The Interpretation of Exhaled Nitric Oxide Values in Children With Asthma Depends on the Degree of Bronchoconstriction and the Levels of Asthma Severity

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BACKGROUND: The clinical implications of fractional exhaled nitric oxide (F_{ENO}) measurements in childhood asthma are unclear. We aimed to evaluate the relationship between the level of exhaled nitric oxide and pre-bronchodilator FEV_1 and the change in FEV_1 after bronchodilator in children with asthma. METHODS: This was a retrospective, cross-sectional study. We evaluated data from medical documentation of children with asthma with special attention to F_{ENO} results, asthma severity, FEV_1 (% predicted), and bronchial reversibility test. RESULTS: Four hundred and five subjects (age 6–18 y) completed the study. Median levels of F_{ENO} increased linearly with subjects' age ($P = .03$). We found a nonlinear trend of pre-bronchodilator FEV_1 across 4 quartiles of F_{ENO} in episodic and mild asthma; we observed lower pre-bronchodilator FEV_1 in children with higher F_{ENO}, but only up to the F_{ENO} value of 35.4 ppb; in children with F_{ENO} value > 35.4 ppb, pre-bronchodilator FEV_1 was increased. We found a linear increasing trend of change from baseline (after 400 μg of salbutamol) in FEV_1 across F_{ENO} categories in children with moderate asthma. CONCLUSIONS: Our results suggest a need to measure F_{ENO} before as well as after spirometry. Consequently, in children with asthma with bronchial obstruction, we suggest assessing F_{ENO} after short-acting β_2 agonists as well. (ClinicalTrials.gov registration NCT00815984.) *Key words: F_{ENO}; FEV_1; asthma; children; airway caliber; glucocorticosteroids; asthma severity.* [Respir Care 2014;59(9):1404–1411. © 2014 Daedalus Enterprises]

Introduction

The current concept of asthma pathogenesis underlines a chronic inflammatory process, which causes air flow obstruction and bronchial hyper-responsiveness.[1] The exact pathophysiological role of nitric oxide (NO) in the airways and lungs is complex.[2-4] On the one hand, it may act as a pro-inflammatory mediator predisposing to the development of airway hyper-responsiveness; on the other hand, under physiological conditions, it acts as a weak mediator of smooth muscle relaxation and protects against airway hyper-responsiveness.[2-7] Recently, it has been proved that F_{ENO} results are in disagreement with other measurements of asthma control in children with asthma,

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namely spirometry, children's asthma control test, and conventional clinical assessment.[8] Green et al[8] showed that mean F_{ENO} in pediatrician-judged uncontrolled asthma was double that of controlled asthma. F_{ENO} correlates with bronchial reactivity[9] and decreases with anti-inflammatory asthma therapy, such as inhaled corticosteroids (ICS) and anti-leukotrienes, in children.[10] F_{ENO} values can be affected by several factors.[2] We are aware of the fact that most children with asthma have normal FEV_1 outside acute attacks; however, to date, no study has assessed the influence of the degree of baseline bronchoconstriction on F_{ENO} results in children.[11,12] Current guidelines suggest the use of cut points rather than reference values when interpreting F_{ENO} results, but this recommendation is weak, based on evidence of low quality.[2] Therefore, our analysis was focused on investigating the relationship between F_{ENO} measurements and the degree of bronchoconstriction. Specifically, we evaluated the relationship between the level of exhaled nitric oxide and pre-bronchodilator FEV_1 and the change in FEV_1 after bronchodilator in children with asthma.

QUICK LOOK

Current knowledge

Exhaled nitric oxide concentrations have been shown to increase in a variety of disorders associated with lung inflammation. The usefulness of measuring exhaled nitric oxide in monitoring disease progression or disease management has not been proven.

What this paper contributes to our knowledge

Exhaled nitric oxide concentrations increased linearly with age, but the relationship of exhaled nitric oxide and FEV_1 demonstrated only a nonlinear trend. Exhaled nitric oxide should be measured before and after the FEV_1 measurement. Exhaled nitric oxide alone was not a discriminator of lung inflammation or degree of dysfunction.

Methods

Study Design

The study was retrospective and cross-sectional, and was performed at the Department of Pediatrics and Allergy, Medical University of Lodz, N Copernicus Hospital (Lodz, Poland). We evaluated data from medical documentation of 943 children (age 6–18 y) with symptoms suggestive of asthma, who attended our out-patient allergy clinic from January 2008 to March 2009. We included subjects with at least 2 y of clinical observation, whose asthma was either confirmed or excluded. Children with asthma and allergic rhinitis (64%) were also included. The 405 subjects analyzed had F_{ENO} measurement, spirometry, and bronchial reversibility test performed at the same visit. The diagnosis and the severity of asthma and allergic rhinitis were universally established by the medical doctors (all doctors involved were from our out-patient allergy clinic) according to standard definitions of both diseases in the latest guidelines.[1,13] Diagnosis of asthma was universally established by allergy specialists on the basis of the symptoms of asthma, the findings of the physical examination of the respiratory system, and the improvement in the pre-bronchodilator $FEV_1 \geq 12\%$ after the administration of salbutamol (200 μg) in all patients.[1] Medical documentation of the subjects was analyzed with special attention to the results of F_{ENO}, spirometry, bronchial reversibility test, and allergic rhinitis diagnosis, as well as allergen sensitization and treatment. Non-atopic children with asthma who showed normal F_{ENO} values were excluded from the analysis. We analyzed the mean doses of inhaled glucocorticosteroids, which were assessed throughout the period of 3 months preceding the measurements of F_{ENO} and spirometry (the dose of inhaled glucocorticosteroid was stable throughout that period in children with asthma). Children had been on inhaled glucocorticosteroids since the diagnosis of asthma. Inhalation technique was routinely checked at each visit by allergy specialists in our out-patient allergy clinic. All patients with asthma in this study were controlled. For the purpose of this study, we categorized our patients according to the level of treatment into the following study groups: an episodic/low steroid daily use (episodic asthma), a medium steroid daily use (mild asthma), or a high steroid daily use (moderate asthma). Such an approach allowed us to obtain almost equal sample size in the study groups; therefore, it facilitated statistical analysis. The healthy group consisted of patients in whom asthma and allergic rhinitis were excluded and who were free of any kind of current illnesses. All tests among the healthy subjects were performed during differential diagnosis of asthma. Subjects from the healthy group were children with no asthma and with no atopy according to a negative prick test for common inhalant and food allergens; none had respiratory tract symptoms nor were treated with any drug in the 2 months preceding the evaluation of the results. The study was approved by the medical ethical committee of the Medical University of Lodz, Lodz, Poland. All parents or guardians of the patients gave their oral and written consent for the evaluation of data from medical documentation of their children. The study was registered at www.ClinicalTrials.gov as NCT00815984.

Allergen Sensitization

All subjects underwent skin prick test with common inhalant and food allergens (allergy profile)*: Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria*, *Cladosporium*, cat dander, dog dander, mixed grass pollen, rye, birch, hazel, ribwort, alder, motherwort, feather, cocoa, milk, egg, and peanut. Positive (histamine chloride, 10 mg/mL) and negative (glycerol) controls (extracts from Nexter-Allergopharma, Reinbek, Germany) were also used. A positive skin prick test reaction was defined as a mean wheal diameter $>$ 3 mm in excess of the negative control. Atopy was defined as a positive skin test response to any of the 18 allergens tested.

Nitric Oxide Measurement

In our out-patient allergy clinic, F_{ENO} was measured before a forced flow-volume curve measurement and bronchial reversibility test in all subjects on the same day (in the morning, between 9 AM and 11 AM). The NO measurements were performed according to the European Respiratory Society/American Thoracic Society recommendations,[14] with a chemiluminescence analyzer (model 280i nitric oxide analyzer, Sievers, Boulder, Colorado) and defined in parts per billion. The analyzer provides an online continuous measurement of NO in a single exhalation with a detection range of 0.1–500 ppb. Environmental NO was measured before and after each test, and it never exceeded 5 ppb. Dead space and nasal NO (which are reflected by the NO concentration peak during exhalation) and NO from the lower respiratory tract (determined by the plateau value after the peak) were recorded automatically by using the manufacturer's software. Three F_{ENO} measurements of the plateau phase were obtained, with $<$ 10% variation. The mean value of 3 successive, reproducible recordings was retained for statistical analysis.

FEV_1

Pulmonary function testing was performed with a Master Screen unit (Erich Jaeger GmbH, Hochberg, Germany). Predicted values for all lung function variables were based on a previous study of healthy controls.[15-18] Flow-volume curves were performed according to the American Thoracic Society standards.[17] The highest of 3 successful measurements was taken and analyzed. The results were expressed as the percentage of a predicted value. All the subjects were able to perform spirometry adequately.

Bronchial Reversibility Test

Reversibility test was performed after the administration of salbutamol (400 μg), according to the latest American Thoracic Society guideline.[18] The percentage of change from baseline in FEV_1 after salbutamol and pre- and post-bronchodilator FEV_1 values were included in the analysis.

Statistics

The analysis was performed in 4 different subgroups: (1) episodic asthma, defined as episodic use of low dose, 100–200 μg of steroid dose equivalent to budesonide (metered-dose inhaler) daily; (2) mild asthma, defined as medium dose, 200–400 μg of steroid dose equivalent to budesonide (metered-dose inhaler) daily; (3) moderate asthma, defined as high dose, $>$ 400 μg of steroid dose equivalent to budesonide (metered-dose inhaler) daily; and (4) healthy subjects. To assess the relationship between FEV_1 (as a dependent variable) and F_{ENO} level, an analysis of variance (ANOVA) was implemented. Additionally, a test for a linear and nonlinear trend was used. The above relationship was adjusted for the effect of age, sex, presence of allergic rhinitis, allergy profile, and asthma severity. The analysis of variance was implemented to assess the relationship between FEV_1 (as a dependent variable) and F_{ENO} level categorized according to quartile range; such covariates as age, sex, presence of allergic rhinitis, allergy profile, and asthma severity were also included. The above analysis was performed separately in healthy subjects, in children with episodic asthma, and in children with mild and moderate chronic asthma. All statistical analyses were performed using Statistical Package for the Social Sciences 11.5 (SPSS, Chicago, Illinois). $P < .05$ was considered of statistical significance.

Results

Data obtained from 405 subjects (children with asthma and healthy controls with all the required tests results) were included in the analysis. Baseline characteristics of the subjects are shown in Table 1. Non-atopic children with asthma who showed normal F_{ENO} values were excluded from the analysis. Subjects from the control group were non-asthmatic and non-atopic according to a negative prick test; none had respiratory tract symptoms or were treated with any drug in the 2 months preceding the evaluation of the results.

Median levels of F_{ENO} increased linearly with the subjects' age ($P = .03$). We found a nonlinear trend of pre-bronchodilator FEV_1 across 4 quartiles of F_{ENO} in children with asthma without ICS drug (ANOVA, quadratic term: $P = .03$) and in children with asthma treated with a low dose of ICS (ANOVA, quadratic term: $P = .049$) (Table 2, Fig. 1); we observed lower pre-bronchodilator FEV_1 in children with higher F_{ENO}, but only up to the F_{ENO} value of 35.4 ppb (Table 2, Fig. 1); in children with F_{ENO} value $>$ 35.4 ppb, pre-bronchodilator FEV_1 was increased (Ta-

Table 1. Baseline Characteristics of Subjects

	Healthy Subjects (n = 135)	Episodic Asthma (n = 65)	Mild Asthma (n = 116)	Moderate Asthma (n = 89)
Age (y, mean ± SD)	11.0 ± 4.7	10.1 ± 4.0	10.0 ± 4.4	10.9 ± 3.5
Male gender (n, %)	72 (53.3)	47 (72.3)	83 (71.6)	63 (70.8)
Height (cm, mean ± SD)	148.7 ± 14.6	150.3 ± 1.2	149.5 ± 14.8	151.3 ± 16.1
Allergic rhinitis	0	31	78	64
Allergy profile (n, %)				
Non-atopy	135 (100)	11 (16.9)	19 (16.4)	19 (21.3)
Seasonal only	NA	6 (9.2)	22 (19.0)	11 (12.4)
Perennial	NA	47 (72.3)	69 (59.5)	58 (65.2)
Food	NA	1 (1.5)	6 (5.2)	1 (1.1)

NA = not applicable

Table 2. Pre-Bronchodilator FEV_1 According to Four Categories of F_{ENO} Level (Defined by Lower/Upper Quartile) in Healthy Subjects and in Children with Episodic, Mild, and Moderate Asthma

	Healthy Subjects (n = 135; mean ± SD)	Episodic Asthma (n = 65; mean ± SD)	Mild Asthma (n = 116; mean ± SD)	Moderate Asthma (n = 89; mean ± SD)
FeNO (ppb)				
< Lower quartile (< 12.8)	105.5 ± 12.2	104.8 ± 9.7	102.6 ± 14.1	103.6 ± 14.1
Lower quartile to median (12.8–19.1)	104.8 ± 11.4	96.9 ± 13.1	98.6 ± 16.7	101.6 ± 14.5
Median to higher quartile (19.1–35.4)	104.7 ± 11.8	94.7 ± 16.7	94.6 ± 14.5	100.0 ± 11.9
> Higher quartile (> 35.4)	108.6 ± 14.0	102.3 ± 11.7	96.6 ± 13.8	94.7 ± 6.8
Test for trend (ANOVA)*				
Linear term	.43	.53	.29	.039
Quadratic term	.38	.03	.049	.56

* P values are adjusted for age, sex, allergy profile, and anti-asthma therapy.
F_{ENO} = fractional exhaled nitric oxide
ppb = parts/billion
ANOVA = analysis of variance

ble 2, Fig. 1). In children with moderate asthma, the above trend had linear characteristics (ANOVA, linear term: $P = .039$) (Table 2, Fig. 1). In healthy children (without asthma), we did not observe any significant changes in pre-bronchodilator FEV_1 across 4 quartiles of F_{ENO} (ANOVA, linear term: $P = .43$; quadratic term: $P = .39$).

We found a linear increasing trend of change from baseline (after 400 μg of salbutamol) in FEV_1 across F_{ENO} categories in children with moderate asthma (ANOVA, linear term: $P = .02$) (Table 3, Fig. 2). In other groups, we did not observe any significant trends of change from baseline (after 400 μg of salbutamol) in FEV_1 across 4 quartiles of F_{ENO}: (1) healthy children (ANOVA, linear term: $P = .36$; quadratic term: $P = .51$); (2) children with asthma without ICS drug (ANOVA, linear term: $P = .09$; quadratic term: $P = .58$); and (3) children with asthma treated with a low dose of ICS (ANOVA, linear term: $P = .84$; quadratic term: $P = .16$).

We were not able to demonstrate any significant correlation between F_{ENO} value and post-bronchodilator FEV_1 (Table 3, Fig. 2).

Discussion

The current analysis is the first to demonstrate a relationship between the degree of bronchoconstriction and the level of exhaled nitric oxide in a large group of children with asthma. We found a nonlinear trend of pre-bronchodilator FEV_1 across 4 different categories of F_{ENO} values in pediatric subjects with episodic and mild asthma. We observed lower pre-bronchodilator FEV_1 values in children with higher F_{ENO}, but only up to the F_{ENO} cutoff point of 35.4 ppb; in children with F_{ENO} value > 35.4 ppb, pre-bronchodilator FEV_1 was increased. Our study showed that, in children with moderate asthma, the above trend was linear.

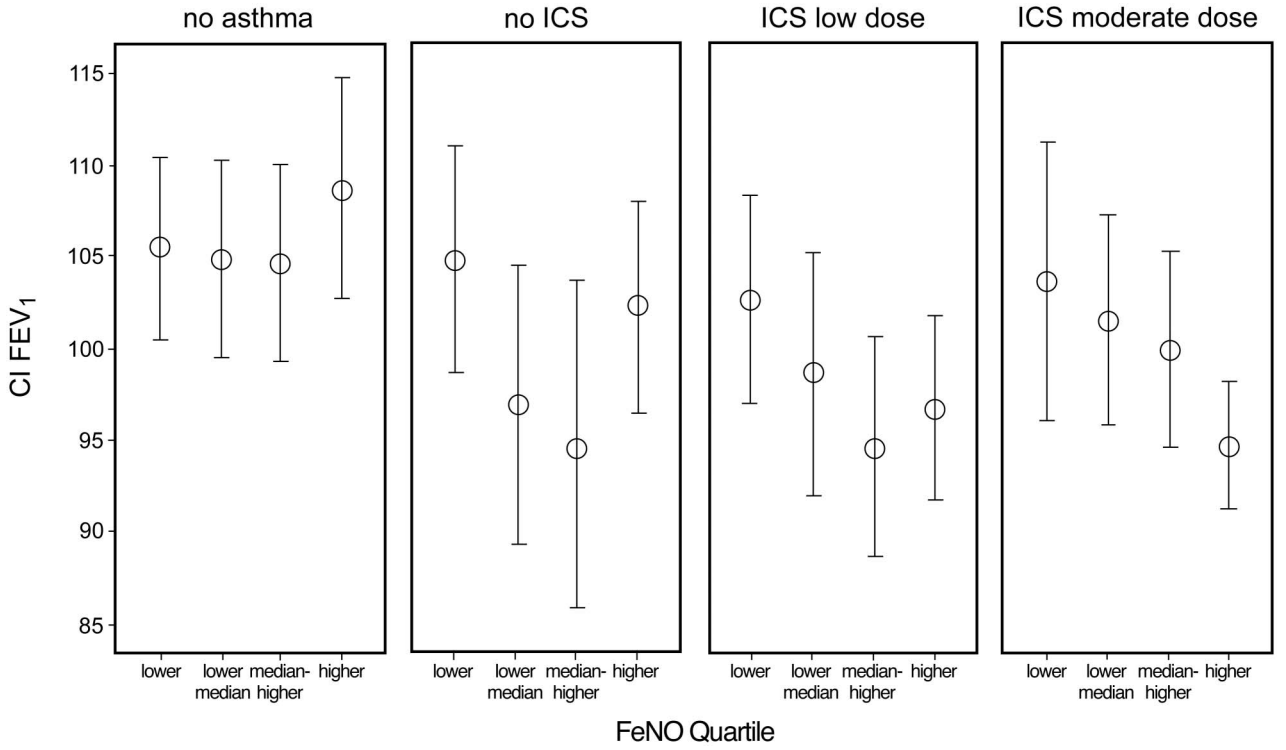

Fig. 1. Pre-bronchodilator FEV_1 according to 4 categories of fractional exhaled nitric oxide (F_{ENO}) level (defined by lower/upper quartile) in healthy subjects, in children with asthma without current inhaled corticosteroid therapy (ICS), and in children with asthma treated with low or moderate ICS dose. Data are presented as mean with 95% CI.

Table 3. Change from Baseline after 400 μg of Salbutamol in FEV_1 According to Four Categories of F_{ENO} Level (Defined by Lower/Upper Quartile) in Healthy Subjects and in Children with Episodic, Mild, and Moderate Asthma

	Healthy Subjects (n = 135; mean ± SD)	Episodic Asthma (n = 65; mean ± SD)	Mild Asthma (n = 116; mean ± SD)	Moderate Asthma (n = 89; mean ± SD)
F_{ENO} (ppb)				
< Lower quartile (< 12.8)	4.8 ± 4.8	4.6 ± 2.7	4.5 ± 7.0	2.5 ± 3.1
Lower quartile to median (12.8–19.1)	7.2 ± 7.7	8.2 ± 6.1	11.4 ± 18.7	4.6 ± 5.4
Median to higher quartile (19.1–35.4)	3.7 ± 2.3	12.2 ± 14.0	5.7 ± 3.3	3.7 ± 3.4
> Higher quartile (> 35.4)	3.5 ± 5.5	12.1 ± 11.2	7.0 ± 9.1	9.4 ± 10.0
Test for trend (ANOVA)*				
Linear term	.36	.09	.84	.02
Quadratic term	.51	.58	.16	.28

* P values are adjusted for age, sex, allergy profile, and anti-asthma therapy.
ppb = parts/billion
F_{ENO} = fractional exhaled nitric oxide
ANOVA = analysis of variance

We showed that, in episodic and mild asthma, there were 2 trends of FEV_1 in relation to F_{ENO}: (1) a decreasing linear trend in case of values up to 35.4 ppb, (probably as a consequence of inflammation process) and (2) an increasing trend in case of values exceeding 35 ppb (probably as a direct bronchodilator effect of F_{ENO}). This hypothesis seems to be confirmed by the fact that healthy subjects had F_{ENO} results > 35.4 ppb and higher FEV_1 compared with subjects with asthma. This nonlinear trend suggests that higher F_{ENO} may induce bronchodilator response but only in healthy subjects and in episodic and mild chronic asthma. It is within the bounds of possibility that the distinct response of bronchi to higher F_{ENO} concentration in moderate asthma could be explained by a more intense inflammation process, resulting in a poor response to a natural autogenic bronchodilator such as

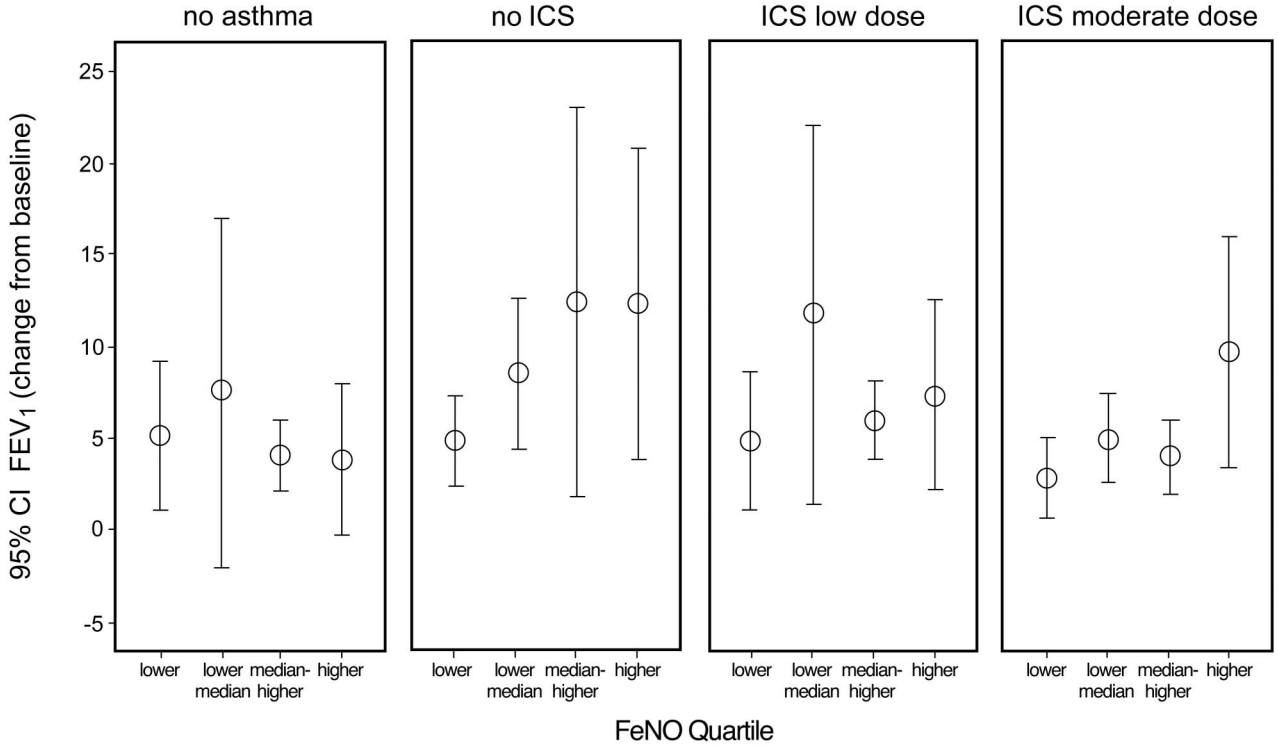

Fig. 2. Change from baseline after 400 μg of salbutamol in FEV_1 according to 4 categories of fractional exhaled nitric oxide (F_{ENO}) level (defined by lower/upper quartile) in healthy subjects, in children with asthma without current inhaled corticosteroid therapy (ICS), and in children with asthma treated with low or moderate ICS dose. Data are presented as mean with 95% CI.

nitric oxide. A limited number of previous studies in children are similar to our results.[19,20] The study by Cordeiro et al[19] showed that the highest diagnostic accuracy of asthma can be achieved by the combination of F_{ENO} ($>$ 27 ppb) and/or the presence of bronchodilator reversibility. Moreover, it showed that an increased F_{ENO} level was positively correlated with the presence of respiratory symptoms and air flow reversibility; however, in their study, all subjects were steroid naive.[19] We conducted a similar analysis, and we found a linear increasing trend of change from baseline in bronchial reversibility test using FEV_1 values across 4 F_{ENO} categories of children with moderate asthma. In contrast with our study, in the study by Cordeiro et al,[19] the patients were not categorized into different asthma groups nor was the exact number of participants provided. The authors of yet another study identified 4 clusters of subjects with well-controlled asthma versus uncontrolled asthma, associated with increased airway tone; F_{ENO} did not differ in these 4 clusters.[20] The authors concluded that F_{ENO} is independently linked to ICS-dependent inflammation and bronchomotor tone but does not help to identify a clinically relevant phenotype of children with asthma.[20] We showed that, in children with episodic/mild asthma, F_{ENO}, in a certain concentration of $>$ 35.4 ppb may act as a significant bronchodilator. To our knowledge, there exists no other study to date (using our model) that fully supports our results. Moreover, there is no general consensus about correlation between F_{ENO} and respiratory function in children.[2,21-34]

The main limitation of our study is the retrospective design. We gathered data from the subjects' medical documentation, which could partly have influenced the accuracy of our results. The same could be true for a relatively wide range of the subjects' age (from 6 to 18 y). However, all subjects participating in this study remain under the regular care of specialists from our clinic, including physical examination, lung function measurements, and other necessary tests, which excludes any doubts concerning the heterogeneity of diagnostic and therapeutic procedures. Therefore, all lung function tests were performed according to guidelines.[14-18]

Another limitation of our study is that, because it was a retrospective study, we analyzed medical data of different phenotypes of asthma. However, the various groups of children with asthma that we defined in our study do not seem to differ significantly as far as their lung function is concerned, presumably because they are all reasonably controlled on their ICS. In turn, this could have affected the results we obtained regarding both F_{ENO} and lung function. We suspect that it would be best to study subjects off ICS to remove its effect on both parameters. When interpreting our results, it should be kept in mind that even if

a certain medication, such as short-acting β_2 agonist, does not affect NO production, it might affect the apparent level of NO through other mechanisms such as changes in airway caliber, which so far has been shown only in the studies of adults, as cited in the guideline.[31] Until now, studies in children have revealed the opposite, namely that F_{ENO} does not significantly change after long- or short-acting bronchodilators, which have no known anti-inflammatory effect.[32,33] We carefully noted that, in many of the clinical studies in adults, it is advised to perform F_{ENO} measurements before any other lung function measurement and even before usage of short-acting β_2 agonists,[34,35] and the same order of measurements and procedures is followed by most of the studies in children.[19,20,36] It does not seem justified in the context of our results in children with asthma. Therefore, our results reveal important new clinical aspects regarding the order of measurements in children with asthma. The regulation of both exhaled NO and bronchomotor tone is intriguingly complex in childhood asthma,[2] and it is also possible that slightly higher or lower FEV_1 in those subjects with the highest F_{ENO} values may not serve as an indicator of physiological interactions. It has been well documented that F_{ENO} falls by approximately 20% after forced expiratory maneuvers in adults, probably as a consequence of depleted tissue stores.[31] Thus, the standard recommendation of performing F_{ENO} measurement before spirometry may continue to seem a logical choice; nevertheless, clinicians must be aware of the multiple factors influencing F_{ENO} and draw sensible conclusions.[2] The authors are also aware that the bronchodilator effect of salbutamol is determined not only by inflammation but also genetic variability of β_2 receptor expression[1]; hence, there is a possibility that $\Delta(FEV_1)$ in subjects without a significant bronchodilator effect may not always be a valid variable.

Conclusions

Pediatricians and allergists expect that F_{ENO} is an inflammometer but not a lung function indicator. It is reasonable to use a ratio, $F_{ENO}/FEV_{1,}$ which could probably overcome doubts concerning the adequate measurement of inflammation by F_{ENO}; however, it still requires validation. The conclusion of our study is that, independent of the influence of FEV_1 on F_{ENO} value, such relationship has obvious clinical implications, which suggest a need to measure F_{ENO} before and after spirometry and, consequently, in children with symptomatic asthma, to assess F_{ENO} after short-acting β_2 agonists as well.

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