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FINAL CLEAN VERSION

Title page

The interpretation of exhaled nitric oxide values in children with asthma depends on the degree of bronchoconstriction and the levels of asthma severity.

Running title: Bronchoconstriction and exhaled nitric oxide

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Abbreviations

AR - allergic rhinitis

AHR – airway hyper-responsiveness

BRT - bronchial reversibility test

ERS/ATS – European Respiratory Society/American Thoracic Society

GINA – Global Initiative for Asthma

FeNO - fractional exhaled nitric oxide

FEV_1 - forced expiratory volume in the first second

ICS - inhaled corticosteroids

SABA – short-acting Beta2-agonists

SPT – skin prick tests

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ABSTRACT

Introduction: The clinical implications of FeNO measurements in childhood asthma are unclear.

Aim: 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: It was a retrospective, cross-sectional study. We evaluated data from medical documentation of children with asthma with special attention to FeNO results, asthma severity, FEV_1 (% predicted), and bronchial reversibility test (BRT).

Results: Four hundred and five subjects (aged 6-18) completed the study. Median levels of FeNO increased linearly with subjects' age (p=0.025). We found a non-linear trend of pre-bronchodilator FEV_1 across four quartiles of FeNO in episodic and mild asthma; we observed lower pre-bronchodilator FEV_1 in children with higher FeNO, but only up to the FeNO value of 35.4 ppb; in children with FeNO value higher than 35.4 ppb, pre-bronchodilator FEV_1 was increased. We found a linear increasing trend of change from baseline (after 400 mcg of salbutamol) in FEV_1 across FeNO categories in children with moderate asthma.

Conclusions: Our results suggest a need to measure FeNO before as well as after spirometry. Consequently, in children with asthma with bronchial obstruction we suggest assessing FeNO also after short-acting Beta2-agonists.

Key words: FeNO; FEV_1; asthma; children; airway caliber; glucocorticosteroids; asthma severity

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Introduction

The current concept of asthma pathogenesis underlines a chronic inflammatory process, which causes airflow obstruction and bronchial hyper-responsiveness (AHR).[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 hyperresponsiveness (AHR); on the other hand, under physiological conditions, it acts as a weak mediator of smooth muscle relaxation and protects against AHR.[2-7] Recently it has been proved that FeNO results are in disagreement with other measurements of asthma control in children with asthma, namely spirometry, children Asthma Control Test and conventional clinical assessment.[8] Green RJ et al. showed that mean FeNO in pediatrician-judged uncontrolled asthma was double that of controlled asthma.[8] FeNO correlates with bronchial reactivity[9] and decreases with anti-inflammatory asthma therapy, such as inhaled corticosteroids (ICS) and anti-leukotrienes, in children.[10] FeNO 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, so far no study has assessed the influence of the degree of baseline bronchoconstriction on FeNO results in children.[11,12] Current guidelines suggest the use of cut points rather than reference values when interpreting FeNO results, but this recommendation is weak, based on evidence of low quality.[2] Therefore, our analysis was focused on investigating the relationship between FeNO 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.

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Methods

Study design

It was a retrospective, cross-sectional study. We evaluated data from medical documentation of 943 children (aged 6-18) with symptoms suggestive of asthma, who attended our Allergic Outpatient Clinic from January 2008 to March 2009. We included subjects with minimum 2-years of clinical observation, whose asthma was either confirmed or excluded. Children with asthma and allergic rhinitis (64%) were also included. 405 analyzed subjects had FeNO 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 allergic outpatient 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 the patients.[1] Medical documentation of the subjects was analyzed with special attention to the results of FeNO, spirometry, bronchial reversibility test, and allergic rhinitis diagnosis, as well as allergen sensitization and treatment. Non-atopic children with asthma who showed normal FeNO values were excluded from the analysis. We analyzed the mean doses of inhaled glucocorticosteroids, which were assessed throughout the period of three months preceding the measurements of FeNO 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 Allergic Outpatient 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

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daily use ("episodic" asthma), a medium steroid daily use ("mild" asthma), a high steroid daily use ("moderate" asthma). Such an approach allowed us to obtain almost equal sample size in the study groups and 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, 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 on: www.ClinicalTrials.gov, NCT00815984.

Allergen sensitization

All subjects underwent skin prick test (SPT) 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, Reinbeg, Germany) were also used. A positive SPT reaction was defined as a mean wheal diameter greater than 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 Allergic Outpatient Clinic, FeNO was measured prior to a forced flow-volume curve measurement and bronchial reversibility test in all subjects on the same day (in the morning, between 9 a.m. and 11 a.m.). The NO measurements were performed according to the

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European Respiratory Society/ American Thoracic Society (ERS/ATS) recommendations,[14] with a chemiluminescence analyzer (model 280i nitric oxide analyzer; Sievers, Boulder, CO, USA) and defined in parts per billion. The analyzer provides an on-line continuous measurement of NO in a single exhalation with a detection range of 0.1 to 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 FeNO measurements of the plateau phase were obtained, with less than 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 (400ug), according to the latest American Thoracic Society guideline.[18] The percent of change from baseline in FEV_1 after salbutamol, pre- and post-bronchodilator FEV_1 values were included in the analysis.

Statistics

The analysis was performed in four different subgroups: i) episodic asthma – episodic use of low dose, 100-200 μg of steroid dose equivalent to budesonide (MDI) daily, ii) mild asthma –

medium dose, 200-400 μg of steroid dose equivalent to budesonide (MDI) daily, iii) moderate asthma – high dose, >400 μg of steroid dose equivalent to budesonide (MDI) daily and iv) healthy subjects. To assess the relationship between FEV_1 (as a dependent variable) and FeNO level, an analysis of variance (ANOVA) was implemented. Additionally, a test for a linear and non-linear trend was used. The above relationship was adjusted for the effect of age, sex, the presence of allergic rhinitis, the allergy profile and asthma severity. The analysis of variance was implemented to assess the relationship between FEV_1 (as a dependent variable) and FeNO level categorized according to quartile range; such covariates as age, sex, the 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 (SPSS) 11.5. $P<0.05$ 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 FeNO 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 nor were treated with any drug in the 2 months preceding the evaluation of the results.

Median levels of FeNO increased linearly with the subjects' age (p=0.025). We found a non-linear trend of pre-bronchodilator FEV_1 across four quartiles of FeNO in children with asthma without ICS drug (ANOVA, quadratic term: p=0.029) and in children with asthma treated with a low dose of ICS (ANOVA, quadratic term: p=0.049) (Table 2, Figure 1); we observed lower pre-bronchodilator FEV_1 in children with higher FeNO, but only up to the FeNO value

of 35.4 ppb (Table 2, Figure 1); in children with FeNO value higher than 35.4 ppb, pre-bronchodilator FEV_1 was increased (Table 2, Figure 1). In children with moderate asthma, the above trend had linear characteristics (ANOVA, linear term: p=0.039) (Table 2, Figure 1). In healthy children (without asthma) we did not observe any significant changes in pre-bronchodilator FEV_1 across four quartiles of FeNO (ANOVA, linear term: p=0.426; quadratic term: p=0.386).

We found a linear increasing trend of change from baseline (after 400 mcg of salbutamol) in FEV_1 across FeNO categories in children with moderate asthma (ANOVA, linear term: p=0.017) (Table 3, Figure 2). In other groups we did not observe any significant trends of change from baseline (after 400 mcg of salbutamol) in FEV_1 across four quartiles of FeNO: i) healthy children (ANOVA, linear term: p=0.357; quadratic term: p=0.506); ii) children with asthma without ICS drug (ANOVA, linear term: p=0.092; quadratic term: p=0.576); and iii) children with asthma treated with a low dose of ICS (ANOVA, linear term: p=0.842; quadratic term: p=0.158)

We were not able to demonstrate any significant correlation between FeNO value and post-bronchodilator FEV_1 (Table 3, Figure 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 non-linear trend of pre-bronchodilator FEV_1 across four different categories of FeNO values in pediatric subjects with episodic and mild asthma. We observed lower pre-bronchodilator FEV_1 values in children with higher FeNO, but only up to the FeNO cut off point of 35.4 ppb; in children with FeNO value higher than 35.4 ppb, pre-bronchodilator FEV_1 was increased. Our study showed that in children with moderate asthma, the above trend was linear.

We showed that in episodic and mild asthma, there were two trends of FEV_1 in relation to FeNO: i) a decreasing linear trend in case of values up to 35.4 ppb , (probably as a consequence of inflammation process) and ii) an increasing trend in case of values exceeding 35 ppb (probably as a direct bronchodilator effect of FeNO). This hypothesis seems to be confirmed by the fact that healthy subjects had FeNO results above 35.4 ppb and higher FEV_1 compared to subjects with asthma. This non-linear trend suggests that higher FeNO 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 FeNO 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 nitric oxide. A limited number of previous studies in children are similar to our results.[19-20] The study of Cordeiro et al. showed that the highest diagnostic accuracy of asthma can be achieved by the combination of FeNO (>27 ppb) and/or the presence of bronchodilator reversibility.[19] Moreover, it showed that an increased FeNO level was positively correlated with the presence of respiratory symptoms and airflow 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 BRT using FEV_1 values across four FeNO categories of children with moderate asthma. In contrast with our study, in the study of Cordeiro et al. the patients were not categorized into different asthma groups nor was the exact number of participants provided.[19]. The authors of yet another study identified four clusters of subjects with asthma with well-controlled asthma *vs.* uncontrolled asthma, associated with increased airway tone; FeNO did not differ in these four clusters.[20] The authors concluded that FeNO is independently linked to ICS-dependant 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, FeNO, in a certain concentration of more than 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 FeNO 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). However, all patients 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, since 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 FeNO, and lung function. We suspect that it would be best to study subjects off ICS in order 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 SABA, 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 FeNO 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 FeNO measurements before any other lung function measurement and even before SABA usage[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 FeNO values may not serve as an indicator of physiological interactions. It has been well documented that FeNO 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 FeNO measurement before spirometry may continue to seem a logical choice; nevertheless, clinicians must be aware of the multiple factors influencing FeNO 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 beta-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 FeNO is an inflammometer but not a lung function indicator. It is reasonable to use a ratio FeNO/FEV_1, which could probably overcome doubts concerning the adequate measurement of inflammation by FeNO; however, it still requires validation. The conclusion of our study is that independently of the influence of FEV_1 on FeNO value, such relationship has obvious clinical implications which suggest a need to measure FeNO before as well as after spirometry and, consequently, in children with asthma with bronchial obstruction, to assess FeNO also after SABA.

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Legend to the figures

Figure 1 Pre-bronchodilator FEV_1(forced expiratory volume in one second) according to four categories of FeNO 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 presented as mean with 95% confidence intervals.

Figure 2 Change from baseline after 400mcg of salbutamol in FEV_1(forced expiratory volume in one second) according to four categories of FeNO 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 presented as mean with 95% confidence intervals.

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Table 1 Baseline characteristics of patients.

	healthy subjects n=135	episodic asthma n=65	mild asthma n=116	moderate asthma n=89
Age [years], 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±15,2	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	-	6(9,2)	22(19,0)	11(12,4)
parennial	-	47(72,3)	69(59,5)	58(65,2)
food	-	1(1,5)	6(5,2)	1(1,1)

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Table 2 Pre-bronchodilator FEV_1 (forced expiratory volume in one second) according four categories of FeNO level (defined by lower/upper quartile) in healthy subject, in children with episodic, mild and moderate asthma.

FeNO [ppb]	healthy subjects n=135		episodic asthma n=65		mild asthma n=116		moderate asthma n=89	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<lower quartile (<12.8)	105.5	12.2	104.8	9.7	102.6	14.1	103.6	14.1
lower quartile-median (12.8-19.1)	104.8	11.4	96.9	13.1	98.6	16.7	101.6	14.5
median-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	.426		.534		.290		**.039**	
quadratic term	.383		**.027**		**.049**		.564	

* p-level adjusted for age, sex, allergy profile and anti-asthma therapy

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Table 3 Change from baseline after 400 mcg of salbutamol in FEV_1 (forced expiratory volume in one second) according four category of FeNO level (defined by lower/upper quartile) in healthy subject, in children with episodic, mild and moderate asthma.

FeNO [ppb]	healthy subjects n=135		episodic asthma n=65		mild asthma n=116		moderate asthma n=89	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<lower quartile (<12.8)	4.8	4.8	4.6	2.7	4.5	7.0	2.5	3.1
lower quartile-median (12.8-19.1)	7.2	7.7	8.2	6.1	11.4	18.7	4.6	5.4
median-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	.357		.092		.842		**.017**	
quadratic term	.506		.576		.158		.280	

* p-level adjusted for age, sex, allergy profile and anti-asthma therapy

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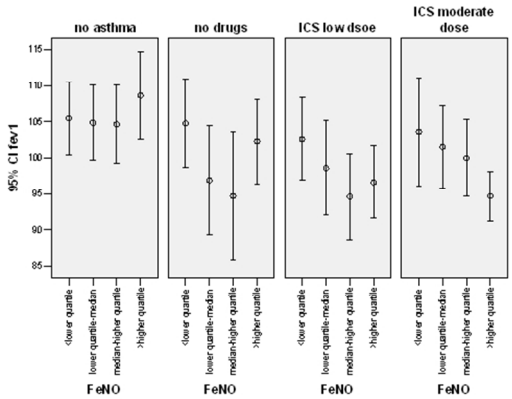

Figure 1 Pre-bronchodilator FEV1(forced expiratory volume in one second) according to four categories of FeNO level (defined by lower/upper quartile) in healthy subjects, in children with asthma without current inhaled corticosteroid therapy (ICS) and in children with asthma treated with low or moderate ICS dose. Data presented as mean with 95% confidence intervals.
254x190mm (96 x 96 DPI)

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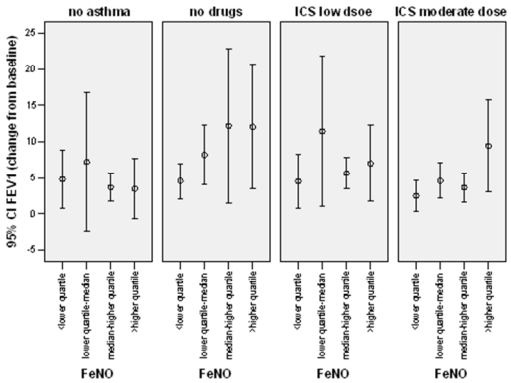

Figure 2 Change from baseline after 400mcg of salbutamol in FEV1(forced expiratory volume in one second) according to four categories of FeNO level (defined by lower/upper quartile) in healthy subjects, in children with asthma without current inhaled corticosteroid therapy (ICS) and in children with asthma treated with low or moderate ICS dose. Data presented as mean with 95% confidence intervals.
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