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# The effect of passive smoking on exhaled nitric oxide in asthmatic children

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A B S T R A C T

To date, some studies suggest that passive smoking (PS) may be an important determinant of FeNO levels in children but sill there is a need of investigations using objective methods of PS exposure.

The aim of our study was to examine the effect of PS, measured by urine cotinine levels, on FeNO and lung function (FEV1) in allergic and non-allergic asthmatic children.

*Methods:* It was a prospective, non-interventional study. 140 children, aged 4–17, newly diagnosed with asthma were recruited into two study groups (exposed group, unexposed group), according to smoking exposure/unexposure based on the questionnaire. There was one study visit. Subjects underwent a medical history (including history of atopy), physical examination, spirometry, FeNO and urinary cotinine measurement.

*Results:* 70 individuals had been exposed to tobacco smoke. The patients exposed to tobacco smoke were characterized by statistically significantly higher urine concentration of cotinine, 10,80 ng/mL, than their counterparts who had not been exposed to tobacco smoke, 1,56 ng/mL (P = 0,019). In the group of individuals unexposed to tobacco smoke the mean value of FeNO was 34,99 ppb, while in the group of patient who had been exposed to tobacco smoke, the corresponding mean value was significantly lower, it amounted to 22,41 ppb (P = 0,001) (Table 1). As regards to FEV1 measurements, there were not any statistically significant differences by study groups unexposed/exposed to tobacco smoke (P = 0,179 and P = 0,074, respectively). FeNO levels (ppb) in the studied patients allergic to cat, grass or trees, exposed to tobacco smoke were significantly lower than in those children unexposed to tobacco smoke.

Our results suggest a clinically important issue, that FeNO results should be interpreted in the context of environmental tobacco smoke exposure. Additionally allergy to cat dander, grass or tree may be potential confounding factor, which should be taken into consideration.

## 1. Introduction

The current concept of asthma pathogenesis underlines a chronic inflammatory process that causes air flow obstruction and bronchial hyperreactivity [1]. The measure of fractional concentration of exhaled nitric oxide (FeNO) may be considered a marker of common asthma endotype characterized by Th2-mediated airway inflammation, eosinophilia and responsiveness to inhaled steroids [2]. Since FeNO is being used by some clinicians as a treatment follow-up parameter in allergic asthmatics, it should be cleared, which factors can influence NO production.

Even if both active and passive smoking is decreasing, there are still children exposed to passive smoke. To date, studies suggest that smoking may be an important determinant of FeNO levels and this effect was found after both active and passive smoking (PS). Healthy adults exposed passively to environmental tobacco smoke had reduced FeNO levels [3]. Exposure to domestic tobacco smoke is widespread and children with asthma are particularly susceptible to it's detrimental effects [4]. Some authors indicate passive smoking as a major determinant of FeNO in asthmatic children [5] but sill there is a need of investigations using objective methods of PS exposure. Children's exposure to PS can be measured by the report of an adult in the child's household and testing the child's or a household member's biomarker, such as cotinine, especially when the biomarker is measured while a person receives an ongoing exposure to tobacco smoke [6,7]. The aim of our study was to examine the effect of passive smoking, on FeNO level and lung function (FEV1) in allergic and non-allergic asthmatic children. An assessment of patients' tobacco exposure was supposed to be accomplished by means of estimating urine cotinine levels.

## 2. Methods

### 2.1. Study design

It was a prospective, real life, non-interventional study. All children, aged 4–17, newly diagnosed with asthma in our Allergic Outpatient Clinic, between January 2017 and February 2018, able to perform spirometry and FeNO, were included into the study. A diagnosis of

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**Table 1**
Descriptive statistics for analyzed measurable traits in the studied patients by exposition to tobacco smoke.

| Analyzed trait | Unexposed | | Exposed | | Level of statistical significance |
|---|---|---|---|---|---|
| | M* | 95% CI** | M* | 95% CI** | |
| Age (years) | 8.74 | 7.91–9.57 | 8.63 | 7.87–9.38 | P = 0.840 |
| FEV1 best (L) | 2.04 | 1.83–2.25 | 1.86 | 1.69–2.02 | P = 0.179 |
| FEV1 (%predicted) | 104.97 | 102.09–107.86 | 100.57 | 96.65–104.50 | P = 0.068 |
| FeNO (ppb) *** | 26.98 | 22.96–31.70 | 17.98 | 15.49–20.87 | **P = 0.001** |
| Cotinine in urine (ng/mL) | 1.56 | 1.12–1.99 | 10.80 | 2.75–18.84 | **P = 0.019** |
| Number of cigarettes smoked daily in the domicile | – | – | 29.26 | 23.68–34.83 | – |

*M – mean; ** CI – confidence interval; *** – Geometric means were provided.
Bold indicates p-value considered to be significant under 0.5.

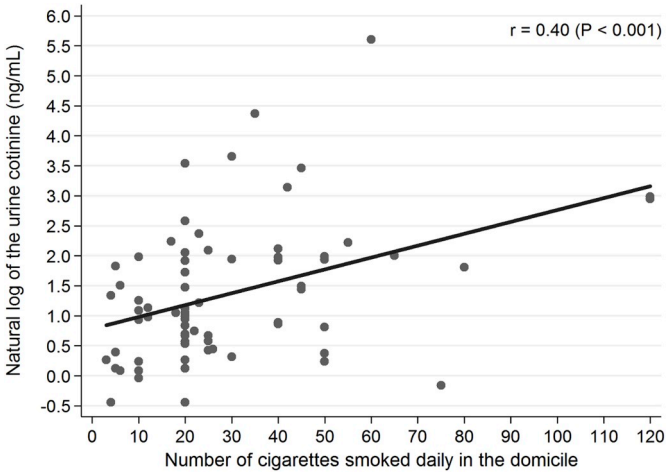


**Fig. 1.** Relation between urine cotinine concentration (ng/mL) and the number of cigarettes smoked daily in the domicile.

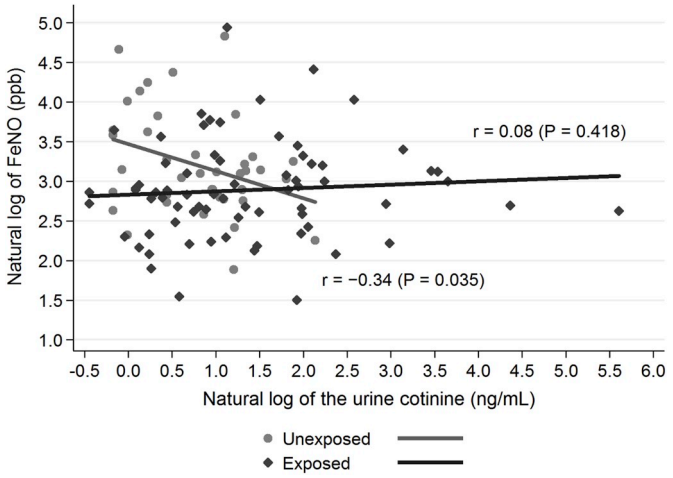


**Fig. 2.** Relation between FeNO (ppb) and urine cotinine concentration (ng/mL).

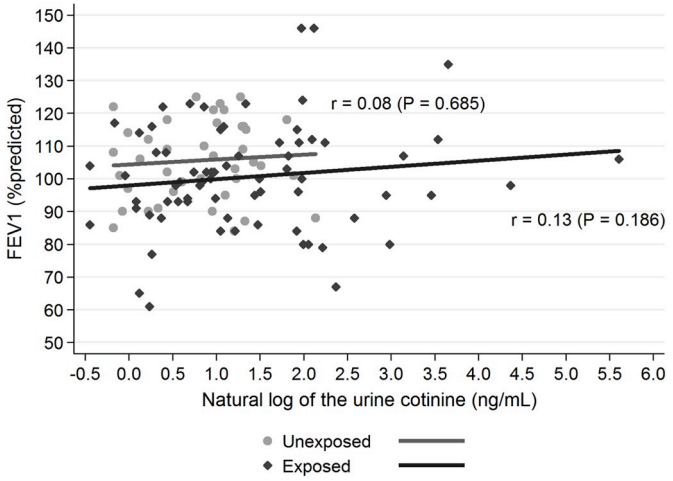


**Fig. 3.** Relation between FEV1 (%predicted) and urine cotinine concentration (ng/mL).

asthma was established by the allergist doctors following GINA guidelines [1,8]. Parents/caregivers were asked if their children are exposed to tobacco smoke at home, and how many cigarettes they smoke daily. Children were considered to be exposed to PS when at least 1 cigarette per day was declared to be smoked at home [5]. Patients were recruited into two study groups (exposed group, unexposed group), according to smoking exposure/unexposure, until the number of 70 children in each group was reached. There was one study visit. Subjects underwent a medical history (including history of atopy), physical examination, spirometry, FeNO and urinary cotinine measurement. The sequence of measurements was spirometry and FeNO. All parents or legal guardians gave their written consent for the evaluation of data from medical documentation of their children. Patients were classified as atopic based on history and skin prick testing/sIgE. In patients with polysensitization, clinically meaningful allergen was defined. In patients with cat dander sensitization, there were children with any other sensitizations as well as monosensitized to cat.

The study was approved by the Medical Ethics Committee of the Medical University of Lodz, approval number RNN/76/14/KE.

### 2.2. Pulmonary function tests

Spirometry was done with a Master Screen unit (Erich Jaeger Gmbh-Hochberg, Germany). Flow and volume were measured with a pressure-screen-type pneumotachograph, calibrated daily. Measurements were carried out in a familiar and quiet room. Standing height and weight were assessed: subjects were measured without shoes. During measurements, children were instructed to sit upright, and a nose clip and a non-compressible mouthpiece were used. When needed, an adult accompanied the subject during testing. Predicted values for all lung function variables were based on a previous study of healthy controls, provided by the lung function test equipment manufacturer. All pulmonary function tests were performed by one trained research technician, according to the American Thoracic Society/European Respiratory Society standards [9]. The highest of 3 successful measurements was taken and analyzed. The results were expressed as the percentage of a predicted value.

### 2.3. Nitric oxide measurement

The NO measurements were performed according to the European

**Table 2**
Descriptive statistics for FeNO in the studied patients by exposition to tobacco smoke and presence of separate monovalent allergy.

| Allergen | Exposition to tobacco smoke | Unexposed | | Exposed | | Level of statistical significance* | |
|---|---|---|---|---|---|---|---|
| | Allergy status | M ** | 95% CI | M ** | 95% CI | By exposition to tobacco smoke[a] | By presence of allergy[b] |
| House dust mites | Non-allergic | 24.61 | 20.22–29.96 | 16.08 | 13.14–19.68 | **P = 0.001** | P = 0.087 |
| | Allergic | 32.54 | 24.38–43.44 | 20.92 | 16.67–26.25 | P = 0.123 | |
| Trees | Non-allergic | 23.39 | 19.85–27.56 | 15.93 | 13.63–18.60 | **P = 0.003** | **P = 0.024** |
| | Allergic | 43.70 | 29.87–63.94 | 27.20 | 18.80–39.35 | **P = 0.029** | |
| True grasses | Non-allergic | 22.26 | 18.58–26.69 | 14.80 | 12.55–17.45 | **P = 0.002** | **P = 0.039** |
| | Allergic | 35.98 | 27.25–47.52 | 24.04 | 18.49–31.25 | P = 0.176 | |
| Moulds | Non-allergic | 26.53 | 22.46–31.32 | 17.43 | 14.88–20.42 | **P = 0.001** | P = 0.303 |
| | Allergic | 32.32 | 13.41–77.85 | 22.54 | 12.23–41.52 | **P = 0.007** | |
| Cat dander | Non-allergic | 22.89 | 19.59–26.75 | 16.28 | 13.85–19.13 | **P = 0.003** | **P = 0.003** |
| | Allergic | 52.05 | 35.82–75.63 | 28.04 | 20.09–39.13 | **P = 0.047** | |
| Dog dander | Non-allergic | 25.98 | 22.19–30.41 | 17.01 | 14.60–19.82 | **P = 0.003** | P = 0.179 |
| | Allergic | 62.81 | N/A | 34.19 | 18.99–61.54 | **P = 0.029** | |
| Mugwort | Non-allergic | 26.99 | 22.73–32.04 | 17.43 | 15.00–20.25 | **P = 0.001** | P = 0.787 |
| | Allergic | 26.85 | 15.01–48.04 | 23.55 | 8.89–62.37 | P = 0.529 | |

*The statistical significance was estimated by using multivariate models; all the calculations were controlled for the study subjects' age and gender.
Bold indicates p-value considered to be significant under 0.5.
a. p-values for between-group differences by exposition to tobacco smoke, in the allergic and non-allergic subjects separately, were shown.
b. p-values for differences between the allergic versus non-allergic patients, obtained in a multifactor analysis including interaction terms, were shown.
** Geometric means were provided.

Respiratory Society/American Thoracic Society (ERS/ATS) recommendations [10,11], with a chemiluminescence analyzer (model280i 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 0.1–500 ppb. Environmental NO was measured before and after each test and it never exceeded 5 ppb. All subjects were tested in a sitting position, without wearing a nose clip. The subjects exhaled at a constant flow rate (50 mL/s) from total lung capacity to residual volume without breath holding. They maintained a constant mouth pressure (17 cm $H_2O$) by monitoring a visual display in order to eliminate contamination from nasal NO. 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.

### 2.4. Cotinine level assessment

A urine sample was collected from all patients. A 50 mL volume of morning urine was collected into a 100 mL polypropylene container (Bene, Poland). All urine samples were transported to the laboratory in a cool box and stored at −20 °C until analysis. Urinary cotinine levels in ng/mL (marker of tobacco smoke exposure) were determined employing the method described and optimized by Stragierowicz et al. (2013) [12].

### 2.5. Statistical methods

The investigated traits were described by way of measures of location – mean, median and quartiles, along with measures of dispersion – interquartile range, standard deviation, standard error of mean, 95% confidence interval, and minimum-to-maximum values. The categorical variables were depicted by using absolute values and percentages.

Mixed-effects linear regression or logistic regression models were fitted in order to test the significance of differences in the investigated parameters between the two study groups. When dealing with non-normally distributed variables, robust standard errors were employed within a specific regression model. All the regression equations were controlled for the studied patients' age and gender.

A level of $P < 0.05$ was considered statistically significant. All the statistical computations were carried out by means of Stata/Special Edition, release 14.2 (StataCorp LP, College Station, Texas, USA).

## 3. Results

We included 140 children, 60 girls and 80 boys into the analysis. Among them, 70 individuals had been exposed to tobacco smoke, 33 girls (47,14%) and 37 boys (52,86%), whereas 70 study participants had not been exposed to tobacco smoke, 27 girls (38,57%) and 43 boys (61,43%) (P = 0,306). The mean age of the study participants amounted to 8,69 years (±SD = 3,32 years). There was no statistically significant difference concerning the studied patients' age by study group (P = 0,840). Descriptive statistics for the analyzed parameters in the studied patients by exposition to tobacco smoke are presented in Table 1.

The patients exposed to tobacco smoke were characterized by statistically significantly higher urine concentration of cotinine, 10,80 ng/mL, than their counterparts who had not been exposed to tobacco smoke, 1,56 ng/mL (P = 0,019) (Table 1). There was a correlation between the number of cigarettes smoked daily in the domicile and patients' urine cotinine levels (Fig. 1).

In the group of individuals unexposed to tobacco smoke the mean value of FeNO was 34,99 ppb, while in the group of patient who had been exposed to tobacco smoke, the corresponding mean value was significantly lower, it amounted to 22,41 ppb (P = 0,001) (Table 1). As regards to FEV1 measurements, there were not any statistically significant differences by study groups unexposed/exposed to tobacco smoke (P = 0,179 and P = 0,074, respectively). There were not any statistically significant correlation between FeNO level and FEV1 and urine cotinine levels (P > 0.3) (Fig.s 2 and 3).

Descriptive statistics for the values of FeNO in the studied patients, by exposition to tobacco smoke and presence of any allergy are presented in Table 2. The prevalence of investigated types of allergy was equalized between the study groups. The association between allergy profile (allergy to all measured allergens) and FeNO level, and FEV1 were measured. FeNO levels (ppb) in the studied patients allergic to cat, grass or trees, exposed to tobacco smoke were significantly lower than in those children unexposed to tobacco smoke. FeNO (ppb) in the studied patients by exposition to tobacco smoke and presence of allergy to

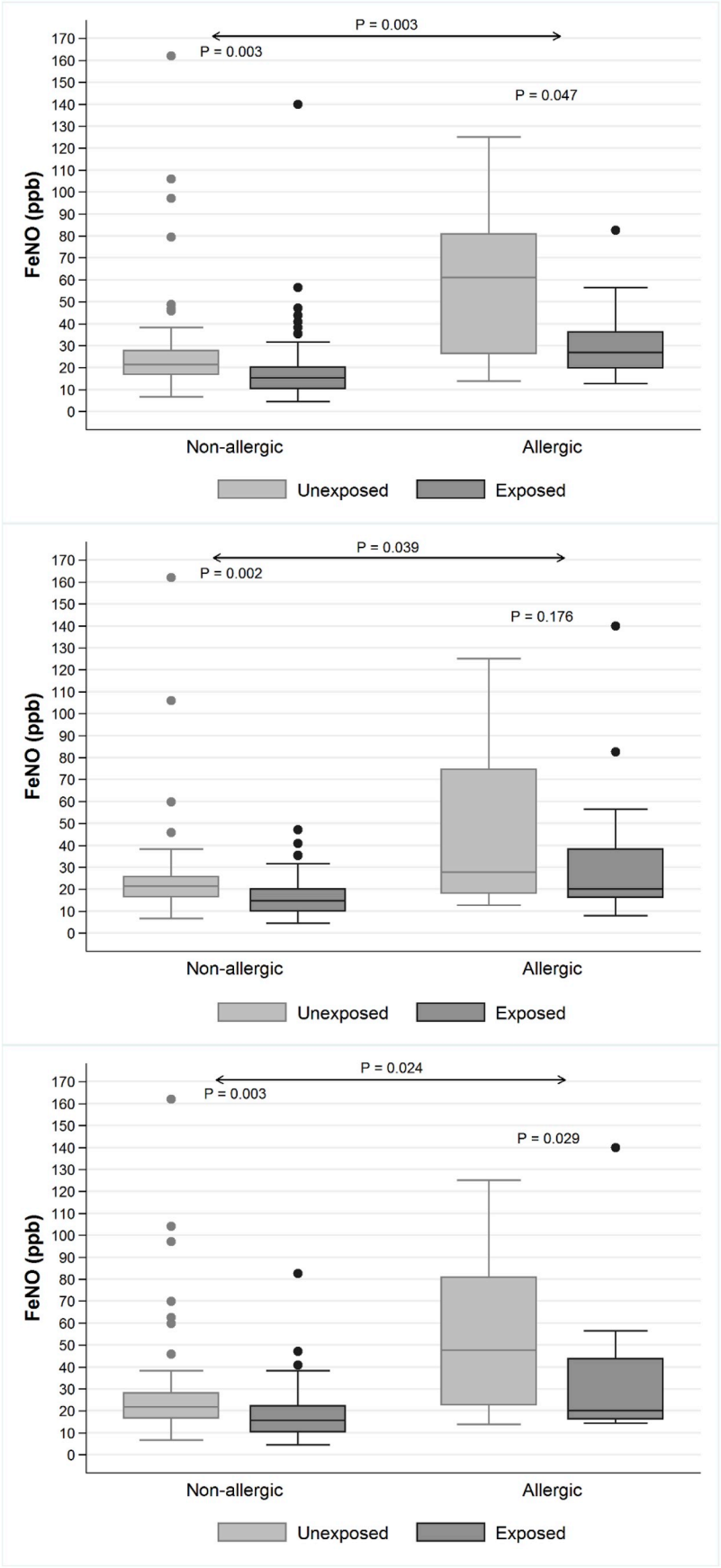


**Fig. 4.** FeNO (ppb) in the studied patients by exposition to tobacco smoke and presence of allergy to (a) cat dander, (b) grasses, and (c) trees.
P-values were obtained through multifactor analyses and were controlled for the studied patients' gender and age. The P-values indicate the interaction between studied variables: presence of investigated monovalent allergy (the upper-middle inscription) and exposition to tobacco smoke within one particular study group, i.e., the non-allergic exposed versus the allergic subjects.

(a) cat dander, (b) grasses, and (c) tress are presented in Fig. 4a,b,c respectively.

The presence of allergy did not coincide with a change in the study participants' respiratory function, with reference to the $FEV_1$ best-to-predicted value (%), both overall, and in the separate study groups.

## 4. Discussion

We conducted the present study to establish the effect of passive smoking (PS) on FeNO level in asthmatic children. Our main findings showed that children with asthma exposed to PS had significantly lower levels of FeNo compared with unexposed to PS, regardless of their allergy status. Taking into consideration allergy profile, we observed that in patients allergic to cat, grass and tree allergens, unexposed to PS, FeNo levels were significantly higher than in patients exposed to PS sensitized to above allergens.

In our previous study, we showed that atopy and presence of allergic rhinitis were independently associated with increased FeNO level [13].

Whether other factors may modified FeNo is not clearly stated. Ricciardolo et al. suggest that neonatal respiratory distress should be taken into consideration in this aspect [14]. Taking into account environmental factors, tobacco smoke is one of the widespread triggers of asthma. According to WHO report, more than 700 million children are vulnerable to be passive smokers [15]. A survey showed that 8.7 million adult Poles smoke tobacco daily. The findings demonstrate smoking, as a serious social problem associated with allergic diseases in Poland [6].

A reduction in FeNO levels in smokers was first described almost 25 years ago [7,16]. Malinovschi et al. found that current smokers exhibited lower levels of FeNo in comparison to ex-smokers and never-smokers [17]. Passive smoking is associated with a reduction in FeNo in healthy subjects [3] and in adults suffering from asthma [18]. The same effect was observed in asthmatic children exposed to tobacco smoke at home [19]. The association with smoking was observed in non-asthmatic and asthmatic participants, especially in atopic asthmatic participants. Multivariate analyses showed that environmental tobacco smoke exposure of at least 2 h/d were negatively and significantly associated with FeNO levels independent of age, sex, height [18]. Laoudi et al. showed that passive smoking lowers FeNO, and might be a major determinant of FeNO levels in nontreated allergic asthmatic children [5]. Our study confirms the above observations, children with asthma exposed to PS had significantly lower levels of FeNo compared with unexposed to PS, regardless of their allergy status. In contrast, other authors failed to demonstrate a difference of FeNO levels in asthmatic children whether exposed or unexposed to PS, [20,21]. Sundy et al. observed only a week correlation between FeNO and serum cotinine levels in subjects with cotinine levels between 1 and 25 ng/ml- concentrations consistent with exposure to second hand smoke [22]. We did not find any statistically significant correlation between FeNO level and urine cotinine levels, and between FeNO and the number of smoked cigarettes by parents, perhaps due to smaller nicotine exposure in our patients compared to smokers.

Taking into consideration allergy to seasonal and perennial allergens, we observed that in patients allergic to cat dander, grass or tree, unexposed to PS, FeNo levels were significantly higher than in above patients but exposed to PS, and in comparison with unexposed to PS non-allergic children. The possible mechanisms by which exhaled NO levels are reduced in smoking subjects are a potential downregulation of NO synthase in the lungs by the NO from cigarette smoke [23,24], an inadequate supply of cofactors necessary for NO production, such as tetrahydrobiopterin [25], and an increase in the breakdown of NO [26,27]. This exogenous NO could both inhibit iNOS activity and down regulate its genetic expression [24]. Other hypothesis suggests that changes in local osmolality in the airways due to cigarette smoke could reduce the NO concentration in expired air [28].

As far as we know, our study is the first assessing passive smoking as a determinant of exhaled nitric oxide, in which, objective marker of environmental tobacco smoke exposure was employed. According to our experience, the bias to underreport secondhand smoke exposure could be particularly strong in children caregivers. This opinion was supported by Boyaci et al. [29] and Howrylak et al. [30] who found that caregiver/parents' reports of tobacco exposure were not reliable. The determination of cotinine is recommended for the monitoring of environmental tobacco smoke [31]. We decided to assess the urinary cotinine because it can be used to estimate more precisely the level of exposure to tobacco smoke, especially in nonsmokers and can be obtained easily and non-invasively [32]. The limitation is that the cut-off value for cotinine in urine is still not established for children and we observed an overlap regarding cotinine levels between exposed and unexposed subjects. In our study we performed spirometry before FeNO, what could be some limitation according to recommendations. There was a suggestion that exhaled NO summary measures assessed ≤ 6 minutes after spirometry were lower in children with asthma [33], therefore we performed FeNO 30 minutes after spirometry.

Our results suggest a clinically important issue, that FeNO results should be interpreted in the context of environmental tobacco smoke exposure. Additionally allergy to cat dander, grass or tree may be potential confounding factor, which should be taken into consideration. Especially, since there are observations that FeNO helps to differentiate asthmatics from non-asthmatics [34,35], and FeNO-guided treatment significantly reduces exacerbation rates compared with guidelines-based treatment in children and young adults (GINA) [36].

## Acknowledgements

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