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MATERNAL ALLERGY AS A POTENTIAL SOURCE OF VARIABILITY OF EXHALED NITRIC OXIDE IN CHILDREN NON-SENSITIZED TO COMMON DOMESTIC ALLERGENS

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The goal of the study is to evaluate the importance of maternal atopy as a potential biological source of variability of exhaled FeNO values in healthy children who were non-asthmatic and non-sensitized to common domestic allergens. The study sample consisted of 61 seven-year old children. Fractional exhaled nitric oxide (FeNO) has been measured by NObreath (Bedfont portable device). Children with reported maternal atopy had significantly higher mean FeNO values (geometric mean =10.7 ppb; 95%CI: 6.7–17.1 ppb) than those who denied it (geometric mean =5.2 ppb 95%CI: 3.9–6.9 ppb) (p=0.010). Neither the correlation between FeNO values and gender, respiratory and eczema symptoms, nor ETS exposure in the prenatal and postnatal period or body mass of children were significant. We also found no significant association of FeNO values with the amount of common domestic allergens measured in the households. The results of the ROC analysis suggested 11 ppb as the cut-off point for FeNO to distinguish groups of healthy children with and without maternal atopy. In conclusion, our study provided some evidence suggesting that maternal atopy may affect FeNO level in children independently of asthma and sensitization status to common domestic allergens. The data should be considered in the interpretation of FeNO levels in clinical practice and setting up FeNO screening criteria for identification of eosinophilic airway inflammation.

Key words: *allergy, fractional, exhaled nitric oxide, healthy children, maternal atopy, asthma, bronchial hyperactivity*

INTRODUCTION

Since more than a decade the measurement of fractional exhaled nitric oxide (FeNO) has been recommended as a biomarker of eosinophilic inflammatory processes, which may be useful to monitor airway inflammation in asthma and other lung diseases (1, 2). Nitric oxide (NO) appears to be produced in the airway epithelium as a result of nitric oxide synthase up-regulation, which occurs in inflammation and has multiple important biological functions. In the airways, NO is supposed to be involved in mediating inflammatory processes, dilatation of vascular system and bronchial tract, but the full biological relevance of increased NO production in lungs has not been fully understood (3).

Many studies have shown that higher levels of FeNO are present in adults and children with asthma (4-10) and exhaled NO levels correlate positively with bronchial hyperactivity (11). In subjects with both bronchial hyperactivity and recent symptoms the increased levels of FeNO were observed as well, however, airways NO levels are also increased in other allergic disorders in the absence of asthma (12-14). Atopic children with asthma have much increased levels of exhaled NO compared with nonatopic asthmatics, but atopy exerts an effect on exhaled NO levels regardless of the presence of asthma. It was found that atopic children have raised FeNO levels and it is more strongly associated with sensitization to domestic aeroallergens than sensitization to other allergens (15-19).

Given the importance of FeNO as an indicator of airway eosinophilic inflammation, it is essential to define the range of normal values in healthy subjects as it is necessary for the interpretation of findings in clinical settings. A number of recent publications on the reference normal values for children showed a great deal of discrepancies, which may result not only from genetic traits, age, presence of atopy, or anthropometric characteristic of subjects (20-24). Actually, there is a debate to identify all relevant biologic factors, which should be accounted for when deriving reference values helpful in clinical practice (25-31).

The main goal of this study is to evaluate the association of maternal atopy as a potential biological source of variability of FeNO values in healthy non-asthmatic and non-sensitized children to common domestic allergens. The secondary purpose of the paper is to assess the magnitude of the effect possibly resulting from maternal allergy on reference FeNO values in children.

MATERIAL AND METHODS

Study population

The study sample of children at the age of 7 years were participants of the ongoing longitudinal investigation on the

health impact of prenatal exposure to outdoor/indoor air pollution in infants and children from the Cracow inner city area (principal investigator of the study is Prof. F.P. Perera from Columbia Center for Children's Environmental Health, Mailman School Public Health, Columbia University). The detailed description of the study design was presented elsewhere (32, 33). Upon enrollment, a detailed questionnaire was administered to mothers to elicit information on demographic data, house characteristics, medical and reproductive history, nutritional habits, and smoking practices of others present in the home. Maternal atopy was defined as reported medical diagnosis of eczema, asthma or hay fever. Gestational age at birth denotes the interval between the last day of the mother's LMP and the date of birth. Prenatal environmental tobacco smoke (ETS) was defined by the cord blood cotinine levels above median and postnatal ETS was assumed if at least one of the household members was an active smoker over the follow-up period. The Jagiellonian University Bioethical Committee approved the research.

Out of 90 asymptomatic children with FeNO tests, for whom we collected regular data on the occurrence of respiratory symptoms and chest diseases and medical diagnosis of asthma in the course of seven-year follow-up from birth, 77 of them underwent SPT for common house aeroallergens at age 5. Furthermore, the children included in the final analysis had to fulfill following criteria: gestational age above week 36, no former or present diagnosis of asthma, no respiratory infections at present or within the past 4 weeks and no anti-allergic medication. In the initial sample we found 5 children with medical diagnosis of asthma and 12 children with sensitization to at least one common domestic allergen. After excluding children with asthma and sensitization to common domestic aeroallergens from total sample of children who underwent SPT, the final study sample consisted of 61 children, among whom 12 (19.7%) with reported maternal atopy. The children underwent measurements of FeNO at the age of 7 years. Aeroallergen concentrations in house dust was measured at age of 6 years. Atopic status of children was defined as the sensitization to at least one common aeroallergen (Der f1, Der p1, Can f1 and Fel d1) measured by the skin prick testing (SPT) at the age of 5 years.

Cord blood cotinine measurement

Newborns at delivery provided cord blood specimen, which before laboratory analysis were stored at –70°C. The serum cotinine concentration was measured at CDC (Center for Disease Control) using a sensitive isotope-dilution high-performance liquid chromatographic/atmospheric pressure ionisation tandem spectrometric (LC/MS/MS) procedure (34). Limits of detection (LOD) were below 0.050 ng/mL. About 25% of specimens had cotinine levels below the LOD.

FeNO measurement

Fractional exhaled nitric oxide (FENO) has been measured by NObreath (Bedfont newer portable device) in children who were free from any chest symptoms, rhinitis or eczema for at least one month prior to the examination and were not on any medical therapy. The instrument has a electrochemical sensor (the accuracy ±5 ppb NO and detection range: 5–300 ppb) and a NO ambient filter. The instrument was set to zero every month and serviced every 12 months. On three consecutive occasions in the morning, children first inhaled ambient air to near total lung capacity and then exhaled for 16 s at a constant exhalation flow rate (50 mL/sec) through a mouthpiece into the device. The instrument has a color touch screen with visual prompts for subjects whilst taking the test. As a visual feedback, an eye level flow indicator (a small bullet in a plastic tube) helps the subjects keeping a constant flow during exhalation. The measurement is non invasive, requires little technical expertise and is easy to perform in older children. Out of three expiratory efforts the highest value was considered in the analysis.

Dosimetry of house dust allergens

When the children reached age of 6, house dust samples were collected from kitchen floors and from children's bedrooms and the mattresses. Floors were sampled over a 2-min period, from a 2 m^2 frame; in bedrooms, samples were collected adjacent to the bed, and in the kitchen where the child used to spend time. Parents were requested not to clean the mattresses, sweep or vacuum these floors for 48 h prior to sampling. The same vacuum cleaner was used to collect dust samples from all household sites, and trained staff performed the dust collection. To avoid cross-contamination between samples from different sites, vacuum cleaner parts were cleaned with wet cloths and dried after each sampling. All dust samples were sealed in plastic bags and sent to the laboratory of the Department of Clinical Immunology at the Polish-American Institute of Pediatrics (Jagiellonian University Medical College), where they were stored at 4°C, under desiccant, until they were extracted. Extracted dust samples were assayed for common domestic aeroallergens (Der f1 and Der p1), by ELISA (Indoor Biotechnologies, Chester, United Kingdom). House dust cumulative domestic allergens (μg/g) was used to define the exposure status of the household.

Ascertainment of atopic status

All 5-year olds who completed the follow-up underwent SPT for 4 common aeroallergens (Dermatophagoides pteronyssimus, Dermatophagoides farinae, dog and cat hair). The results were read after 15 min by measuring the largest diameter of the wheal. Sensitization status was ascertained as a wheal-reaching diameter 3 mm and greater than the histamine control for at least one of the tests.

Statistical analysis

Statistical analysis was performed in order to assess a possible association between maternal allergy and FeNO in seven-year olds. As the distribution of FeNO values were positively skew, in the statistical analysis we used log-transformed values which normalized the distribution. To assess the significance of difference of FeNO in subgroups of children the t-test for log-transformed FeNO values was performed. In the preliminary analysis the effects of potential confounders (child's gender, past respiratory symptoms, body mass index, exposure to common domestic allergens, prenatal and postnatal environmental tobacco smoke) were also checked. Finally, to assess whether the production of FeNO is different in the group of children with and without maternal atopy and to select a reasonable cut off value, we analyzed the ROC curve as well. All statistical analyses were performed with the STATA software (version 12.1).

RESULTS

Table 1 presents the characteristics of the study sample grouped by the maternal atopy. Beside FeNO levels, the groups did not differ in terms of basic characteristic. Children without reported maternal atopy had significantly lower mean FeNO values (geometric mean =5.2 ppb; 95%CI: 3.9–6.9 ppb) than

Table 1. Characteristics of healthy non-asthmatic and non-sensitized children included in the analysis (grouped by the medical diagnosis of maternal atopy). [a]/ Common domestic allergens: house mites (Dermatophagoides pteronyssimus and Dermatophagoides farinae).

Variables	Maternal atopy (-) N=49	Maternal atopy (+) N=12	Total N=61	P for difference
Gender of child: Boys n (%)	24 (49.0)	6 (50.0)	30 (49.2)	
Girls n (%)	25 (51.0)	6 (50.0)	31 (50.8)	1.0000
Cord blood cotinine level:				
0.00–0.06 n (%)	24 (53.3)	7 (58.3)	31 (54.4)	
0.06–0.12 n (%)	14 (31.1)	4 (33.3)	18 (31.6)	0.8145
>0.12 n (%)	7 (15.6)	1 (8.3)	8 (14.06)	
Missing date	4	0	4	
ETS (1-7yr): No n (%)	37 (78.7)	7 (63.6)	44 (75.9)	
Yes n (%)	10 (21.3)	4 (36.4)	14 (24.1)	0.5084
Missing date	2	1	3	
Eczema (1-7yr): No n (%)	14 (29.8)	3 (27.3)	17 (29.3)	
Yes n (%)	33 (70.2)	8 (72.7)	41 (70.7)	1.0000
Missing data	2	1	3	
Cough days (1-7 yr):				
0-125 n (%)	24 (51.1)	5 (45.5)	29 (50.0)	
>125 n (%)	23 (48.9)	6 (54.5)	29 (50.0)	1.0000
Missing data	2	1	3	
Wheezing days (1-7 yr):				
0 n (%)	29 (61.7)	4 (36.4)	33 (56.9)	
≥1 n (%)	18 (38.3)	7 (63.6)	25 (43.1)	0.2343
Missing data	2	1	3	
BMI at age 7 yrs	16.42	16.21	16.37	
Mean SD	1.969	1.733	1.912	0.7411
Missing data	1	0	1	
FeNO (ppb) at age 7 yrs:				
0–10 n (%)	40 (81.6)	5 (41.7)	45 (73.8)	
11–20 n (%)	7 (14.3)	5 (41.7)	12 (19.7)	0.0175
≥21 n (%)	2 (4.1)	2 (16.7)	4 (6.6)	
Common domestic allergens[a]:				
(µg/g) 0–2 n (%)	20 (51.3)	3 (33.3)	23 (47.9)	
>2–10 n (%)	5 (12.8)	1 (11.1)	6 (12.5)	0.5426
>10 n (%)	14 (35.9)	5 (55.6)	19 (39.6)	

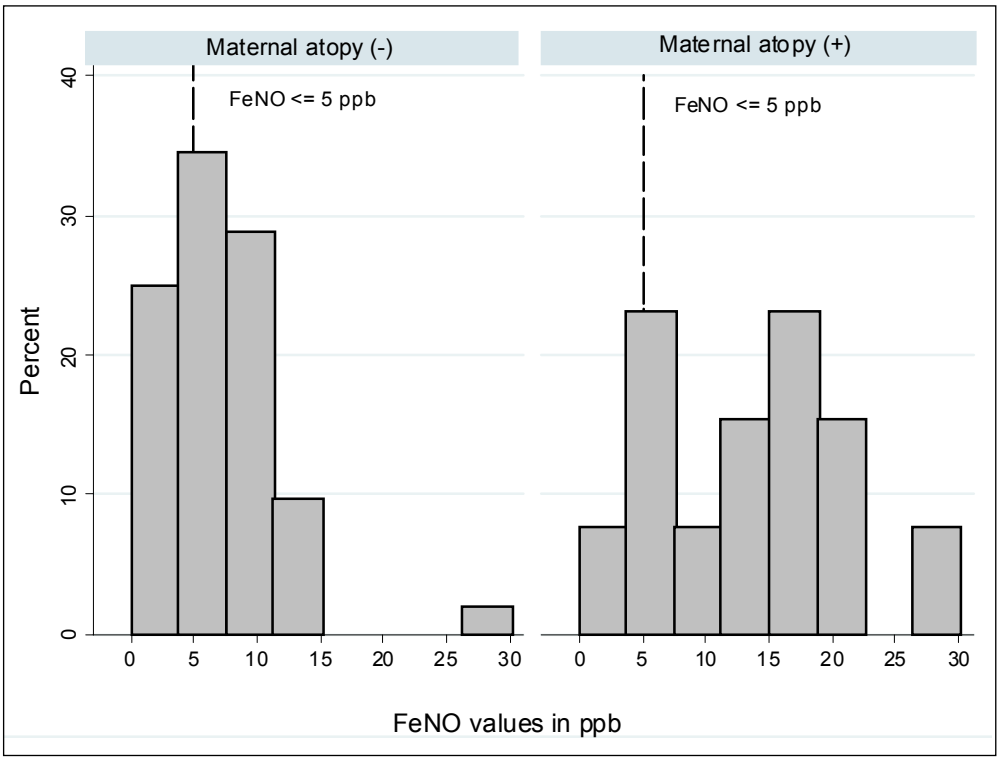

Fig. 1. Distribution of FeNO values in groups of children grouped by the atopic status of mothers.

Table 2. Logistic multivariable regression model for maternal atopy based on FeNO measurements in children without asthma and non-sensitized to common domestic allergens.

Predictors	Odds Ratio	z	P>z	[95% Conf.	Interval]
FeNO log-transformed	2.66	2.03	0.042	1.04	6.85
Eczema reported in the last year	0.85	-0.14	0.888	0.08	8.67
Constant	0.034	-2.98	0.003	0.004	0.316

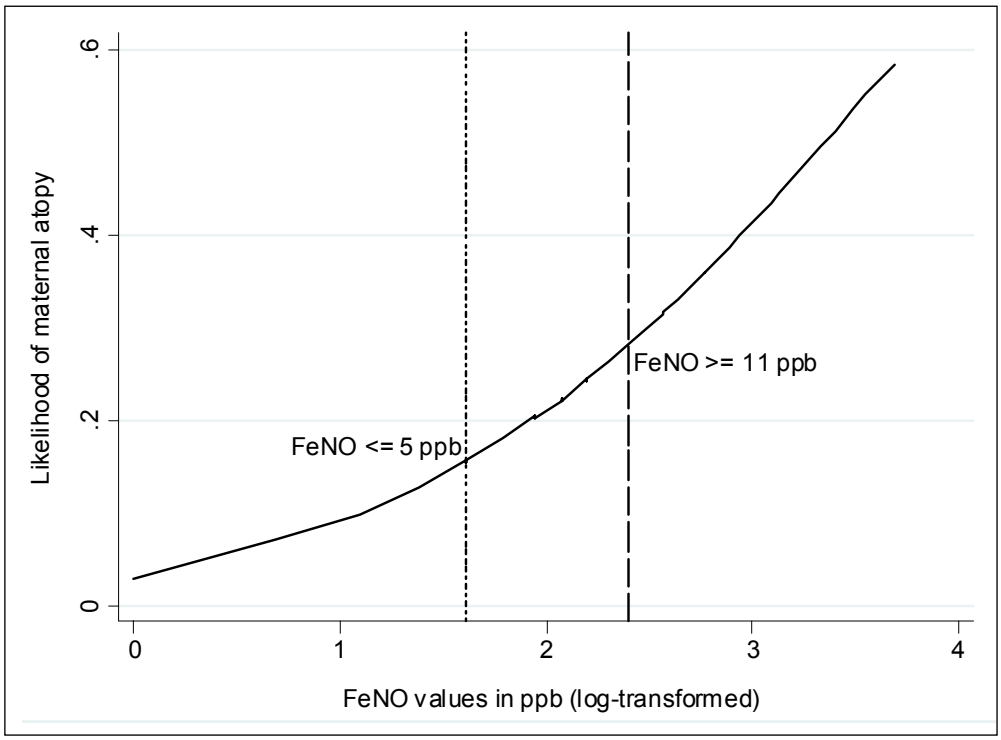

Fig. 2. Fitted likelihood of self-reported maternal atopy in non-sensitized children to common house dust allergens by FeNO values (estimates based on logistic regression model, Lowess smoothing).

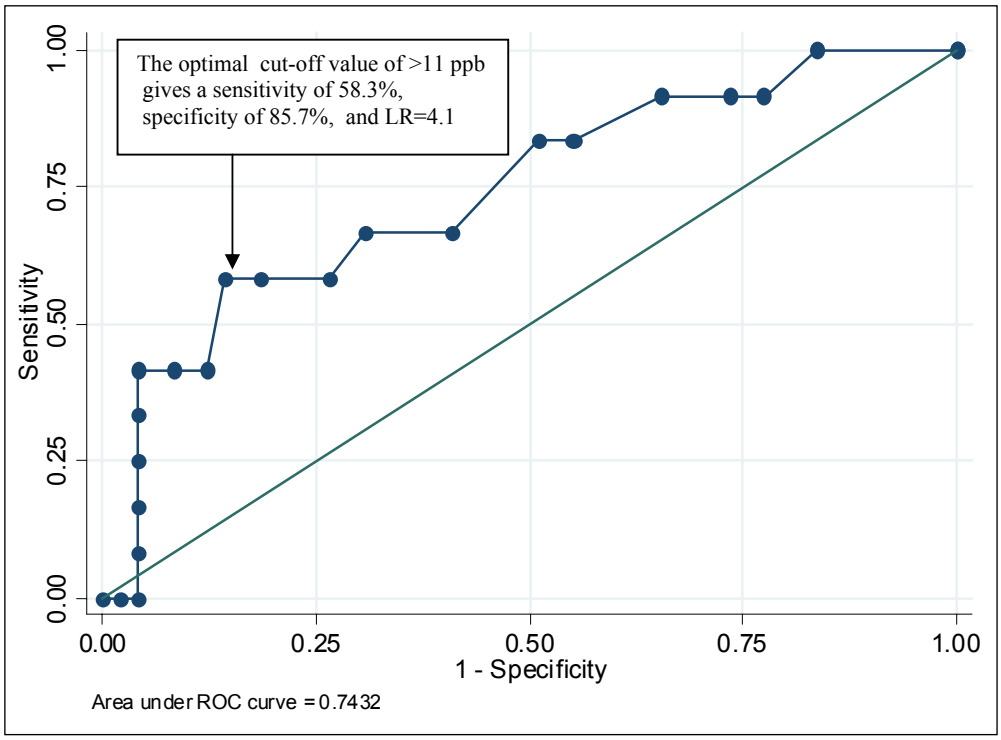

Fig. 3. Receiver operating characteristics (ROC) curve highlighting optimal cut-off points of FeNO for distinguishing children with maternal allergy and without it. The area under ROC curve (AUC) is the probability that a random true-positive child has the test value higher than a random true-negative person.

those who reported maternal atopy (geometric mean =10.7 ppb; 95%CI: 6.7–17.1 ppb) (p=0.010). Percent of very low FeNO values (<=5 ppb) was much higher in children having atopic mothers than in those with non- atopic mothers (34.7 *vs*. 8.3, chi=3.220, p=0.073). *Fig. 1* shows the detailed distribution of FeNO grouped by the atopic status of mothers.

Likelihood of maternal atopy related to FeNO values was presented in *Table 2*. It does show that OR more than doubled with one unit of log FeNO values. The estimates of OR for the occurrence of eczema in the last year appeared to be insignificant. *Fig. 2* is visual presentation of the association between maternal atopy and FeNO levels (log-transformed). Neither associations between FeNO values and gender of children, the respiratory symptoms in the past, nor ETS exposure in the prenatal and postnatal periods or body mass of children were significant. We also found no significant association of FeNO values with the amount of common domestic allergens collected in the households of children as well.

The results of the ROC analysis to choose the optimal FeNO value for the discrimination of children with and without maternal atopy was presented in *Fig. 3*. It suggests that the optimal cut-off point for FeNO level that provided the best combination of specificity and sensitivity was >11 ppb. This provides the estimates of 58.3% sensitivity (95%CI: 27.7–84.8%) and 85.7% specificity (95%CI: 72.8–94.1%), with an AUC (area under curve) of 0.743 (p=0.004). The likelihood ratio (LR of 4.1) indicates that a child with FeNO level >11 ppb is more than 4 times as likely to have atopic mother than one with lower test value.

DISCUSSION

This is the first study that found higher FeNO values in older children of atopic mothers and provide evidence for the potential importance of maternal factors in the development of airway disease. Previous studies were mainly concerned with utility of FeNO measurement for screening of asthma and atopy and none of the studies in older children or adults were concerned with maternal atopy as a biologic source of FeNO variability. It is worthwhile to mention that the children under study were asymptomatic over at least one month preceding the testing and did not suffer from asthma or sensitization to common domestic allergens.

The ROC curve analysis showed that that FeNO level >11 ppb discriminates healthy children with and without maternal atopy. A child with FeNO test above 11 ppb would be more that 4 times as likely to have atopic mother than an individual with lower test value. As the upper normal level of FeNO in healthy seven-year olds with negative history of maternal atopy was 6.9 it may point to the normal cut-off point for healthy non-symptomatic children. However, the relative low detection rate of FeNO and small study sample did not allow us to further the issue. Another limitation of the study is relatively low representation of healthy non-sensitized children without eczema and wheeze.

The biological meaning of increased FeNO level in children of mothers with reported maternal allergy is yet unclear. It may be assumed that the inducible nitric oxide synthase in those children is up-graded or is secondary to recurrent immunological stimulation by respiratory infections taking place in the past. The latter hypothesis, however, is not likely as we could not confirm an association between past history of respiratory symptoms (cough/wheezing spells) and FeNO values. We rather think that the effect of maternal allergy-related factors on FeNO phenotype may be established in early fetal development and could have persisted throughout later life. The biologic meaning of family allergy influencing FeNO phenotype has to be clarified by ongoing genetic studies taking into consideration gene-environment interactions.

Our findings on the effect of maternal atopy on FeNO is consistent with the results of the study by Frey *et al*. (35). The authors in a prospective study determined levels of exhaled NO in 98 healthy unsedated infants (35 from mothers with atopy) with prenatal and postnatal environmental exposures and found that prenatal and postnatal tobacco exposure was associated with much higher exhaled NO level in children of atopic mothers than that in non-atopic mothers. Authors hypothesized that in the early phase of immunologic development, before the onset of infections and allergic disease, the effect of prenatal or early postnatal environmental factors on exhaled NO was modified by the presence of maternal factors in the development of airway disease.

In our study FeNO have been measured by the new electronic portable device (NObreath, Bedfont), which gives reproducible values and was found appropriate for both healthy and asthmatic children. Moreover, the results of the NObreath analyser has also been found directly comparable with other standard stationary techniques used in clinical studies. For example, Pisi *et al*. (36) in a cohort of asthmatic patients compared FeNO values obtained by the Bedfont NObreath analyzer with those of the standard stationary chemiluminescence analyzer (NIOX, Aerocrine AB, Solna, Sweden), and found a strong relationship between the FeNO values obtained by these two devices ($r=0.95$, $p<0.001$). The within-patient repeatability was excellent in both devices (intraclass correlation coefficients for NIOX and NObreath values were 0.925 and 0.967, respectively). In another study Antus *et al*. (37) compared FeNO levels measured by the NObreath device (Bedfont) with a chemiluminescence detector (Logan, Logan Research) in healthy volunteers. They also confirmed an excellent linear relationship between FeNO measurements performed by the two devices ($r=0.923$, $p<0.001$).

In conclusion, our study provided some evidence suggesting that maternal atopy may affect FeNO level in children independently of asthma and sensitization status to common domestic allergens. The data documenting effects of maternal allergy on FeNO level in children should be considered in the interpretation of FeNO levels in clinical practice and setting up FeNO screening criteria for identification of eosinophilic airway inflammation. As our results are preliminary, they have to be verified in the bigger prospective population-based cohort studies.

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