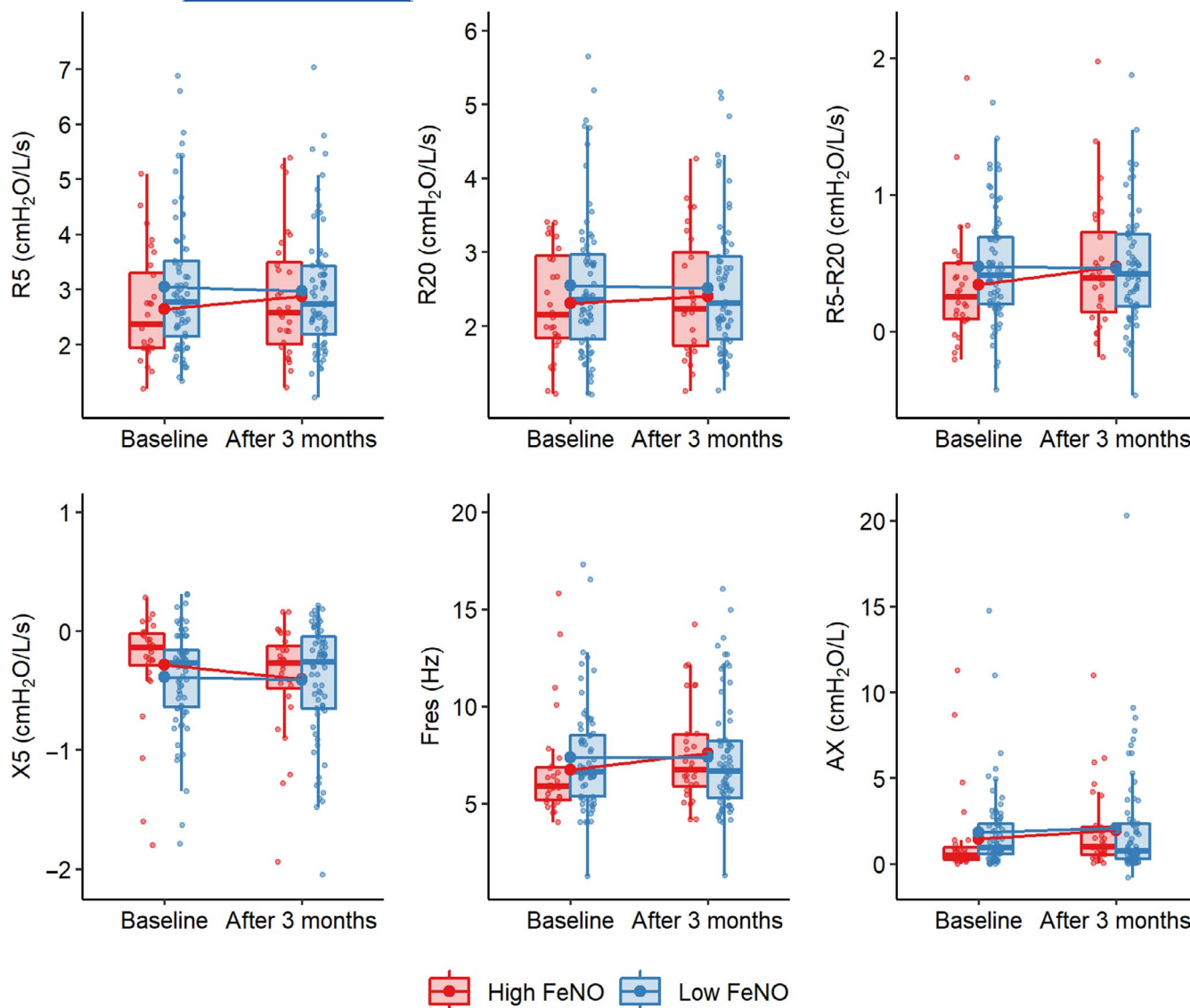


FIGURE 1 Changes in oscillometric parameters at baseline and after 3 months. R5, R5-R20, X5, Fres, and AX significantly worsened in the high FeNO group. Boxes and thick lines in the box indicate interquartile range and median, respectively. Whiskers extend to most extreme data points that are no more than 1.5 times the interquartile range from the box. Points on the box edges connected by solid lines represent the group means. Abbreviations: AX, area under the reactance; FeNO, fractional exhaled nitric oxide; Fres, resonant frequency; R5 and R20, respiratory system resistance at 5 and 20 Hz, respectively; X5, respiratory system reactance at 5 Hz

change despite no change in spirometry, reflecting subtle changes in airway caliber. R5-R20 is also a marker of frequency dependence, and its interpretation is controversial. A recent task force report stated that R5-R20 increases due to the shunting of applied flow oscillations into the upper airway (cheeks, pharynx, etc), while it suggests the possibility that R5-R20 may reflect small airway caliber.⁶ We previously reported that R5-R20 correlated with FEV1 and the N₂ phase III slope of single-breath N₂ washout in asthma patients and speculated that R5-R20 was associated with small airway obstruction and ventilation inhomogeneity.⁷ We also found the correlations between X5, Fres, and AX and the N₂ phase III slope of single-breath N₂ washout, suggesting that not only R5-R20 but also other parameters reflect small airway narrowing or obstruction.⁷

A large part of FeNO is produced in the peripheral airways and therefore FeNO might paradoxically decrease if the small airway caliber reduces despite no changes in airway inflammation.⁸ Thus, we speculate that the decrease in FeNO in the high FeNO group might be induced by small airway narrowing as reflected by the increase in R5 and R5-R20 in this study. In the previous study⁷ (mean FeNO 53.2 ppb), we found the correlations between oscillometric parameters and alveolar NO, which were stronger than with FeNO. The ATS FeNO guidelines recommend FeNO <25 ppb as the level of no airway inflammation.¹ Based on these, the patients in the high FeNO group had persistent airway inflammation for more than 3 months. Because the inclusion criteria stated the medications were not changed for at least 3 months prior to the study. Thus, although asthma was controlled based on the ACT scores, we

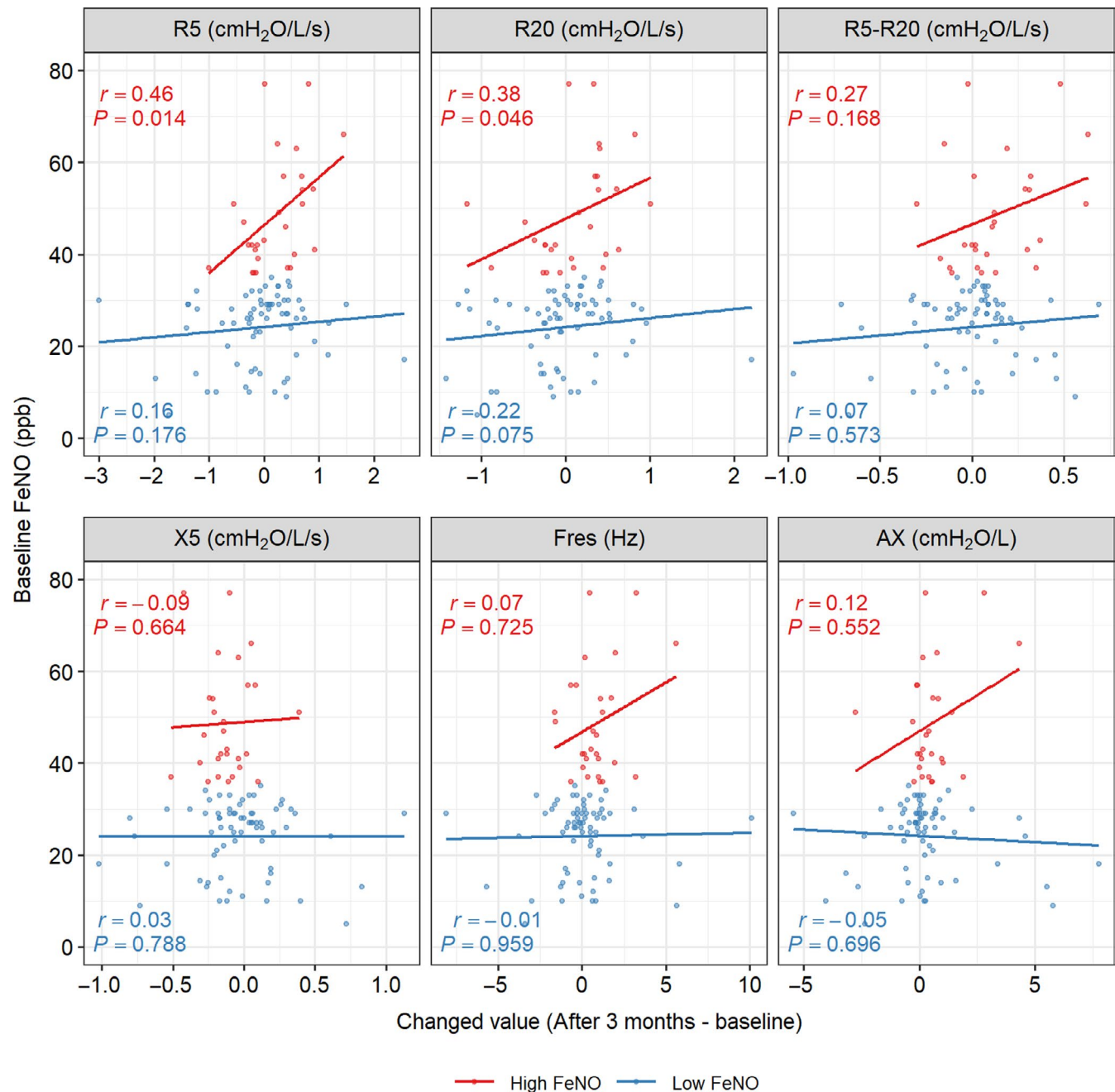


FIGURE 2 Correlations between baseline FeNO levels and changes in oscillometric parameters. Baseline FeNO levels correlated with the changes in R5, R20, and R5-R20, but not in X5, Fres, or AX in the high FeNO group. There was no correlation in the low FeNO group. Abbreviations: AX, area under the reactance; FeNO, fractional exhaled nitric oxide; Fres, resonant frequency; R5 and R20, respiratory system resistance at 5 and 20 Hz, respectively; X5, respiratory system reactance at 5 Hz

speculate that high FeNO levels, reflecting not totally controlled airway inflammation, might induce the worsening of oscillometry. Furthermore, higher baseline FeNO related to changes in resistance but not reactance.

There are several limitations. One might think the changes in FeNO levels and oscillometry after 3 months were due to the regression toward the mean effect. However, these changes can be explained thoroughly by the mechanisms stated above. Since the patients of this study had well controlled asthma and good lung

function, attention has to be paid for the generalizability. Further studies warrant the effect of high FeNO levels on asthma pathophysiology and the interpretation of R5-R20.

CONFLICT OF INTEREST

The authors have nothing to disclose.

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Yoshinari Endo¹ 
Toshihiro Shirai¹ 
Keita Hirai^{2,3} 
Taisuke Akamatsu¹
Eishi Kato⁴
Hidehiko Furui⁵
Tetsuo Hiramatsu⁶

¹Department of Respiratory Medicine, Shizuoka General Hospital, Shizuoka, Japan

²Department of Clinical Pharmacology and Genetics, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

³Laboratory of Clinical Pharmacogenomics, Shizuoka General Hospital, Shizuoka, Japan

⁴Hanasaki Clinic, Kiyosu, Japan

⁵Furui Clinic, Tarui, Japan

⁶Hiramatsu Clinic, Komaki, Japan

Correspondence

Toshihiro Shirai, MD, PhD, Shizuoka General Hospital, 4-27-1
Kita-Ando, Aoi, Shizuoka 420-8527, Japan.
Email: toshihiro-shirai@i.shizuoka-pho.jp

ORCID

Yoshinari Endo  <https://orcid.org/0000-0003-1857-6369>

Toshihiro Shirai  <https://orcid.org/0000-0002-2352-7580>

Keita Hirai  <https://orcid.org/0000-0001-8027-3508>

REFERENCES

1. Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med*. 2011;184(5):602-615.
2. Matsunaga K, Hirano T, Oka A, Ito K, Edakuni N. Persistently high exhaled nitric oxide and loss of lung function in controlled asthma. *Allergol Int*. 2016;65(3):266-271.
3. Shirai T, Kurosawa H. Clinical application of the forced oscillation technique. *Intern Med*. 2016;55(6):559-566.
4. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2015. www.ginasthma.org
5. Dweik RA, Sorkness RL, Wenzel S, et al. Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med*. 2010;181(10):1033-1041.
6. King GG, Bates J, Berger KI, et al. Technical standards for respiratory oscillometry. *Eur Respir J*. 2020;55(2):1900753.
7. Shirai T, Mori K, Mikamo M, et al. Respiratory mechanics and peripheral airway inflammation and dysfunction in asthma. *Clin Exp Allergy*. 2013;43(5):521-526.
8. Michils A, Haccuria A, Michiels S, Van Muylem A. Airway calibre variation is a major determinant of exhaled nitric oxide's ability to capture asthma control. *Eur Respir J*. 2017;50(2):1700392.

SUPPORTING INFORMATION

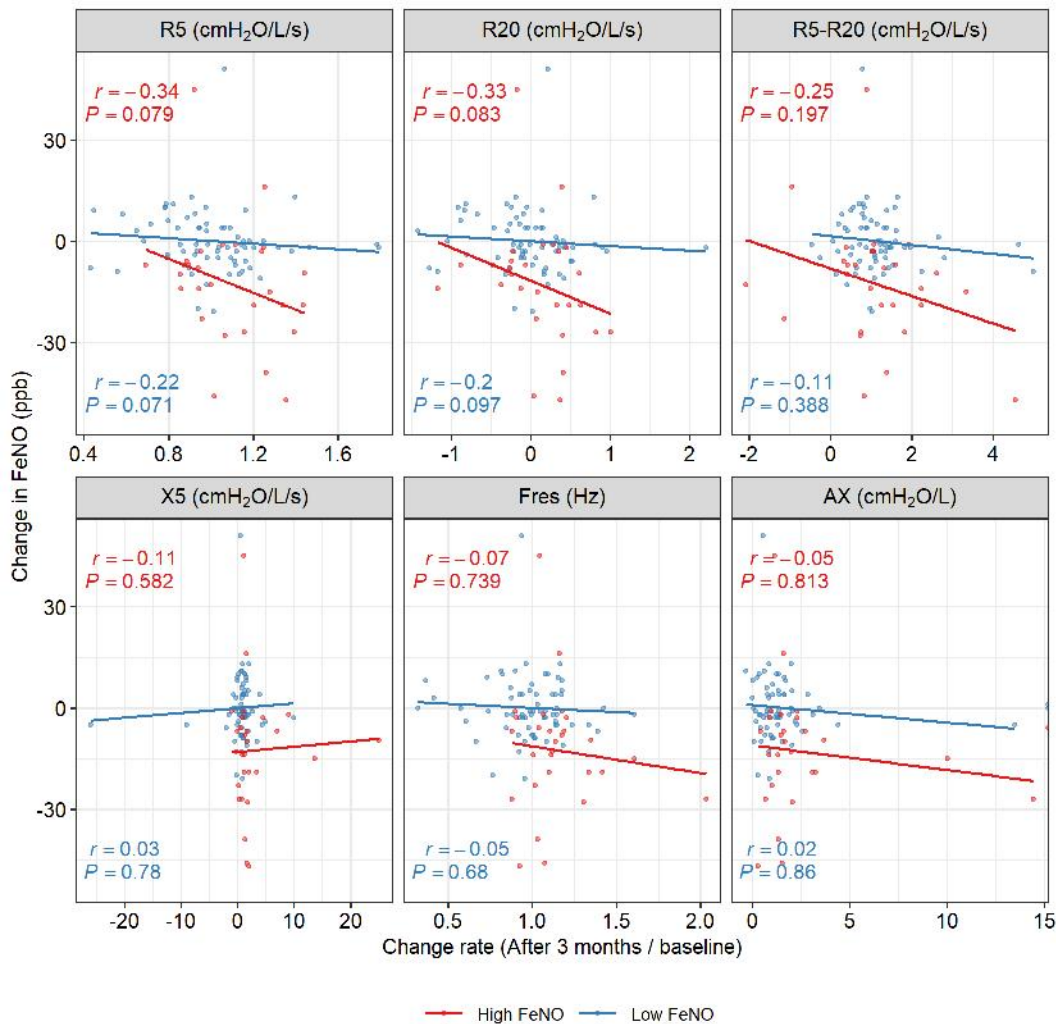
Additional supporting information may be found online in the Supporting Information section.

Measurement

FeNO was measured with NIOX VERO (Aerocrine AB, Solna, Sweden). Spirometry and oscillometry (whole-breath value) were performed with CHESTAC-8800 and MostGraph-01 (Chest M.I. Co. Ltd, Japan), respectively.

Statistical analysis

Categorical variables are summarized as frequencies and percentages, and continuous variables are expressed as median with interquartile range. The Mann-Whitney U test was used to compare continuous variables, and Fisher's exact test was used to compare categorical variables between the two groups. Wilcoxon signed-rank test was used to compare the changes in clinical parameters between baseline and after 3 months. Correlations between variables were performed using the Spearman's correlation coefficient. Univariate linear regression analysis followed by multivariate linear regression analysis were used to determine the factors influencing changes in R5 and R5-R20. P values < 0.05 were considered statistically significant. R software (version 3.6.2, R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analyses.



1 Table E1. Clinical characteristics of the patients at baseline

Variables	All (N = 97)	Low FeNO (N = 69)	High FeNO (N = 28)	P value*
Age (years)	50 (41, 63)	50 (41, 62)	49 (41, 67)	0.971
Gender, male	45 (46.4)	31 (44.9)	14 (50.0)	0.661
Body mass index (kg/m ²)	21.9 (19.8, 25.2)	21.9 (19.8, 24.9)	21.7 (19.9, 26.2)	0.682
Rhinitis or rhinosinusitis	49 (50.5)	33 (47.8)	16 (57.1)	0.503
Smoking status				0.814
Non-smoker	63 (64.9)	45 (65.2)	18 (64.3)	
Ex-smoker	28 (28.9)	19 (27.5)	9 (32.1)	
Current smoker	6 (6.2)	5 (7.2)	1 (3.6)	
Pack-years	0 (0, 4)	0 (0, 2)	0 (0, 8.9)	0.952
FEV ₁ % predicted	104.2 (89.7, 111.7)	102.3 (89.2, 110.9)	106.2 (95.9, 119.6)	0.208
FeNO (ppb)	29.0 (24.0, 37.0)	27.0 (18.0, 29.0)	44.5 (39.8, 54.9)	<0.001
Asthma Control Test	25 (24, 25)	25 (24, 25)	25 (24, 25)	0.124
Treatment				
ICS use	97 (100.0)	69 (100.0)	28 (100.0)	1.000
ICS dose, low/middle/high	21/69/7	13/51/5	8/18/2	0.520
LABA	87 (89.7)	62 (89.9)	25 (89.3)	1.000
LAMA	3 (3.1)	3 (4.3)	0 (0.0)	0.554
LTRA	34 (35.1)	25 (36.2)	9 (32.1)	0.816

2 Data are shown as median (interquartile range) or frequency (percentage).

3 Abbreviations: FeNO, fraction of exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; ICS, inhaled corticosteroid; LABA,

4 long-acting β_2 -agonists; LAMA, long-acting muscarinic antagonist; LTRA, leukotriene receptor antagonist.

5 *Mann-Whitney U test or Fisher's exact test

6 Table E2. Changes in clinical parameters between baseline and after 3 months

Variables	Low FeNO			High FeNO		
	Baseline	P value*		Baseline	After 3 months	P value*
Asthma	25 (	25 (24, 25)	0.570	25 (24, 25)	25 (24, 25)	0.167
Control Test						
FeNO (ppb)	27.0 (	23.0 (17.0, 28.0)	0.987	44.5 (39.8, 54.9)	32.5 (27.0, 41.2)	<0.001
FEV ₁ %	102.3 (	99.4 (88.0, 110.5)	0.097	106.2 (95.9, 119.6)	106.9 (96.6, 114.8)	0.243
predicted						
R5	2.77 (	2.73 (2.18, 3.43)	0.816	2.37 (1.94, 3.30)	2.58 (2.00, 3.50)	0.032
(cmH ₂ O/L/s						
)						
R5 z-score	0.14 (	0.27 (−0.37, 0.74)	0.898	−0.26 (−0.54, 0.65)	0.17 (−0.44, 0.74)	0.069
R20	2.35 (	2.30 (1.81, 2.94)	0.693	2.15 (1.84, 2.94)	2.22 (1.73, 2.99)	0.176
(cmH ₂ O/L/s						
)						
R20 z-score	0.41 (	0.33 (−0.38, 0.89)	0.902	0.19 (−0.56, 0.97)	0.32 (−0.34, 0.99)	0.274
R5-R20	0.41 (	0.42 (0.18, 0.71)	0.876	0.25 (0.09, 0.50)	0.39 (0.14, 0.72)	0.008
(cmH ₂ O/L/s						
)						
R5-R20 z-	−0.31 (	−0.30 (−1.02, 0.39)	0.978	−0.85 (−1.63, −0.11)	−0.37 (−1.24, 0.35)	0.029
score						
X5	−0.27 (	−0.26 (−0.65, −0.05)	0.356	−0.14 (−0.29, −0.03)	−0.27 (−0.48, −0.13)	<0.001
(cmH ₂ O/L/s						
)						
X5 z-score	0.11 (	0.15 (−0.70, 0.74)	0.325	0.47 (0.07, 0.84)	0.12 (−0.38, 0.49)	<0.001
Fres (Hz)	6.62 (	6.64 (5.29, 8.22)	0.915	5.86 (5.15, 6.82)	6.72 (5.88, 8.54)	0.002
Fres z-score	−0.24 (	−0.27 (−0.82, 0.37)	0.869	−0.58 (−0.94, −0.13)	−0.24 (−0.59, 0.44)	<0.001
AX	0.94 (	0.72 (0.27, 2.35)	0.446	0.48 (0.24, 0.94)	0.97 (0.54, 2.13)	<0.001
(cmH ₂ O/L)						
AX z-score	0.00 (	−0.05 (−0.63, 0.64)	0.847	−0.40 (−0.89, 0.02)	0.00 (−0.35, 0.55)	0.002

7 Data are shown as median (interquartile range) or frequency (percentage).

- 8 Abbreviations: AX, area under the reactance; FeNO, fraction of exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; Fres,
- 9 resonant frequency; R5 and R20, respiratory system resistance at 5 and 20 Hz, respectively; X5, respiratory system reactance at 5 Hz.
- 10 *Wilcoxon signed-rank test

11 Table E3. Univariate and multivariate linear regression analysis of factors influencing changes in R5 and R5-R20

Variables	Univariate		Multivariate	
	Coefficient	P value	Coefficient (95% CI)	P value
<u>Object variable: Change in R5</u>				
Age (per 10 years)	0.147	0.003	0.135 (0.043, 0.228)	0.005
Gender males	−0.024	0.880		
Body mass index (per 10 kg/m ²)	0.355	0.064		
Atopy	−0.001	0.998		
Smoking (per 10 pack-years)	0.143	0.027		
ICS dose (low vs middle to high)	0.237	0.213		
Asthma Control Test (per 1 point)	0.031	0.366		
FeNO (per 10 ppb)	0.143	0.008	0.128 (0.027, 0.228)	0.014
FEV1 (per 10 % predicted)	0.056	0.176		
Intercept			−1.077 (−1.630, −0.523)	<0.001
<u>Object variable: Change in R5-R20</u>				
Age (per 10 years)	0.028	0.117		
Gender males	−0.001	0.988		
Body mass index (per 10 kg/m ²)	0.052	0.448		
Atopy	0.040	0.576		
Smoking (per 10 pack-years)	0.032	0.172		
ICS dose (low vs middle to high)	0.106	0.117		
Asthma Control Test (per 1 point)	0.002	0.879		
FeNO (per 10 ppb)	0.057	0.003	0.057 (0.021, 0.094)	0.003
FEV1 (per 10 % predicted)	0.013	0.376		
Intercept			−0.151 (−0.277, −0.025)	0.021

- 12 Abbreviations: FeNO, fraction of exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; ICS, inhaled corticosteroid; R5 and
- 13 R20, respiratory system resistance at 5 and 20 Hz, respectively.

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