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Factors Associated with Elevated FeNO in Infants with Recurrent Respiratory Symptoms

Anne Kotaniemi-Syrjänen, MD, L. Pekka Malmberg, MD, Kristiina Malmström, MD, Anna S. Pelkonen, MD, Mika J. Mäkelä, MD.

Department of Allergology, Helsinki University Central Hospital, Helsinki, Finland.

Corresponding author:

Dr Pekka Malmberg

Skin and Allergy Hospital

P.O. Box 160

FIN-00029 HUS

FINLAND

Email: pekka.malmberg@hus.fi

Fax: +358 9 471 86560

Tel: +358 9 471 86217

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ABSTRACT

Fraction of exhaled nitric oxide (FeNO) has been proposed as a non-invasive marker of eosinophilic bronchial inflammation in active asthma, supposed to reflect responsiveness to corticosteroid therapy. There are several factors influencing FeNO, and its role in early childhood respiratory disorders is still to be established. Between 2004 and 2008, 444 children aged <3 years with recurrent lower respiratory tract symptoms were referred to a tertiary centre for further investigation. 136 full-term, steroid-free, infection-free infants, median age of 16.4 months (range 4.0-26.7), successfully underwent measurement of FeNO, lung function tests, and a dosimetric methacholine challenge test.

The median level of FeNO was 19.3 ppb (interquartile range 12.3-26.9 ppb). Elevated FeNO (≥27 ppb, the highest quartile) was associated with maternal history of asthma (adjusted odds ratio (OR) 3.2; 95% confidence interval (CI) 1.3-8.1; p=0.012), and increased airway responsiveness (the provocative dose of methacholine causing a 40% fall in maximal expiratory flow at functional residual capacity (PD_{40} $V'_{max,FRC}$) ≤0.30 mg) (adjusted OR 4.1; 95% CI 1.4-12.7; p=0.012). Atopy, blood eosinophilia, and lung function were not associated with elevated FeNO.

In conclusion, maternal history of asthma, and increased airway responsiveness are associated with elevated FeNO in infants with recurrent lower respiratory tract symptoms.

Key words: atopy, fraction of exhaled nitric oxide, increased airway responsiveness, lung function tests, recurrent lower respiratory tract symptoms, wheezing

INTRODUCTION

Fraction of exhaled nitric oxide (FeNO) is a widely used non-invasive biomarker of active asthma in adults and school children [1]. Elevated FeNO has been proposed as a marker of eosinophilic inflammation in bronchial mucosa, supposed to imply a favourable response to corticosteroid therapy [1]. In addition, increased airway responsiveness has been related to elevated FeNO [2-4].

At preschool age, children with probable asthma present with elevated FeNO [5]. However, the role of FeNO in respiratory disorders of early childhood is not established: wheezing illnesses of infancy are usually not associated with eosinophilic bronchial inflammation [6,7], and although increased airway responsiveness may play a role in recurrent lower respiratory tract symptoms of early childhood [8], the studies on the relationship of FeNO and increased airway responsiveness in infants are scarce [9]. In addition, there are several characteristics influencing FeNO values: i.e. used methods [10], the height of the child [2], the presence of atopic eczema [11,12], skin test reactivity [2,3,7,12,13], acute respiratory symptoms [14-16], and the tobacco smoke exposure [16-18].

The aims of the present study were to evaluate the relationship between FeNO and increased airway responsiveness, and to clarify whether there are any associations between elevated FeNO and anthropometrics and exposure to risk factors for respiratory morbidity in infants with recurrent lower respiratory tract symptoms.

METHODS

Study subjects

Between 18th August 2004 and 26th November 2008, altogether 444 children aged <3 years were referred to the Department of Allergology, Helsinki University Central Hospital for investigation of recurrent lower respiratory tract symptoms (including wheeze, dry or productive cough, and/or shortness of breath). Of these children, 187 successfully underwent (in a respective order) measurement of FeNO, lung function testing by whole body plethysmography and the rapid thoracic compression technique, and the dosimetric methacholine challenge test. The data of the remaining 257 cases were excluded because of missing (n=220) or technically unacceptable (n=37) measurements of FeNO, lung function, and/or airway responsiveness. In addition, preterm (gestational age <36 weeks) infants, those on corticosteroid medication (during one month prior to testing), and those with any symptoms of acute respiratory infection (during 2 weeks prior to testing) were excluded, leaving 136 children for final analyses. Excluded children did not differ from included infants in any aspects of baseline characteristics. However, excluded children more often had abnormal results in baseline lung function measured by rapid thoracic compression technique ($p=0.021$). On the other hand, there were no differences in FeNO levels between included and excluded children ($p=0.161$).

All the tests were performed as part of the recruitment phase of an intervention study of infant asthma after informed consent to perform these tests was obtained from the parents. In addition, the parents were asked for further written consent to allow the use of the FeNO measurements, lung function and methacholine challenge test results, skin prick test results, and related clinical data for research

purposes. The intervention study, including measurement of FeNO, performance of the baseline lung function tests, and the methacholine challenge test during the recruitment phase, was approved by the local ethics committee.

During the hospital visit, the clinical data were collected with a standardized form designed for the intervention study by interviewing the parents, and by reviewing the medical records of the children. A small amount of blood, <1% of the circulating blood volume, was collected from a fingertip for analysis of peripheral blood eosinophil count.

Measurement of FeNO

FeNO was assessed with a modification of online single-breath measurement [19]. During sedation, the babies breathed spontaneously room air through a mask covering the nose and the mouth, and a pneumotach attached to the mask. Rapid thoracoabdominal compression technique was applied to generate a forced expiration starting from end-inspiration. The expired air was led to a chemiluminescence analyzer (Niox, Aerocrine, Sweden) via a shutter mechanism of the pneumotach and a 3-way valve, and a dynamic resistor that restricted the expiratory flow to 50 ml/s. By using a 3-way valve, sampling of the exhaled air occurred between the mask and the shutter system, at immediate proximity of mask. The effective dead space consisted mainly of the mask (10 ml). FeNO was measured from the end-expiratory sample by using the plateau phase of the NO profile. Repeated exhalations were performed in order to obtain three reproducible FeNO measurements (variation <10%, maximum of 5 ppb). The mean value of these measurements was recorded as well as the ambient NO (NO_{amb}) during the test.

Only measurements with NO_{amb} ≤5 ppb (83% of the measurements) were included in the analyses.

Lung function tests and the dosimetric methacholine challenge

Lung function tests and the dosimetric methacholine challenge were performed according to the protocols used in our hospital [8]. All infants were studied when they were free from signs of acute respiratory infection, and beta 2 –agonists were withheld for 12 hours prior to lung function and challenge tests. In brief: during lung function testing, the sedated infant was lying supine with the head supported in the midline and the neck slightly extended to minimize airway or glottic obstruction. All measurements were recorded and calculations performed with commercial paediatric pulmonary function equipment (Babybody Masterscreen, Jaeger GmbH, Würtzburg, Germany). Functional residual capacity (FRC) was measured by whole body plethysmography. The maximal partial expiratory flow volume (PEFV) was obtained using the rapid thoracic compression technique (i.e. tidal squeeze) by rapid inflation of a thoracoabdominal jacket at the beginning of expiration. Flow was measured at the infant's nose and mouth with a pneumotachometer attached to a face mask. The compression pressure was progressively increased until there was no further increase in forced expiratory flow at functional residual capacity ($V'_{max,FRC}$), and the mean $V'_{max,FRC}$ of 3 technically acceptable PEFV curves obtained at that compression pressure was recorded. The baseline lung function results were expressed as z-scores, which are equivalent to the number of standard deviations by which the measured value deviates from the height- and gender-corrected reference value.

For the dosimetric methacholine challenge, a calibrated nebuliser (Salter Labs 8900, Arvin, CA) was connected to an automatic, inhalation-synchronised dosimeter (Spira Electro II, Spira Respiratory Care Center Ltd, Finland). The dosimeter was set to be triggered by an inhaled volume of 20 ml, after which a methacholine chloride dose of 50 µg was nebulised within 0.2 seconds in an air volume of 25 ml, in each breath. By calculating the number of breaths with nebulised methacholine, a rapid dosage scheme with four non-cumulative dose steps was applied (0.1, 0.3, 0.9 and 1.8 mg), with $V'_{max,FRC}$ being recorded after each dose. At each phase, the applied compression pressure was the same as that achieved the highest flows at baseline. The provocative dose of methacholine causing a 40% fall in $V'_{max,FRC}$ (PD_{40} $V'_{max,FRC}$) was determined from the dose-response curves. In cases where the maximal dose was reached and $PD_{40}V'_{max,FRC}$ could not be determined from the dose-response curves, for statistical purposes, $PD_{40}V'_{max,FRC}$ was defined as twice the highest dose of methacholine, 3.60 mg.

During lung function measurements and the challenge test, oxygen saturation and heart rate were continuously monitored with a pulse oximeter. Following the challenge test, the children received inhaled salbutamol (0.6 mg) (Ventoline Evohaler 0.1 mg/dos) via Nebunette (manufactured by AstraZeneca Liquid production Sweden AB), and the measurement of $V'_{max,FRC}$ was repeated 15 min after the salbutamol inhalation.

Skin prick tests

Sensitisation to common food and/or inhalant allergens, including egg white, cow's milk, wheat, soy bean, cod, shrimp, peanut, birch pollen, timothy grass pollen, dog epithelial dander, cat epithelial dander, house dust mite *Dermatophagoides*

pteronyssinus was tested by skin prick tests (SPT). SPT positivity was defined as a wheal with a diameter of ≥3 mm against at least one of the tested allergens [20]. Physiologic saline was applied as a negative control, and no reaction was allowed against to its injection.

Definitions

Food allergy was defined as a diagnosis confirmed by a positive food challenge. Atopic eczema was defined as a current diagnosis made by a paediatrician or a dermatologist. Atopy was defined as the presence of atopic eczema and/or SPT positivity. A parental history of asthma or allergy was defined as physician-diagnosed asthma or allergy, respectively, in either of the parents. A maternal history of asthma or allergy was defined as physician-diagnosed asthma or allergy, respectively, in the mother of the child.

Blood eosinophilia was defined as eosinophils accounting for ≥4% of the total white blood cells [21].

Lung function parameters which were regarded abnormal were FRC z-score of ≥2 [8], and $V'_{max,FRC}$ z-score of ≤ -2 [8]. FeNO values of ≥27 ppb, i.e. highest quartile, were considered elevated. $PD_{40}V'_{max,FRC}$ ≤0.30 mg was considered as increased airway responsiveness to methacholine, $PD_{40}V'_{max,FRC}$ 0.31-0.90 mg as intermediate responsiveness to methacholine, and $PD_{40}V'_{max,FRC}$ ≥0.91 mg as no/mild responsiveness to methacholine.

Statistics

To evaluate the statistical differences between the groups, χ^2- test or Fisher's exact test (if the expected frequency for any cell was <5) were used to analyse categorical data, and Kruskal-Wallis test or Mann-Whitney U test were applied to analyse continuous data. Correlations between continuous variables were determined by Spearman's rank correlation test. Logistic regression analysis was performed to calculate the adjusted odds ratios (OR) and related 95% confidence intervals (CI) in a multivariate setting, as follows: a dichotomous variable indicating elevated FeNO was included in the analysis as a dependent variable, and explanatory (those with $p<0.05$ in univariate analyses) or confounding variables (selected by clinical importance, i.e. atopy and height) were included as covariates. Enter-method including all covariates simultaneously in the model was applied. Two-tailed tests were used in all analyses. P-values less than 0.05 were considered statistically significant. The data were analysed using IBM SPSS 19.0 for Windows.

RESULTS

The median age of the study children was 16.4 months (range 4.0 to 26.7 months), and the median height 80.7 cm (range 62.0-94.2 cm). None of the children had major congenital cardiac or other malformations.

The median duration of recurrent lower respiratory tract symptoms was 7 months (range 2 to 26 months), and cough was most commonly (72%) reported as a main respiratory symptom. However, up to 105 (77%) children had experienced at least one episode of wheezing confirmed by a physician. A parental history of asthma or allergy was present in 107 (79%) children; in the majority (60%), this was a maternal history of asthma or allergy. 57 (42%) children had either atopic eczema, food allergy, and/or SPT positivity. Blood eosinophilia was present in 31 (23%) children.

The median FeNO was 19.3 ppb (interquartile range 12.3 to 26.9 ppb). The median FRC z-score was 0.7 (range -2.2 to 4.8), and the median $V'_{max,FRC}$ z-score -0.8 (range -3.8 to 1.5). FRC was regarded as abnormal in 27 (20%), and $V'_{max,FRC}$ in 23 (17%) children. Median coefficients of variation for FeNO, FRC, and $V'_{max,FRC}$ were 7%, 3%, and 4%, respectively. During the methacholine provocation, the median changes in $V'_{max,FRC}$ and oxygen saturation were -53% and -3%, respectively. Fourty-three (32%) children were found to have increased airway responsiveness to methacholine (i.e. $PD_{40}V'_{max,FRC} \leq 0.30$ mg).

When the relationships between FeNO and baseline parameters were assessed, we found no correlation with age (r_s=0.114, p=0.188), height (r_s=0.148, p=0.087), or percentage of eosinophils in peripheral blood (r_s= -0.052, p=0.583) (Fig. 1), and no association between elevated FeNO (i.e. FeNO of ≥27 ppb) and blood eosinophilia (i.e. eosinophils ≥4% in peripheral blood) (p=0.140). When associations

between elevated FeNO and baseline characteristics were evaluated, a significant association between elevated FeNO and a maternal history of asthma was found (Table 1). However, there were no other statistically significant associations between elevated FeNO and any other baseline characteristics in univariate analyses.

When lung function and methacholine challenge data were evaluated with regard to FeNO, we found no correlations between FeNO and FRC (r_s= -0.121, p=0.163), or between FeNO and $V'_{max,FRC}$ (r_s= -0.083, p=0.339). When analysed as a continuous variable, FeNO was not associated with airway responsiveness to methacholine (Fig. 2). However, there was an association between elevated FeNO and increased airway responsiveness (Fig. 3).

Finally, by performing multivariate logistic regression analysis, we were able to show that adjustment for the clinically most important confounding factors, i.e. atopy and height, did not change the significant results obtained in the univariate analyses. Both the maternal history of asthma and increased airway responsiveness were found to be independently associated with elevated FeNO (Table 2).

DISCUSSION

In infants with recurrent lower respiratory tract symptoms, elevated FeNO was associated with a maternal history of asthma, and with increased airway responsiveness to methacholine. In contrast, no associations were found between elevated FeNO and age, height, gender, atopic manifestations, blood eosinophilia, paternal history of asthma or allergy, parentally reported respiratory symptoms, physician-confirmed wheeze, environmental exposures, or lung function.

In earlier studies, the relationship between increased airway responsiveness and FeNO has been controversial in school children. As regards online FeNO measurements, there are reports with no correlation between airway responsiveness and FeNO values [22,23], as well as reports on the association between FeNO and increased airway responsiveness to methacholine [3], histamine [2], and acetylcholine chloride [4]. Studies on infants are scarce: in a study on the relationship of FeNO and airway responsiveness in infants with eczema, there was a correlation between FeNO and increased airway responsiveness to methacholine [9].

At preschool age, FeNO seemed superior to baseline respiratory function and bronchodilator responsiveness in identifying children with probable asthma in our earlier study [5]. In wheezy infants, anti-inflammatory therapy has been shown to reduce levels of FeNO [24-26]. Whether FeNO plays any role as a predictor of later childhood asthma in symptomatic infants is not known. In recently published studies, elevated FeNO predicted decline in lung function in infants with recurrent wheezing [27], and risk of future wheezing both in healthy neonates [28] and wheezy infants [27], although no correlation was found between FeNO and lung function measures at baseline [27,28]. In line with recent findings, we could not find any association between baseline lung function and FeNO levels. On the other hand, there was an

association between increased airway responsiveness to methacholine and elevated FeNO in infants with recurrent lower respiratory tract symptoms, regardless of atopy and height.

Parental asthma is a well known risk factor for asthma in offspring [21], and reduced lung function has been found in children with prolonged breastfeeding with asthmatic and atopic mothers [29]. As well, the maternal history of asthma or atopy has been reported to modify FeNO levels in certain selected cohorts of infants [9,17], that is consistent with our finding on the association between elevated FeNO and a maternal history of asthma. It has been speculated that the milk of asthmatic and/or atopic mothers may differ from the milk of nonasthmatic nonatopic mothers with regard to immunologically active substances and affect the outcome [29]. However, the detailed mechanisms underlying the increased risk related to maternal asthma are still to be elucidated.

In adults and school children FeNO has been proposed as a surrogate marker of eosinophilic airway inflammation [1], and there are reports on the association between elevated FeNO and blood or sputum eosinophilia in school children [2,3,13,30]. According to our previous findings, eosinophilic airway inflammation is rarely seen in infancy [6,7]. However, during infancy, the airways and lungs are in the process of growth, and among other factors, nitric oxide has various functions in the maturating lung [31]. In older subjects, changing expression of inducible nitric oxide synthase (NOS) is thought to explain exhaled nitric oxide variability, whereas in infancy, constitutional NOSes may also contribute to exhaled nitric oxide concentrations [31]. In a recent article on normative data for exhaled nitric oxide in healthy infants [32], an upper limit for normal exhaled nitric oxide was determined as 26.1 ppb. In line with that, FeNO level of ≥27 ppb was regarded

elevated in symptomatic infants in the present study. However, we could not find a correlation between elevated FeNO and blood eosinophilia, and we presume that in infants with recurrent lower respiratory tract symptoms, elevated FeNO may rather reflect other than eosinophilic airway inflammation. In asthmatic subjects, certain viruses, like rhinoviruses, have been found to increase production of exhaled nitric oxide by activating inducible NOS in the airways, and to induce airway hyperresponsiveness simultaneously [33]. However, so far there is lack of studies on the relationship of specific viral infections and FeNO levels in infants. Nevertheless, it has been postulated that there might be a causal relationship between recurrent lower respiratory tract symptoms in infancy, development of increased airway responsiveness, and airways inflammation later in childhood [34].

The clinical usefulness of FeNO in infants and older children has been hampered by several characteristics that have been pointed out to influence FeNO values: i.e. used methods [10], height [2], presence of atopic eczema [11,12], skin test reactivity [2,3,7,12,13], acute respiratory symptoms [14-16], and tobacco smoke exposure [16-18]. In the present study, the used method for FeNO measurement mimics the single breath online method used in older children and adults by standardizing the between-subjects variation in tidal flow. However, the results are not directly comparable with those obtained by using the raised volume thoracoabdominal compression technique [10], due to different lung volume at which the measurement occurs. The FeNO levels may also be confounded with NO_{amb} [35], and in order to avoid that effect, cases with measurements of NO_{amb} >5 ppb were excluded from the analyses. To eliminate the effect of acute respiratory infection to FeNO values, we excluded subjects with acute respiratory illness symptoms within the past two weeks from the final analyses. Because of avoiding performing

methacholine challenge test in children with clinically evident bronchial obstruction, excluded children had more often abnormal results in baseline lung function measured by rapid thoracic compression technique. However, there were no differences in FeNO levels between included and excluded children.

As the study children represented the child population referred to a university hospital clinic because of the recurrent symptoms, there was no selection based on certain respiratory symptoms or severity of symptoms, rendering the study population heterogeneous. However, our goal was not to compare FeNO in children representing different clinical entities, but to evaluate the association between FeNO and airway responsiveness in symptomatic infants, and such a study design does not necessitate symptom-based selection. As the study subjects were selected by their recurrent lower respiratory tract symptoms, the result could have been different if the study subjects had been compared with nonselected healthy children. For ethical reasons, it was not possible to recruit healthy children for such extensive investigations requiring sedation, and consequently we needed to perform all analyses within the study group.

In conclusion, in infants with recurrent lower respiratory tract symptoms, elevated FeNO values are related to increased airway responsiveness and to the maternal history of asthma. These findings may have implications in clinical practice when therapeutic measures are considered for infants with recurrent lower respiratory tract symptoms. In future, studies in symptomatic infants evaluating FeNO as a predictor of later childhood asthma would clarify whether FeNO will be suitable as a biomarker for monitoring early childhood wheezing illnesses.

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TABLES

Table 1. FeNO with regard to baseline characteristics. Results of the univariate analyses.

	FeNO		
	<27 ppb	**≥27 ppb**	
Baseline characteristics [n (%)]	**n=104**	**n=32**	**p [a]**
Boys / Girls	74 (71%) / 30 (29%)	23 (72%) / 9 (28%)	0.937
Parental history of asthma	40 (38%)	16 (50%)	0.246
Maternal history of asthma	23 (22%)	14 (44%)	0.016
Parental history of allergy	77 (74%)	25 (78%)	0.641
Maternal history of allergy	61 (59%)	17 (53%)	0.580
Exposure to environmental tobacco smoke	34 (33%)	9 (28%)	0.604
Furry animals at home	31 (30%)	10 (31%)	0.876
Atopic eczema	28 (27%)	4 (13%)	0.093
Skin prick test positive	27/102 (26%)	7 (22%)	0.602
Atopy	41 (39%)	8 (25%)	0.137
Food allergy	25 (24%)	7 (22%)	0.801
Parentally reported main respiratory symptom			
Dry cough	57 (55%)	16 (50%)	0.633
Productive cough	20 (19%)	5 (16%)	0.645
Wheeze	14 (13%)	5 (16%)	0.774
Shortness of breath	13 (13%)	6 (19%)	0.389
Physician-confirmed wheeze	79 (76%)	26 (81%)	0.533

FeNO, fraction of exhaled nitric oxide.

[a] Analyses were performed by using χ^2-test or Fisher's exact test.

Table 2. Baseline characteristics and factors with regard to elevated FeNO (≥ 27 ppb). Results of the multivariate logistic regression analysis.

Parameter	**OR** [a]	**95%CI** [a]	**p** [a]
Maternal history of asthma	3.2	1.3; 8.1	0.012
Atopy	0.4	0.1; 1.1	0.064
Height	1.0	1.0; 1.1	0.252
Airway responsiveness to methacholine			
Increased	4.1	1.4; 12.7	0.012
Intermediate	1.4	0.4; 4.7	0.547
No / mild	1.0	-	-

[a] Adjusted for the maternal history of asthma, atopy, height, and airway responsiveness to methacholine.

FIGURE LEGENDS

Fig. 1. FeNO with regard to the percentage of blood eosinophils of the total white blood cells. No correlation was found between FeNO and the percentage of blood eosinophils (p=0.583). In addition, there was no association between elevated FeNO (i.e. ≥27 ppb, expressed as a horizontal line) and blood eosinophilia (i.e. ≥4%, expressed as a vertical line) (p=0.140).

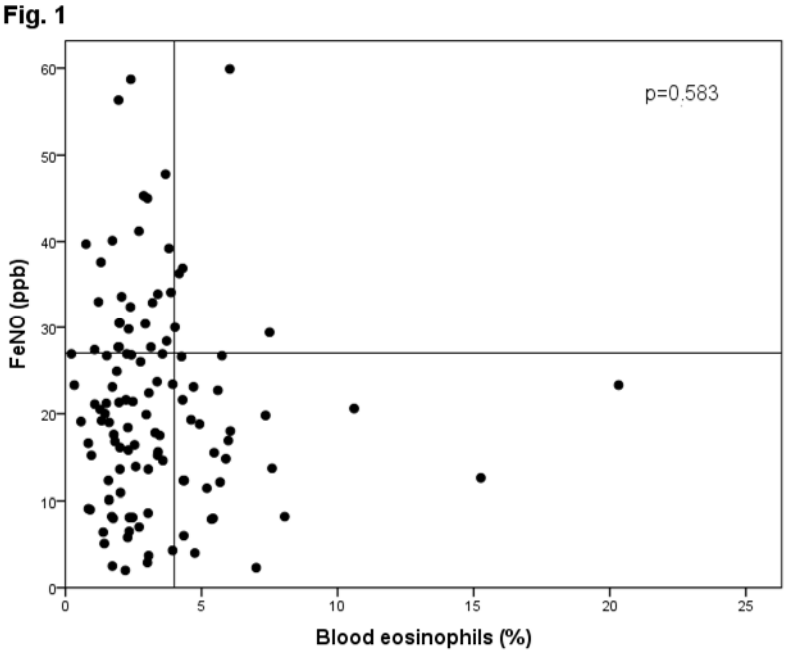

Fig. 2. FeNO presented with regard to airway responsiveness to methacholine. The range of FeNO was wide in all three levels of airway responsiveness, and no statistically significant associations were seen between FeNO and airway responsiveness (p=0.165).

Fig. 2

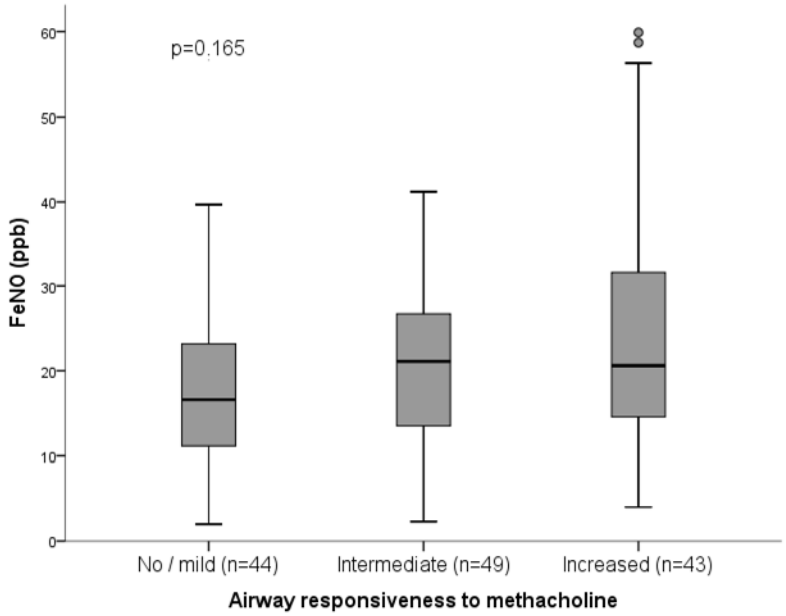

Fig. 3. Elevated FeNO (i.e. ≥27 ppb) with regard to airway responsiveness. Increased airway responsiveness to methacholine (i.e. PD_{40} $V'_{max,FRC}$ ≤0.30 mg) was associated with elevated FeNO when compared to no/mild airway responsiveness to methacholine (i.e. PD_{40} $V'_{max,FRC}$ ≥0.91 mg). *** p=0.047

Fig. 3