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Small airway dysfunction mediates the relationship between Fractional Exhaled Nitric Oxide and asthma control

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ABSTRACT

Background: Most physiological production of Fractional exhaled Nitric Oxide (FeNO) occurs in the small airways, but studies on the relationship between FeNO and small airway dysfunction (SAD) in asthma are scant.
Objective: To investigate the relationship between asthma control, changes of FeNO in relation to airway bronchodilation (BD), and SAD.
Methods: Baseline conventional spirometry, impulse oscillometry, and FeNO pre- and post-BD (salbutamol 400 μg) were tested on consecutive community-treated adult patients with asthma. Results were stratified by FeNO response (change in FeNO [ΔFeNO]), being FeNO "responder" if the increase is greater than 10% post-BD compared with the basal values and "nonresponder" if less than or equal to 10%.
Results: When measured, post-BD FeNO greater than 25 parts per billion was found in an additional 31.5% of patients. Of the 92 patients included, 61% were classified as FeNO "responders" and 39% as "nonresponders." A significant moderate-to-strong correlation was observed between ΔFeNO and R5R20, a functional marker of SAD (R = 0.52, $P < .0001$), whereas the correlations between spirometry markers and ΔFeNO were not significant ($P > .05$). Both R5R20 and ΔFeNO inversely correlated with asthma control ($P < .0001$). Using causal mediation analysis modeling, the effect of asthma control on ΔFeNO was mediated by SAD, with a strong indirect effect of asthma control on ΔFeNO mediated by SAD (β value: −7.04, 95% CI: −11.80 to −3.53, $P < .0001$), without a significant direct effect (β value: −4.96, 95% CI: −9.15 to 0.11, $P = .056$).
Conclusion: Changes in FeNO values pre-/post-BD can improve the identification of patients with "T_H2 high" asthma. The relationship between ΔFeNO and asthma control is mainly mediated by SAD, highlighting its contribution to asthma control.

Introduction

Fractional exhaled nitric oxide (FeNO) is a known marker of T_H2 airway inflammation[1] and has gained broader application in asthma management over the years.[2,3] FeNO levels may be influenced by several factors identified and reported in current guidelines,[2,4] but reference values are not currently considered. Sex, height, dietary intake, allergic sensitization, obesity, and inhaled corticosteroid use may interfere with the ability of FeNO levels to adequately reflect airway inflammation and asthma control.[4] Recently, it has been found that noninflammatory changes related to airway caliber also affect FeNO levels, which could be problematic for FeNO interpretation in patients with low forced expiratory volume in 1 second (FEV1).[5] In addition, most FeNO production occurs in small conductive airways,[6] suggesting a possible association between small airway dysfunction (SAD) and FeNO levels. Recent studies revealed, with different techniques, that in a fraction of patients, an increase of FeNO in response to inhaled β_2-agonists likely associated with a predominant dilation of the pre-acinar airways,[7-10] suggesting for FeNO a role as a biomarker

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that integrates both airway inflammation and lung function changes[7]; however, none of these used impulse oscillometry (IOS) to assess SAD. IOS, in fact, can sensitively assess the obstruction of small peripheral airways compared with conventional spirometry.[11]

Extensive literature[12-16] has revealed that SAD is highly prevalent in patients with asthma, regardless of disease severity. IOS-defined SAD is independently associated not only with an increase of FeNO level but also with female sex, smoking, older age, asthma-related night awakenings, overweight, and exercise-induced asthma symptoms.[17] We and others also reported the association between SAD and asthma control,[13,17,18] with direct correlations between SAD prevalence and severity with worsening of Global Initiative for Asthma (GINA) categories of asthma control (well controlled [WC], partially controlled [PC], and uncontrolled [UC]).[11,17-19] Regardless of these unidirectional associations between SAD and FeNO, FeNO and asthma control, and SAD and asthma control, however, the reciprocal effect of these relationships has never been explored.

This study was designed to investigate the relationship among asthma control, changes in FeNO levels in relation to airway bronchodilation (BD), and small airway obstruction. To accomplish this, we aimed to evaluate the following: (1) the percent change of FeNO before and after BD (change in FeNO [ΔFeNO]); (2) the associations between ΔFeNO, IOS-defined SAD, and asthma control; and (3) the reciprocal effect of these associations. These aspects have significant clinical relevance because of their potential implications in guiding therapeutic decisions.

Methods

Study Setting and Participants

This cross-sectional, single-center observational study was conducted on 92 community-treated patients with asthma consecutively recruited between June 1, 2021, and June 1, 2023. All patients had a history of asthma combined with a previous positive BD test result or a positive methacholine challenge test result. All patients with stable asthma (without new or worsening symptoms of wheezing, breathlessness, chest tightness, or coughing) at the time of the visit and during the 4 weeks were included in the study. Patients fulfilling the criteria for asthma/chronic obstructive pulmonary disease overlap were excluded. Demographic parameters, clinical characteristics (ie, concurrent atopy and asthma duration), and asthma therapy were recorded. The most recent eosinophil count during the 2 years before the first assessment was also collected. Patients were defined as having atopy if a positive skin prick test result for aeroallergens or specific IgE in the peripheral blood and symptoms consistent with allergic asthma were present. All patients underwent FeNO, IOS, and standard spirometry measurements during the same visit, before BD, and 15 minutes after BD, that is, inhalation of short-acting β_2-agonists (400 mg of salbutamol administered through a metered-dose aerosol device) (eFig 1).[20]

This study was approved by the local institutional review board (number NP3364) and conducted in accordance with the amended Declaration of Helsinki. All patients gave written informed consent for their data to be stored electronically. This study was conducted according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines for cohort, case-control, and cross-sectional studies.

Asthma Control

GINA assessment of asthma control was also recorded; this includes the assessment of the following symptoms in the 4 preceding weeks: presence of daytime asthma symptoms more than twice a week, nighttime asthma symptoms, activity limitation, and use of short-acting β_2-agonist more than twice a week. The resulting asthma control is classified as WC (no symptoms), PC (1-2 symptoms), and UC (≥3 symptoms).[20]

Fractional Exhaled Nitric Oxide

According to the American Thoracic Society guidelines, FeNO was administered in duplicate using HypAir FeNO (MediSoft, Sorinnes Belgium), at a standard flow rate of 50 mL/s.[3,4]

The patients were categorized by the percent change of FeNO values after BD (ΔFeNO ≤ 10% of the basal FeNO value, defined as "FeNO nonresponders," vs ΔFeNO > 10%, defined as "FeNO responders," vs ΔFeNO decrease > 10%, defined as "FeNO reduced"), as previous studies have used this cutoff for FeNO, providing a precedent and rationale for its adoption in our analysis.[10] Considering HypAir FeNO reproducibility (±2 parts per billion [ppb]), patients were considered "responders" only if the ΔFeNO was more than 10% and the change was beyond the inherent variability of the measuring device (2 ppb).

Spirometry and Oscillometry Measurements

A Vyntus PNEUMO–PC Spirometer (VyAire Medical, Mettawa IL, USA) was used to perform spirometry in triplicate in accordance with European Respiratory Society guidelines. We used the MasterScreen IOS Impulse Spirometry system sold by VyAire Medical and the manufacturer's recommended equations.[21] The IOS was routinely calibrated according to the manufacturer.[22]

Respiratory resistance at 5 and 20 Hz (R5 and R20, in kPa × s × L^{-1}) was used as an index of total and proximal airway resistance, respectively. The decrease in resistance from 5 to 20 Hz (R5-R20, in kPa × s × L⁻1) was considered to be an index for the resistance of peripheral airways, and an R5-R20 cutoff of more than 0.07 kPa × s × L^{-1} (a conservative upper limit of normal for R5-R20, as previously reported[17,23,24]) was chosen to define the presence of SAD. The following variables were collected: reactance at 5 Hz (X5, in kPa × s × L^{-1}), reflecting the elastic recoil of the peripheral airways; resonant frequency (Fres, in Hz), defined as the frequency at which the inertial properties of the airway and the capacitance of the lung periphery are equal; and reactance area (AX, the area under the reactance curve, in kPa/L), reflecting the elastic properties of the lung periphery and found to be correlated with resistance at lower frequencies.

Statistical Analysis

Data are summarized as percentages, means, and SDs or median and 25% to 75% IQRs. Variations in the quantitative characteristics were assessed using Student's t test or nonparametric Wilcoxon test for paired data, according to the results of the Shapiro-Wilk test of normality on the differences. Variations in dichotomous variables were assessed using McNemar's χ^2 test in a 2-dimensional contingency table. Spearman's method was used to test for univariate correlations between variables. Multiple comparisons among more than 2 groups were performed with 1-way analysis of variance test or Kruskal-Wallis test, where appropriate, for quantitative variables and the χ^2 test for qualitative variables. An analysis of covariance was conducted to evaluate the effect of age or sex on R5R20 while controlling for %ΔFeNO.

Mediation analysis was performed to investigate the IOS-defined SAD as a mediator variable of the relationship between asthma control as an independent variable and changes in FeNO as a dependent variable. Mediation analyses are used to understand known relationships by exploring how an independent variable influences a dependent variable through an intermediary variable (mediator). The assumptions of causal relationships between dependent and outcome variables, necessary for this analysis to be used, were met. In other words, mediation analysis is a statistical approach used to explore whether the relationship between an independent variable

(asthma control) and an outcome variable (ΔFeNO) is explained by a third variable, known as the mediator (SAD). It quantifies how much of the effect of asthma control on FeNO change is direct vs how much it occurs indirectly through SAD. Mediation was demonstrated when (1) the independent variable (asthma control) significantly affected the mediator (SAD), (2) SAD significantly affected the outcome variable (FeNO change), and (3) the direct effect of the independent variable (asthma control) on the outcome variable (FeNO change) was reduced (or eliminated) when the mediator (SAD) was included in the model. This analysis provides insight into the mechanisms underlying the observed relationships and aids in understanding the causal pathways.

Data were analyzed using the statistical software R (https://www.r-project.org/) and GraphPad Prism (San Diego, California). Statistical significance was set at *P* less than .05.

Results

Fractional Exhaled Nitric Oxide Value Distributions and Clinical Usefulness of Post-Bronchodilation Fractional Exhaled Nitric Oxide on Asthma Phenotyping

We collected data from 100 consecutive community-treated subjects with asthma who underwent FeNO, IOS, and standard spirometry before and after BD (eFig 1).

The cohort was categorized by the percent change of FeNO values after BD (ΔFeNO ≤ 10% of the basal FeNO value, defined as "FeNO nonresponders," vs ΔFeNO > 10%, defined as "FeNO responders," vs ΔFeNO decrease > 10%, defined as "FeNO reduced,"; eTable 1).[10] Given the small number of patients with "FeNO reduced" (8%), with baseline characteristics not significantly different from "FeNO nonresponders," we decided to exclude this small group from the comparisons and perform further analyses, focusing only on the remaining 92 patients.

Distribution of FeNO values pre- or post-BD ranged from 5 to 165 ppb and from 5 to 191 ppb, respectively, with percent change before and after BD (ΔFeNO) between −29% to +443% (Fig 1A and B). According to the pre-BD FeNO value alone, 45% of patients (n = 41) can be classified as "T_H2 low" and 55% (n = 51) as "T_H2 high," respectively. According to the post-BD FeNO value alone, 24% (n = 22) can be classified as "T_H2 low" and 76% (n = 70) as "T_H2 high," respectively. Therefore, the value of FeNO post-BD reveals that 29 additional patients have FeNO greater than or equal to 25 ppb as compared with pre-BD FeNO (from 41 to 70 patients, +31.5%; Fig 1C and D), ideally moving from a "T_H2 low" to a "T_H2 high" asthma phenotype.

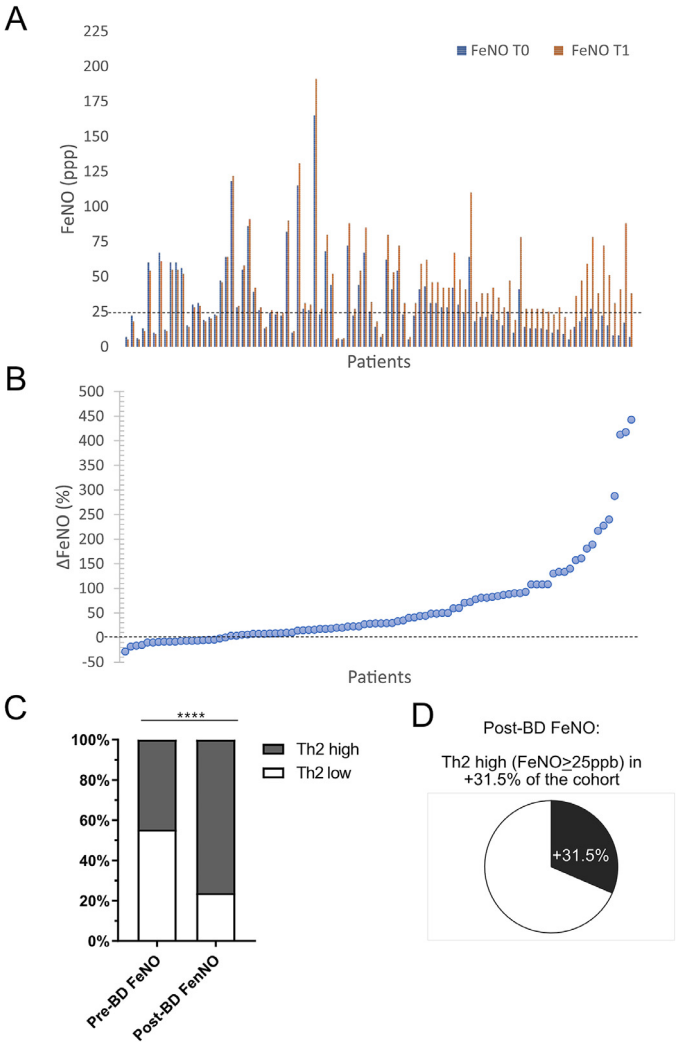

Figure 1. A, Absolute values of FeNO before (blue histograms) and FeNO after (orange histogram) BD with salbutamol 400 μg in the whole study cohort. B, Distribution of %ΔFeNO. C, Changes of patient phenotyping in "T_H2 high" vs "T_H2 low" asthma by FeNO cutoff greater than or equal to 25 ppb, using pre-BD FeNO vs post-BD FeNO. D, Post-BD FeNO increase of 31.5% in the patients categorized as "T_H2 high" asthma, as compared with pre-BD FeNO. BD, bronchodilation; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; ppb, parts per billion.

Characteristics of the Patients at Baseline by Fractional Exhaled Nitric Oxide Responder Status

The baseline characteristics of the 92 patients are presented in Table 1. Approximately half of the cohort were female, the mean age was 44.7 years, the average history of asthma was 11.5 years on average, and almost 60% of the patients had a history of atopy. Overall, FeNO responders were significantly older (mean age, 49.2 vs 37.8 years), and there was a trend toward being less atopic in this group. A higher proportion of patients had a worse control of asthma and more exercise-induced asthma symptoms in FeNO responders vs nonresponders (Table 1). FeNO responders had worse absolute values and percentages of FVC, had worse absolute values of FEV1, were more responsive to BD testing, and had significantly different IOS parameters, indicating SAD (ie, higher R5, R5R20, Ax, and Fres and more negative X5) (Table 1, eFig 2). When focusing on IOS parameters and standard spirometry to assess functional parameter in the study cohort, it became evident that R5-R20, Ax, and X5 were able to detect the presence of SAD in FeNO responders (ie, FeNO > 10%) vs nonresponders (≤10%), whereas forced expiratory flow between 25% and 75% (FEF25%-75%) values were similar between the 2 groups (eFig 2).

Change in Fractional Exhaled Nitric Oxide as a Marker of Small Airway Dysfunction

Because the strongest associations with FeNO categories were observed with IOS parameters, suggesting a higher frequency of SAD in FeNO responders, we performed univariate correlations between R5-R20 (a standardized IOS measure to define SAD) and FeNO before BD (at T0), FeNO after BD (at T1), and ΔFeNO (T1-T0). We observed a significant, moderate-to-strong correlation between ΔFeNO and R5R20 (Rho = 0.52, $P < .0001$), whereas correlations between R5R20 and FeNO before and after BD were not significant (Fig 2 A-C). Corrections for potential effect modifier were tested (ie, age and sex) and did not affect this analysis (median cohort age was 44.7 years: age > 45 years old had Rho = 0.43 with $P = .0042$ and age ≤ 45 years old had Rho = 0.52 with $P = .0002$; sex: males had Rho = 0.48 with $P = .0005$ and females had Rho = 0.52 with $P = .0002$) (eFig 3A-B).

When repeating the same correlation analyses between ΔFeNO and standard spirometry measures for this cohort of patients (eFig 4A-C), and specifically with FEV1/FVC% and FEV1% (conventionally used to assess central airway obstruction) and FEF25%-75% (conventionally used to assess peripheral airway obstruction), we found

Table 1
Features of the Patient Cohort With Asthma by FeNO Variation (ΔFeNO > 10% vs ≤ 10%) From Baseline After Bronchodilation

Patient features	All (N = 92)	FeNO responder 60.9% (n = 56)	FeNO nonresponder 39.1% (n = 36)	*P* value
Demographic, clinical, and laboratory features				
Female sex, n (%)	45 (48.9)	31 (51.7)	14 (38.9)	.184
Age (y), mean (SD)	44.7 (16.6)	49.2 (16.7)	37.8 (13.9)	**.001**
BMI (kg/m^2), median (IQR)	23.5 (5)	23.5 (5.25)	23.5 (5.25)	.544
Current or former smokers (>10 pack-years), n (%)	30 (32.6)	18 (32.1)	12 (33.3)	.999
Asthma duration (y), median (IQR)	11.5 (13)	10 (12.5)	13.5 (11)	.230
Presence of atopy, n (%)	55 (59.8)	29 (51.2)	26 (72.2)	.083
Eosinophils (mm^3), median (IQR)	413.5 (358.8)	450 (277.5)	360 (369.5)	.137
Basal FeNO (ppb), median (IQR)	23 (28.25)	22.5 (27)	23 (36.25)	.996
Patients with FeNO ≥ 25 ppb, n (%)	41 (44.6)	25 (44.6)	16 (44.4)	.999
Post-β_2 FeNO (ppb), median (IQR)	37 (30)	42 (29)	25.5 (36.5)	**<.001**
Post-β_2 FeNO change (ppb), median (IQR)	9.5 (16.25)	16 (9.5)	0.5 (3.25)	**<.001**
Standard spirometry				
FEV1 (% predicted), median (IQR)	77 (15.25)	76 (14.25)	79 (13.25)	.198
FEV1 (L), mean (SD)	2.6 (0.87)	2.41 (0.83)	2.92 (0.86)	**.006**
FVC (% predicted), mean (SD)	88.21 (12.5)	85.9 (12.3)	91.6 (12.4)	**.035**
FVC (L), mean (SD)	3.73 (1.1)	3.46 (1.01)	4.14 (1.12)	**.003**
FEV1/FVC (n), mean (SD)	69.49 (10.2)	69.4 (11.4)	69.7 (8.14)	.880
FEV1/FVC (% predicted), mean (SD)	84.15 (11.1)	84.3 (12.1)	83.7 (9.36)	.841
FEF25-75(%), mean (SD)	51.38 (17.7)	50.6 (19.1)	52.6 (15.6)	.605
FEV1 < 80% predicted, n (%)	54 (58.7)	36 (64.3)	18 (50)	.254
FEV1/FVC < 70%, n (%)	48 (52.2)	31 (55.3)	17 (47.2)	.583
FEF25-75(%) < 65, n (%)	73 (79.3)	44 (78.6)	29 (80.5)	.999
Post-β_2 FEV1 change (L), median (IQR)	0.13 (0.11)	0.15 (0.10)	0.12 (0.09)	**.024**
Impulse oscillometry				
R5 (kPa/(L/s)), mean (SD)	0.49 (0.17)	0.53 (0.19)	0.43 (0.12)	**.005**
R20 (kPa/(L/s)), mean (SD)	0.34 (0.12)	0.34 (0.12)	0.34 (0.09)	.991
X5 (kPa/(L/s)), median (IQR)	−0.15 (0.13)	−0.17 (0.14)	−0.13 (0.07)	**.019**
R5-R20 (kPa/(L/s)), median (IQR)	0.13 (0.15)	0.18 (0.13)	0.07 (0.07)	**<.001**
R5-R20 > 0.07 kPa/(L/s), n (%)	64 (69.6)	48 (85.7)	16 (44.4)	**<.001**
Fres (L/s), mean (SD)	22.1 (7.49)	24.3 (7.64)	18.62 (5.85)	**<.001**
AX (kPa/L), median (IQR)	1.2 (2.19)	2.0 (2.13)	0.65 (0.55)	**<.001**
AX > 1.0 kPa/L, n (%)	52 (56.5)	43 (76.8)	9 (25)	**<.001**
ΔX5 (kPa/(L/s)), median (IQR)	0.03 (0.12)	0.05 (0.17)	0.00 (0.06)	**.005**
Asthma control				
GINA—WC, n (%)	17 (18.5)	5 (29.4)	12 (70.6)	.1456
GINA—PC + UC, n (%)	75 (81.5)	51 (68)	24 (32)	**.0026**
Asthma exacerbations in the previous year, n (%)	36 (39.2)	26 (46.4)	10 (27.8)	.116
Night awakenings due to asthma, n (%)	53 (57.6)	35 (62.5)	18 (50)	.333
EIA symptoms, n (%)	61 (66.3)	27 (48.2)	14 (38.9)	**<.001**
Asthma therapy, n (%)				
ICS	92 (100)	56 (100)	36 (100)	1.000
LABA/ICS	72 (78.2)	44 (78.5)	28 (77.7)	1.000
Extrafine therapy	19 (20.7)	9 (17.3)	10 (29.4)	.290
LAMA	12 (13.04)	8 (14.2)	4 (11.1)	.901
Anti-leukotriene agents	15 (16.30)	10 (17.8)	5 (13.8)	.831

Abbreviations: AX, area of reactance; BMI, body mass index; EIA, exercise-induced asthma; FEF25%-75%, forced expiratory flow between 25% and 75%; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; Fres, resonance frequency; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; LABA, long-acting β-agonist; LAMA, long-acting muscarinic antagonist; PC, partially controlled; ppb, parts per billion; R5, resistance at 5 Hz; R20, resistance at 20 Hz; SAD, small airway dysfunction; UC, uncontrolled; WC, well controlled; X5, reactance at 5 Hz; ΔX5, difference between inspiratory and expiratory X5.
NOTE. Comparisons were analyzed using χ^2 test, Fisher exact tests, Student *t* test, or Mann-Whitney *U* test for independent samples (2-tailed), where appropriate.

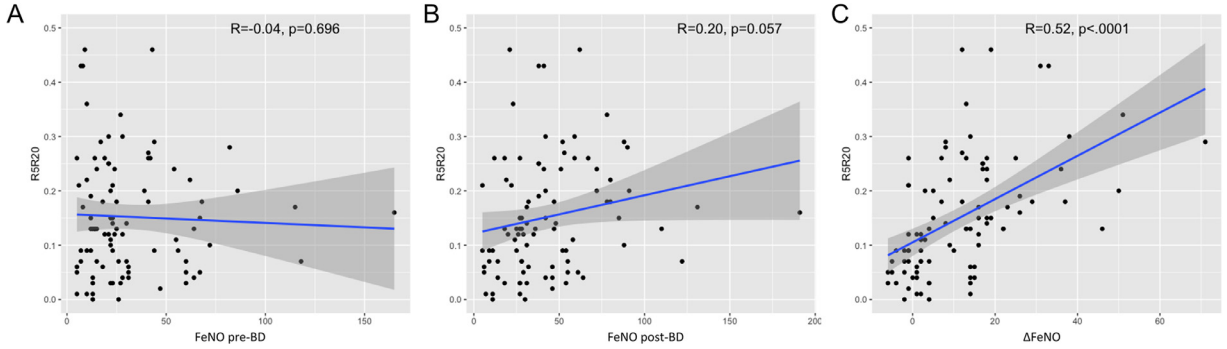

Figure 2. Correlations between R5R20 (the IOS-small airway dysfunction biomarker) and absolute values of FeNO (A) before and (B) after BD with salbutamol 400 μg, and (C) %ΔFeNO in the study cohort. Spearman's correlation was used for this analysis. The *R* and *P* values are reported at the top of each plot. BD, bronchodilation; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; IOS, impulse oscillometry; R5, resistance at 5 Hz; R20, resistance at 20 Hz.

either no correlation or significant weak inverse correlations (Rho = −0.19, *P* = .063; Rho = −0.32, *P* = .013; Rho = −0.25, *P* = .017, respectively), and similar results were found when testing correlation of post-BD FEV1% and ΔFeNO (eFig 5).

Similarly, no correlations were observed between ΔFeNO and clinical baseline continuous variables, namely body mass index, eosinophil levels, and asthma duration, whereas a weak but significant positive correlation was observed with age at clinical evaluation (Rho = 0.30, *P* = .004) (eFig 6A-D).

Asthma Control Correlates With Small Airway Dysfunction and Change in Fractional Exhaled Nitric Oxide

We analyzed the relationships among asthma control (GINA asthma control categories), pulmonary function tests, and IOS parameters (Table 2).

The IOS parameters indicating SAD (ie, R5-R20, X5, and AX) strongly correlated with the GINA categories, revealing WC asthma was associated with SAD and FEF25%-75% (ie, the measure assessing SAD in conventional spirometry) was only weakly associated. No correlation between FEV1/FVC and R20 was observed, indicating central airway involvement, whereas a direct correlation between GINA categories and FEV1 for spirometry was observed, with higher FEV1 values associated with a WC asthma GINA category. Overall, this indicated that SAD was inversely correlated with asthma control and that this association was better detected with the IOS.

To further analyze the relationship between SAD, FeNO, and asthma control GINA categories, we plotted the pre- and post-BD FeNO levels by GINA categories, observing a clear trend in both pre-BD and post-BD FeNO values with worsening of asthma control (Fig 3A and B), more evident with post-BD FeNO (B), with FeNO being higher in UC asthma than in WC. When stratifying the cohort by the presence of SAD (IOS-defined SAD, R5R20 > 0.07), in patients without SAD, we found no difference in FeNO values among the GINA asthma control groups (Fig 3C and D), whereas in the presence of SAD, the trend in FeNO values was evident (Fig 3E and F), indicating that SAD has a direct effect on the relationship between asthma control and FeNO, as hypothesized.

We then correlated asthma control and ΔFeNO (Table 2) and observed an inverse correlation between GINA categories and FeNO values, with WC asthma having lower values of ΔFeNO and with opposite distribution of patients into the GINA categories: FeNO non-responders were more likely to be in WC asthma GINA category (71%) as compared with PC (40%) and UC (20%), whereas FeNO responders were more likely to be in UC asthma category (80%) as compared with PC (60%) and WC (29%).

In the additional 29 patients (representing a 31.5% increase in the cohort) classified as "T_H2 high" only after post-BD FeNO measurement, 27 of 29 exhibited poor asthma control in nearly all cases (PC or UC), with a remarkable frequency of exacerbations in the preceding year.

The Effect of Asthma Control on Change in Fractional Exhaled Nitric Oxide Is Mediated by Small Airway Dysfunction

Because both SAD and ΔFeNO correlated with asthma control, we thus wanted to better investigate the casual relationship of this association. We performed a causal mediation analysis between asthma control as assessed by GINA control categories (independent variable), %ΔFeNO (dependent variable), and IOS-defined SAD as assessed by R5-R20 (mediator variable). The complete analysis of the direct (measured by the average direct effects [AMEs]) and indirect (mediated by SAD as reflected by ΔFeNO; measured by the average causal mediation effects) effects of asthma control on ΔFeNO is presented in Table 3 and eTable 2.

By means of the causal mediation analysis modeling, we observed that the effect of asthma control on ΔFeNO is mediated by SAD (Table 3, Fig 4): there was a strong and significant indirect effect of

Table 2
Asthma Control (GINA Asthma Control Categories), FeNO Values, and Pulmonary Function Tests

	Asthma control and small airway dysfunction				
	Well controlled (n = 17)	Partially controlled (n = 45)	Uncontrolled (n = 30)	*P* values ANOVA test/ Kruskal-Wallis test	*P* values well-controlled vs partially controlled + uncontrolled
Standard spirometry parameters					
FEV1 (% predicted), median (IQR)	84 (6)	76 (14)	74 (17.5)	**.0017**	**.0017**
FEV1 (L), mean (SD)	2.66 (1.17)	2.57 (1.11)	2.25 (0.81)	**.0333**	**.0469**
FEV1 < 80% predicted, n (%)	4 (23.5)	26 (57.8)	24 (80)	**.0008**	**.0028**
FVC (% predicted), mean (SD)	96 (14)	90 (13)	82.5 (16.5)	**.0044**	**.0091**
FVC (L), mean (SD)	4.09 (1.68)	3.59 (1.65)	3.22 (1.07)	.0577	**.0458**
FEV1/FVC (n), mean (SD)	72 (7)	69 (12)	67 (11.92)	.1885	.1285
FEV1/FVC < 70%, n (%)	6 (35.3)	24 (53.3)	18 (60)	.259	.2026
FEV1/FVC (% predicted), mean (SD)	87 (11)	85 (14)	83.5 (14.75)	.5975	.3334
FEF25-75(%), mean (SD)	59 (18)	51 (22)	48.5 (20.5)	**.0334**	**.0463**
FEF25-75(%) < 65, n (%)	13 (76.5)	34 (75.6)	26 (86.7)	.4878	.7458
IOS parameters					
R5 (kPa/(L/s)), mean (SD)	0.37 (0.04)	0.47 (0.24)	0.50 (0.16)	**.0052**	**.0016**
R20 (kPa/(L/s)), mean (SD)	0.31 (0.07)	0.34 (0.14)	0.33 (0.21)	.3615	.5096
X5 (kPa/(L/s)), median (IQR)	0.11 (0.06)	0.15 (0.13)	0.19 (0.14)	**.0069**	**.0017**
R5-R20 (kPa/(L/s)), median (IQR)	0.05 (0.04)	0.13 (0.12)	0.19 (0.13)	**<.0001**	**<.0001**
R5-R20 > 0.07 kPa/(L/s), n (%)	2 (11.7)	34 (75.6)	28 (93.3)	**<.0001**	**<.0001**
Fres (L/s), mean (SD)	14.8 (8.3)	21.4 (8.1)	26.0 (8.3)	**<.0001**	**<.0001**
AX (kPa/L), median (IQR)	0.48 (0.45)	1.13 (1.86)	2.18 (2.34)	**<.0001**	**<.0001**
AX > 1.0 kPa/L, n (%)	1 (5.9)	26 (57.8)	25 (83.3)	**<.0001**	**<.0001**
Asthma control and ΔFeNO					
ΔFeNO values, ppb (SD)	2 (6)	9 (16)	17 (16)	**<.0001**	**.0003**
Patients with FeNO ≤ 10%, n (%)	12 (71)	18 (40)	6 (20)	**.0029**	**.0076**
Patients with FeNO > 10%, n (%)	5 (29)	27 (60)	24 (80)	**.0029**	**.0076**

Abbreviations: ANOVA, analysis of variance; AX, area of reactance; BMI, body mass index; FEF25%-75%, forced expiratory flow between 25% and 75%; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; Fres, resonance frequency; FVC, forced vital capacity; GINA, Global Initiative for Asthma; IOS, impulse oscillometry; ppb, parts per billion; R5, resistance at 5 Hz; R20, resistance at 20 Hz; X5, reactance at 5 Hz.

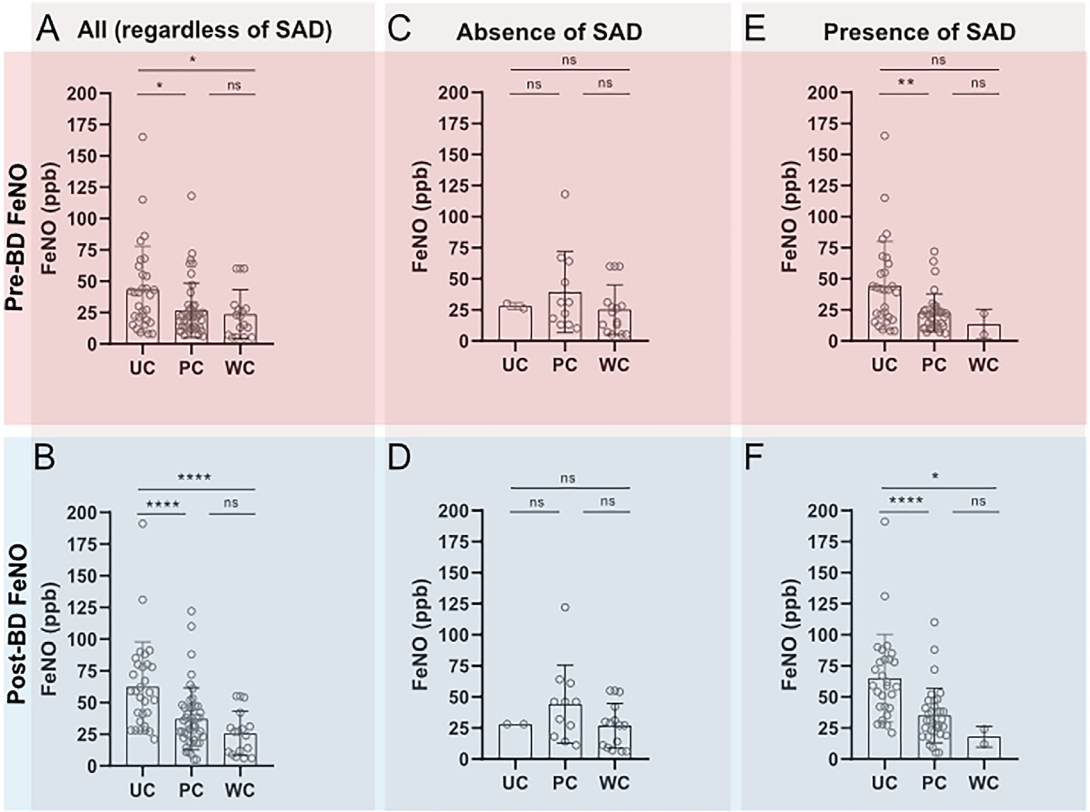

Figure 3. Comparison of pre-BD FeNO (upper quadrants) and post-BD FeNO (lower quadrants) according to GINA asthma control categories, that is, UC, PC, and WC asthma. In the whole cohort of patients, there is a reduction of (A) pre-BD FeNO levels and (B) post-BD FeNO levels by improving GINA categories. When stratifying the cohort by the presence of SAD (IOS-defied SAD, R5R20 > 0.07), in patients without SAD, no difference in FeNO values among GINA categories has been observed with both (C) pre-BD FeNO levels and (D) post-BD FeNO levels. In patients with SAD, the trend in FeNO values among GINA categories is evident with both (E) pre-BD FeNO levels and (F) post-BD FeNO levels. *P* values were determined by (C) 2-tailed Mann-Whitney *U* test and (D) Fisher exact test, respectively. BD, bronchodilation; FeNO, fractional exhaled nitric oxide; GINA, Global Initiative for Asthma; IOS, impulse oscillometry; PC, partially controlled; R5, resistance at 5 Hz; R20, resistance at 20 Hz; SAD, small airway dysfunction; UC, uncontrolled; WC, well controlled.

asthma control on ΔFeNO mediated by SAD (β value: −7.04, 95% CI: −11.80 to −3.53), without a significant direct effect (β value: −4.96; 95% CI: −9.15 to +0.11). This means, in other words, that the modification of ΔFeNO due to asthma control is completely mediated by SAD and SAD is responsible of FeNO change in this cohort.

Table 3
Causal Mediation Analysis Explaining the Relationship Between Asthma Control as Assessed by GINA Control Categories (Independent Variable), %ΔFeNO (Dependent Variable), and IOS-Defined SAD as Assessed by R5-R20 (Mediator Variable): Nonparametric Bootstrap CIs With the Percentile Method

Causal mediation analysis	Estimate (95% CI)	*P* value
ACME	−7.04 (−11.80 to −3.53)	<.0001[a]
ADE	−4.96 (−9.15 to 0.11)	.056
Total effect	−12.00 (-16.16 to −7.58)	<.0001[a]
Prop. mediated	0.59 (0.32-1.02)	<.0001[a]

Abbreviations: ACME, average causal mediation effects: indirect effect of asthma control (GINA categories) onto ΔFeNO through the mediator SAD (R5R20); ADE, average direct effects, direct effect of asthma control (GINA categories) onto ΔFeNO; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; GINA, Global Initiative for Asthma; IOS, impulse oscillometry; prop. mediated, the proportion of the effect of GINA on FeNO passing through the mediator; R5, resistance at 5 Hz; R20, resistance at 20 Hz; SAD, small airway dysfunction; total effect, total effect (direct + indirect) of GINA onto ΔFeNO.
Note. Because ADE is not significant, in such cases, this is defined "complete mediation." This means that the total effect of GINA on ΔFeNO is explained by the mediator.
[a]In all the reported cases, the *P* values were less than $2e^{-16}$.

Discussion

Here, we showed that in community-treated, adult patients with asthma, the FeNO percent change before and after BD (ΔFeNO) strongly correlates with SAD as assessed by IOS. In addition, we also showed that asthma control correlates with SAD and ΔFeNO levels, and that "FeNO nonresponders" (ie, those with ΔFeNO ≤ 10%) were more likely to be in WC asthma GINA category as compared with PC and UC, and that "FeNO responders" (ie, those with ΔFeNO > 10%) were more likely to be in UC asthma category as compared with PC and WC. Overall, these data confirm the previous reports, in which the evaluation of airway inflammation by FeNO was found to correlate with changes in airway caliber induced by BD, adding to previous reports that ΔFeNO is more relevant from a functional perspective than "simple" FeNO measured before BD. Furthermore, we analyze the effects of these associations between ΔFeNO, SAD, and asthma control, and through causal mediation analysis modeling, we observed that the effect of asthma control on FeNO changes is mediated by small airway obstruction mainly through an indirect effect and without any direct effect of asthma control on ΔFeNO in this cohort. These findings provide evidence of how asthma control is linked to FeNO, indicating that FeNO levels in patients with asthma result from both T_H2 inflammation and small airway obstruction, undermining the dogma of direct causality of airway inflammation.

FeNO levels are considered elevated typically above 25 ppb, indicating patients with T2-high asthma. In our analysis, FeNO measurements after BD identified an additional 29 patients with FeNO greater than or equal to 25 ppb (increasing from 41 to 70 patients, representing a 31.5% rise in the cohort), suggesting that these patients could

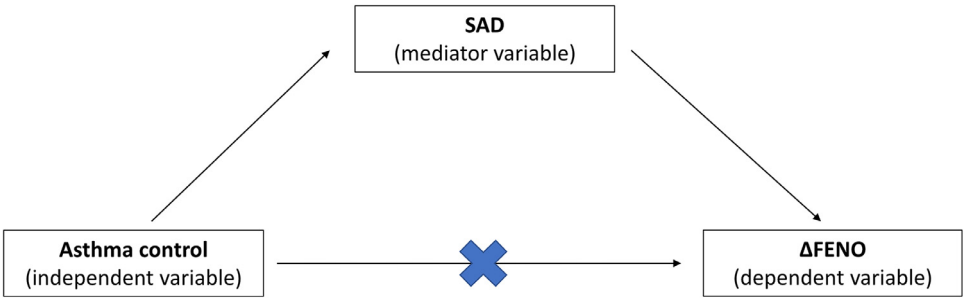

Figure 4. The causal mediation analysis between asthma control as assessed by GINA control categories (independent variable), %ΔFeNO (dependent variable), and IOS-defined SAD as assessed by R5 to R20 (mediator variable). The effect of asthma control on ΔFeNO is mediated by SAD: a strong and significant indirect effect of asthma control on ΔFeNO mediated by SAD (β value: −7.04, 95% CI: −11.80 to −3.53) has been observed, whereas no significant direct effect has been observed (β value: −4.96; 95% CI: −9.15 to +0.11). FeNO, fractional exhaled nitric oxide; %ΔFeNO, percentage change in fractional exhaled nitric oxide; GINA, Global Initiative for Asthma; IOS, impulse oscillometry; R5, resistance at 5 Hz; R20, resistance at 20 Hz; SAD, small airway dysfunction.

potentially be reclassified as the "T2-high" asthma phenotype if such categorization were based on FeNO levels obtained after BD. Interestingly, 28 of 29 of these patients were classified as having GINA PC or UC, revealing an interesting association between scarce asthma control and falsely normal FeNO levels, which could be unmasked by measuring FeNO after BD instead of FeNO after BD, as per clinical routine. This result is straightforward because it allows for a better phenotype of patients with worse asthma control, eventually allowing them to provide better clinical care (eg, quicker access to biologic drugs). Monitoring FeNO levels can aid in defining asthma phenotypes and assessing the efficacy of corticosteroid therapy, including treatment adherence. In addition, FeNO measurement also demonstrates utility in predicting response to biologic therapy, with higher baseline FeNO values predicting better response.[25-27] Considering our data, we believe that the assessment of ΔFENO and FeNO values post-BD can improve the ability of this T_H2 biomarker to identify poor asthma control.

FeNO is widely acknowledged as a T_H2 indicator of airway inflammation because a FeNO value greater than or equal to 25 ppb is conventionally considered to mirror the eosinophilic inflammation in the airways of individuals with asthma,[2] but the fact that blood eosinophils and FeNO provide additive prognostic information is predictable, as both biomarkers provide different and complementary mechanistic information.[28] FeNO is generally measured before BD during spirometry, and clinicians rely on this value to assess airway inflammation. Because an increase in FeNO levels has been associated with poor asthma control, worse lung function, and a higher frequency of asthma exacerbations and lung function decline, along with its simple execution, FeNO use has increased significantly in the last years.[29] Recently, it has been understood that FeNO values could be the result of an intricate interplay of airway eosinophilic inflammation with airway caliber, and FeNO could be influenced by factors unrelated to airway inflammation, such as changes in lung function that occur during the course of asthma. Our study further corroborated these findings indicating ΔFeNO as the value that better displays this relationship. Owing to the use of IOS, a physiological method capable of accurately detecting changes in the small airway compartment, this study revealed strong correlations between IOS parameters reflecting SAD and FeNO changes.

Our work also stratified the patients into FeNO responders and nonresponders[10] based on the variability of FeNO after BD, providing evidence of clinical and functional differences among the different patient groups and revealing their opposite distribution within the GINA asthma control category, that is, WC, PC, and UC. Previously, we revealed that the proportion of patients with SAD distributed in a similar fashion to the distribution of FeNO responders in the current paper, and again herein, we revealed that IOS measurements reflecting SAD behave similarly.[17] Intuitively, these features suggest the influence of SAD between asthma control and ΔFeNO, which preluded our further set of analyses.

To clarify the interplay between ΔFeNO, SAD, and asthma control, we used a causal mediation analysis, modeling the direct and indirect effects of asthma control on ΔFeNO. In the current cohort, we revealed a very strong indirect effect of SAD in asthma control on ΔFeNO, while revealing no significant direct effect of asthma control on ΔFeNO. The directionality of these relationships has never been clearly depicted before; therefore, we hope that our data will contribute to the current knowledge in the field that evolved after the first publications, suggesting different patterns of FeNO response after BD.[7-10]

Our study has several strengths and limitations. We used the definition of physician-diagnosed asthma considering the potential risk of overdiagnosis and the lack of data on treatment adherence. However, this is the gold standard used for several real-life studies,[30,31] and in most cases, the diagnosis is supported by a standard spirometry and/or methacholine challenge. Somebody could argue that the conventional cutoff of FeNO (25 ppb) used in routine practice is approved for pre-BD FeNO and applying it to post-BD FeNO, in the absence of a defined cutoff for post-BD FeNO (as we did to account for the fraction of patients classified as "T_H2 low" and "T_H2 high"), may be an incorrect assumption.

Another limitation is the potentially arbitrary threshold of a 10% ΔFeNO chosen to define the 2 groups. However, we chose this threshold because it has already been used in other publications and is considered to be acceptable.[7,10,32]

Other studies have evaluated small airway involvement using a single breath washout test (S) of He (SHe) and SSF6 (sulfur hexafluoride).[7,10] Even if our study did not go into physiological details or use inert gas breath washout techniques as others, we introduced an easier-to-use tool such as the IOS, accepted as and with a broader clinical use, which allowed us to test a higher number of patients as compared with previous reports.[7,10,32]

Regarding the methodology used, one could argue that the FeNO level can change after bronchodilator administration owing to a reduction in bronchial inflammation, thus losing its value as a biomarker for T_H2 airway inflammation. However, from a physiological perspective, it has been well demonstrated that FeNO production occurs in the small airways.[6] Therefore, in the presence of bronchial obstruction, the evaluation of FeNO post-BD is more reliable when obstruction relief of the central airways by β_2-agonists (post-BD) leads to FeNO diffusion in large airways (avoiding the well-known "back-diffusion" from bronchioles to the alveoli). In addition, a direct action of bronchoconstriction in the peripheral trees coexists,[6-10] which further reduces FeNO production from the small airway epithelium when constricted, could be avoided using BD. In conclusion, FeNO is a marker of airway inflammation linked to the physiology of the small airways; using bronchodilator for testing airways does not

reduce inflammation but just alter the airway caliber, without affecting FeNO role as a biomarker for measuring T_H2 airway inflammation.

Finally, to confirm our causal mediation analysis, the study should be repeated in a larger independent cohort. However, the indirect effect of SAD was so strong ($P < 2e{-}16$) that it was difficult to think that another independent population of patients with asthma with similar features could completely change our observations.

In conclusion, FeNO changes after BD do not consistently result in an increase in the FeNO value, permitting patient stratification into FeNO responders and nonresponders, with FeNO changes after BD strongly correlating with SAD as assessed by IOS. Asthma control correlates with SAD and ΔFeNO levels, and patients classified as FeNO nonresponders were more likely to be in WC asthma GINA category as compared with PC and UC, whereas FeNO responders were more likely to be in UC asthma category as compared with PC and WC. Finally, through causal mediation analysis modeling, we observed that the effect of asthma control on ΔFeNO is mainly mediated through an indirect effect by small airway obstruction, providing evidence of how asthma control is linked to FeNO. Taken altogether, these findings confirmed a role for ΔFeNO in identifying the site of airway obstruction in patients with asthma and highlight how IOS-defined SAD, together with T_H2 inflammation, contributes to determining the correlation between FeNO values and the degree of asthma control in real life.

Data Availability

All data sets generated for this study have been included in this article.

Disclosures

Dr Berti received funding from GlaxoSmithKline (advisory boards and speaker fees) unrelated to the present work. The authors have no conflicts of interest to report.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.anai.2025.01.003.

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Supplementary Data

eTable 1
Features of the Patient Cohort With Asthma (N = 100) by FeNO Variation (10%) From Baseline After Bronchodilation, Divided in the 3 Categories According to Alain Michlis et al, JACI 2016

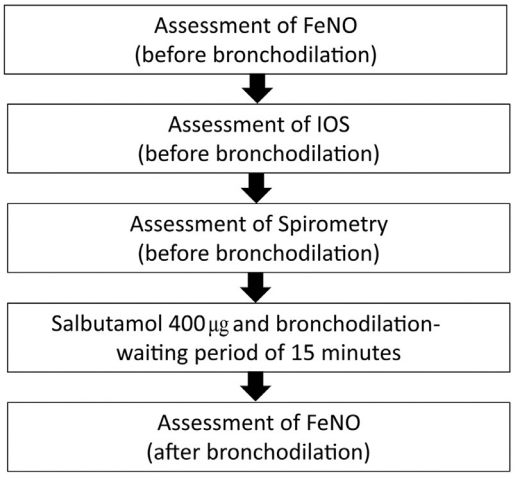
Patient features	FeNO reduced (ΔFeNO > −10%) 8.0% (n = 8)	FeNO responder (ΔFeNO > 10%) 56.0% (n = 56)	FeNO nonresponder (ΔFENO ≤ 10%) 36.0% (n = 36)	*P* value
Demographic, clinical, and laboratory features				
Female sex, n (%)	4 (50)	31 (51.7)	14 (38.9)	.434
Age (y), mean (SD)	38.8 (17.4)	49.2 (16.7)	37.8 (13.9)	.004 a-u
BMI, (kg/m^2), median (IQR)	24 (5.5)	23.5 (5.25)	23.5 (5.25)	.848
Current or former smokers (>10 pack-years), n (%)	2 (25)	18 (32.1)	12 (33.3)	.948
Asthma duration (y), median (IQR)	15 (1.25)	10 (12.5)	13.5 (11)	.278
Presence of atopy, n (%)	5 (62.5)	29 (51.2)	26 (72.2)	.172
Eosinophils (mm^3), median (IQR)	220.5 (95.7)	450 (277.5)	360 (369.5)	.137
Basal FeNO (ppb), median (IQR)	25.5 (11.25)	22.5 (27)	23 (36.25)	.796
Patients with FeNO ≥ 25 ppb, n (%)	4 (50)	25 (44.6)	16 (44.4)	.999
Post-β_2 FeNO (ppb), median (IQR)	14.5 (15.2)	42 (29)	25.5 (36.5)	<.001
Post-β_2 FeNO change (ppb), median (IQR)	10.5 (6.25)	16 (9.5)	0.5 (3.25)	<.001
Standard spirometry				
FEV1 (% predicted), median (IQR)	82 (8)	76 (14.25)	79 (13.25)	.335
FEV1 (L), mean (SD)	2.6 (0.61)	2.41 (0.83)	2.92 (0.86)	.040
FVC (% predicted), mean (SD)	92 (15.0)	85.9 (12.3)	91.6 (12.4)	.093
FVC (L), mean (SD)	3.99 (1.18)	3.46 (1.01)	4.14 (1.12)	.029 a-u
FEV1/FVC (n), mean (SD)	74.5 (12.7)	69.4 (11.4)	69.7 (8.14)	.791
FEV1/FVC (% predicted), mean (SD)	81.3 (15.7)	84.3 (12.1)	83.7 (9.36)	.839
FEF25-75 (%), mean (SD)	54.8 (21.9)	50.6 (19.1)	52.6 (15.6)	.859
Post-β_2 FEV1 change (L), median (IQR)	0.1 (0.06)	0.15 (0.10)	0.12 (0.09)	.011 a-u
Impulse oscillometry				
R5 (kPa/(L/s)), mean (SD)	0.42 (0.10)	0.53 (0.19)	0.43 (0.12)	.025
R20 (kPa/(L/s)), mean (SD)	0.33 (0.06)	0.34 (0.12)	0.34 (0.09)	.994
X5 Hz (kPa/(L/s)), median (IQR)	−0.14 (0.07)	−0.17 (0.14)	−0.13 (0.07)	.037
R5-R20 (kPa/(L/s)), median (IQR)	0.05 (0.09)	0.18 (0.13)	0.07 (0.07)	<.001
Fres (L/s), mean (SD)	17.8 (7.66)	24.3 (7.64)	18.62 (5.85)	<.001
AX (kPa/L), median (IQR)	0.56 (0.91)	2.0 (2.13)	0.65 (0.55)	<.001
ΔX5 Hz (kPa/(L/s)), median (IQR)	0.00 (0.08)	0.05 (0.17)	0.00 (0.06)	.037
Asthma control, n (%)				
GINA—WC	0 (0)	5 (29.4)	12 (70.6)	.014
GINA—PC + UC	8 (100)	51 (68)	24 (32)	.014
Asthma exacerbations in the previous year, n (%)	4 (50)	26 (46.4)	10 (27.8)	.249
Night awakenings due to asthma, n (%)	4 (50)	35 (62.5)	18 (50)	.582
EIA symptoms, n (%)	4 (50)	27 (48.2)	14 (38.9)	<.001
Asthma therapy, n (%)				
ICS	8 (100)	56 (100)	36 (100)	1.000
LABA/ICS	6 (75.0)	44 (78.5)	28 (77.7)	1.000
Extrafine therapy	2 (25.0)	9 (17.3)	10 (29.4)	.370
LAMA	1 (12.5)	8 (14.2)	4 (11.1)	.950
Anti-leukotriene agents	1 (12.5)	10 (17.8)	5 (13.8)	.830

Abbreviations: AX, area of reactance; BMI, body mass index; EIA, exercise-induced asthma; FEF25%-75%, forced expiratory flow between 25% and 75%; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; Fres, resonance frequency; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; LABA, long-acting β-agonist; LAMA, long-acting muscarinic antagonist; PC, partially controlled; ppb, parts per billion; R5, resistance at 5 Hz; R20, resistance at 20 Hz; SAD, small airway dysfunction; UC, uncontrolled; WC, well controlled; X5, reactance at 5 Hz; ΔX5, difference between inspiratory and expiratory X5.
NOTE. Comparisons were analyzed using χ^2 test, Fisher exact tests, Student *t* test, or Mann-Whitney *U* test for independent samples (2-tailed), where appropriate.

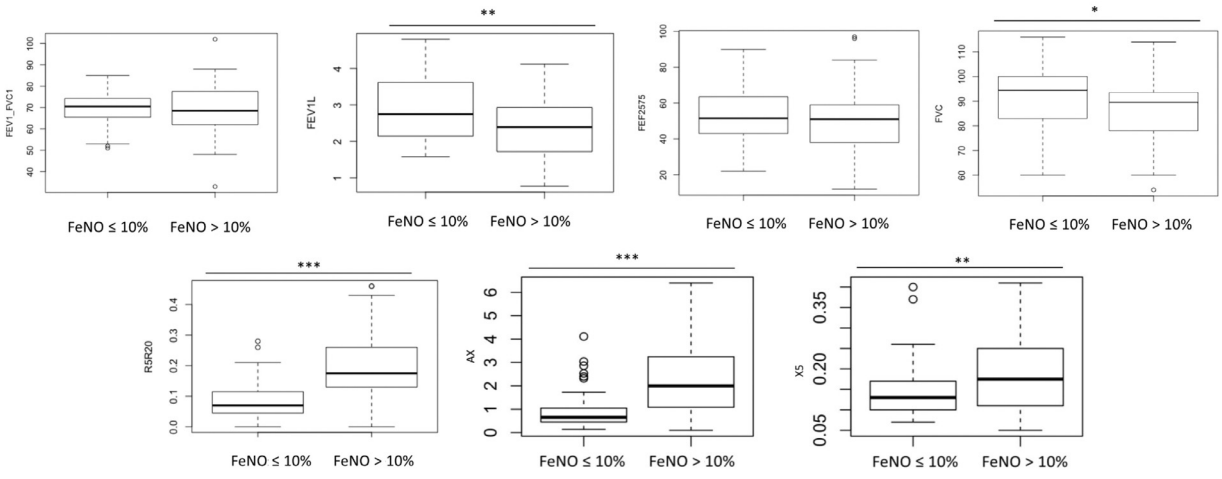
eTable 2
Details of the Causal Mediation Analysis Explaining the Relationship Between Asthma Control as Assessed by GINA Control Categories (Independent Variable), %ΔFENO (Dependent Variable) and IOS-Defined SAD as Assessed by R5-R20 (Mediator Variable)

Step 1: GINA (X) su ΔFeNO (Y)	Step 2: GINA (X) su R5R20 (M)	Step 3: GINA (X) e R5R20 (M) su ΔFeNO (Y)
> fit.totaleffect=lm(Δfeno∼GINA)	> fit.mediator=lm(R5R20∼ GINA)	> fit=lm(varfeno∼GINA+R5R20)
> summary(fit.totaleffect)	> summary(fit.mediator)	> summary(fit)
Coefficients:	Coefficients:	Estimate (SE), *P* values
Estimate (SE), *P* values	Estimate (SE), *P* values Intercept: 0.17 (0.01, 15.3), $P < 2e^{-16}$	Intercept: 3.68 (2.64, 1.40), *P* = .166
Intercept: 14.00 (1.53, 9.14), $P = 1.76e^{-14}$	**GINA-WC, Estimate (95% CI):**	**GINA-WC, Estimate (95% CI):**
GINA-WC, Estimate (95% CI):	**−0.12 (−0.17; −0.07), $P < 1.96e^{-05}$**	**−4.96 (−12.04; 2.12), *P* = .168**
−12.00 (−19.08; −4.92), *P* = .00112		**R5R20, Estimate (95% CI):**
		59.29 (33.66; 84.91), $P = 1.41^{e-05}$
Causal mediation analysis		
Nonparametric Bootstrap CIs with the Percentile Method		
Estimate (95% CI), *P* value		
ACME: −7.04 (−11.80 to −3.53), $P < 2e^{-16}$		
ADE: −4.96 (−9.15 to 0.11), *P* = .056		
Total effect: −12.00 (−16.16 to −7.58); $P < 2e^{-16}$		
Prop. mediated: 0.59 (0.32-1.02); $P < 2e^{-16}$		

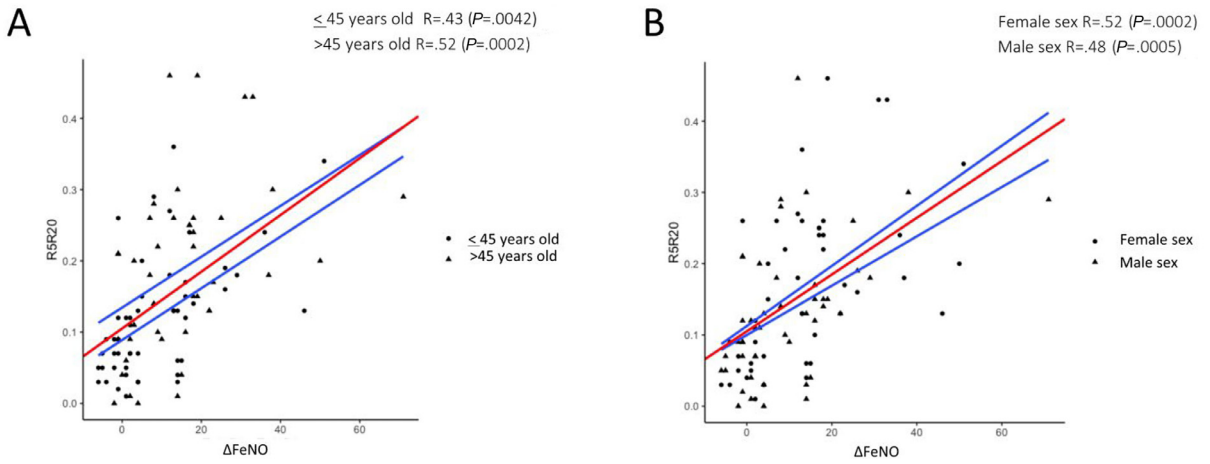
Abbreviations: ACME, average causal mediation effects: indirect effect of asthma control (GINA categories) onto ΔFeNO through the mediator SAD (R5R20); ADE, average direct effects, direct effect of asthma control (GINA categories) onto ΔFeNO; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; GINA, Global Initiative for Asthma; IOS, impulse oscillometry; prop. mediated, the proportion of the effect of GINA on FeNO passing through the mediator; R5, resistance at 5 Hz; R20, resistance at 20 Hz; SAD, small airway dysfunction; total effect, total effect (direct + indirect) of GINA onto ΔFeNO.
NOTE. Because ADE is not significant, in such cases, this is defined "complete mediation." This means that the total effect of GINA on ΔFeNO is explained by the mediator.



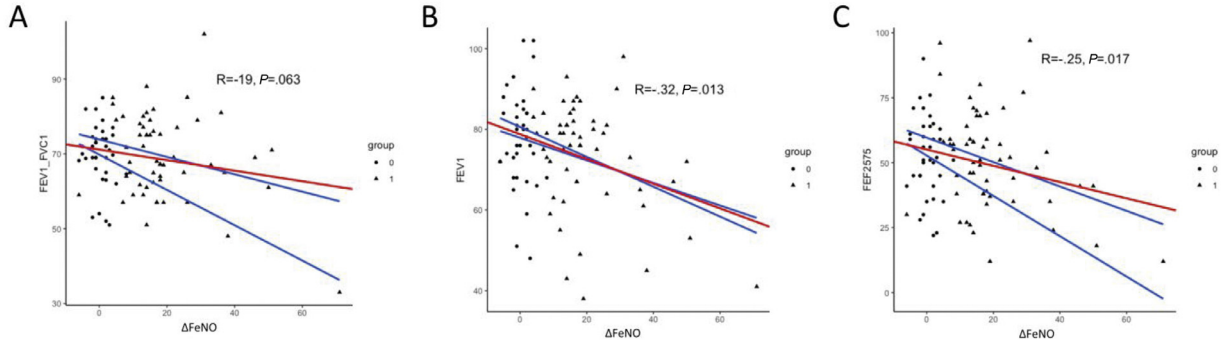
eFigure 1. Representation of the study design. To avoid perturbation during volume testing, current volume testing was performed before forced testing. FeNO, fractional exhaled nitric oxide; IOS, impulse oscillometry.



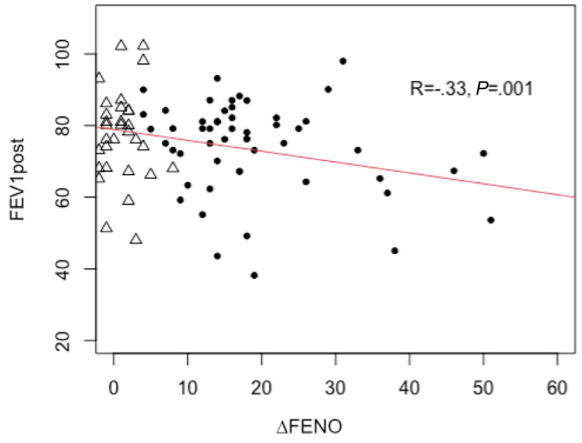
eFigure 2. Standard spirometry and IOS parameters to assess bronchoconstriction in the study cohort. Horizontal lines illustrate the median with 25% to 75% IQR. *P* values were determined a 2-tailed Mann-Whitney *U* test. *P* values in the figures are indicated as * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$. AX, area of reactance; FEF25%-75%, forced expiratory flow between 25% and 75%; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IOS, impulse oscillometry; X5, reactance at 5 Hz.



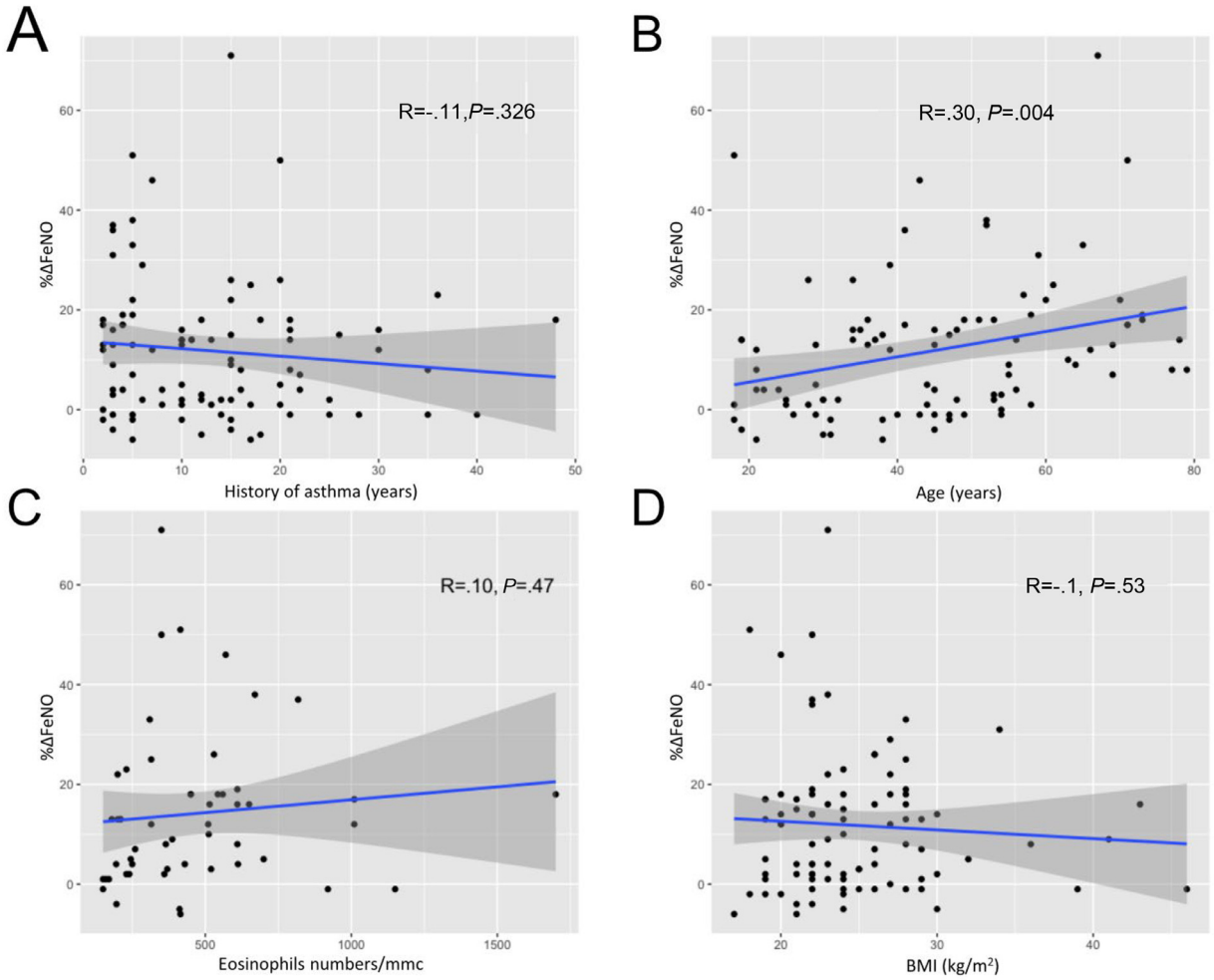
eFigure 3. Scatterplots and correlations between R5R20 (the IOS-SAD biomarker) and %ΔFeNO in the study cohort by potential effect modifiers, that is (A) age (>45 vs <45 years) and (B) sex. Spearman's correlation coefficient was used for analysis. The *R* and *P* values are reported at the top of each plot. Red lines represent the whole group, and blue lines represent the subsets by (A) age and (B) sex. FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; IOS, impulse oscillometry; R5, resistance at 5 Hz; R20, resistance at 20 Hz; SAD, small airway dysfunction.



eFigure 4. Scatterplots and correlations between %ΔFENO and FEV1/FVC% (A), FEV1% (conventionally used to assess central airway obstruction) (B), and FEF25%-75% (conventionally used to assess peripheral airway obstruction) (C) in the study cohort by a potential effect modifier, that is age (>45 vs <45 years). Spearman's correlation coefficient was used for analysis. The *R* and *P* values are reported at the top of each plot. Red lines represent whole groups, and blue lines represent subsets by age. FEF25%-75%, forced expiratory flow between 25% and 75%; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity.



eFigure 5. Scatterplot and correlation between %ΔFENO and post-BD FEV1 in the study cohort by a potential effect modifier, that is age (>45 vs <45 years). Spearman's correlation coefficient was used for analysis. The *R* and *P* values are reported at the top of the plot. BD, bronchodilation; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second.



eFigure 6. Scatterplots and correlations between %ΔFENO and clinical and laboratory features, that is (A) asthma duration in years, (B) age in years, (C) eosinophil levels/mmc, and (D) BMI (kg/m^2). Spearman's correlation coefficient was used for analysis. The *R* and *P* values are reported at the top of each plot. BMI, body mass index; FeNO, fractional exhaled nitric oxide; ΔFeNO, change in fractional exhaled nitric oxide.