

## The association between fractional exhaled nitric oxide (FeNO) and cat dander in asthmatic children

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### ABSTRACT

**Background:** The aim of our study was to assess risk factors of increased FeNO in asthmatic children with no cat at home.

**Methods:** It was a retrospective, cross-sectional study. We evaluated data from medical documentation of children with asthma: FeNO results, allergen sensitization, seasonal allergen exposure, FEV<sub>1</sub>, allergic rhinitis (AR) diagnosis and cat presence at home. We assessed asthma severity using mean doses of inhaled glucocorticosteroids and a management approach based on control according to the newest guidelines of Global Initiative for Asthma (GINA) throughout the last three months before the measurement of FeNO and spirometry.

**Results:** 316 patients (age 6–18) completed the study. Sensitization to cat dander was associated with the highest median value of FeNO concentration compared to other allergens in our patients (28.4 ppb) and co-existing sensitization did not affect FeNO level. Median levels of FeNO increased linearly with patient's age. In asthmatics with AR, the levels of FeNO were increased significantly compared to asthmatics without AR (20.8 vs. 16.3, respectively). We showed that in patients without AR, sensitization to cat allergen was associated with more severe asthma in comparison to other perennial allergy (step 4 vs. other steps according to GINA treatment steps). The above relation was not observed in patients with AR. We did not observe correlation between allergy profile and FEV<sub>1</sub> among patients in neither subgroup nor in general population.

**Conclusions:** We revealed that sensitization to cat dander was associated with the highest increase of FeNO concentration compared to other allergens in patients not having any cat at home ever. We also observed that in patients without allergic rhinitis, sensitization to cat allergen, compared to other perennial allergy, was associated with more severe asthma.

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### Introduction

The current concept of asthma involves a chronic inflammatory process which causes the development of airflow limitation and increased responsiveness to allergens [1]. Fractional exhaled nitric

**Abbreviations:** AR, allergic rhinitis; ERS/ATS, European Respiratory Society/American Thoracic Society; GINA, Global Initiative for Asthma; *Fel d 1* protein, *Felis domesticus* protein 1; FeNO, fractional exhaled nitric oxide; FEV<sub>1</sub>, forced expiratory volume in one second; IFN- $\gamma$ , interferon- $\gamma$ ; IL-5, interleukin 5; iNOS, inducible nitric oxide synthase; NOS, nitric oxide synthases; SPT, skin prick tests.

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oxide (FeNO) has proven to be a reliable noninvasive measure for airway inflammation. The synthesis of NO is mediated by NO synthases (NOS) and only the expression of inducible isoform (iNOS) correlates with the levels of exhaled NO [2–5]. FeNO also correlates with bronchial reactivity and decreases with anti-inflammatory asthma therapy, such as inhaled corticosteroids and antileukotrienes, in children [6,7]. However, it needs to be noted there are some studies indicating that FeNO does not correlate with the measures of airway hyperresponsiveness or pulmonary function values in children [8,9]. When assessing the relationship between NO and asthma, the role of atopy must be considered. Several studies have shown that children with atopic asthma demonstrate higher levels of FeNO than other patients with asthma [10–12]. Moreover, the pattern of allergen sensitization is likely to play an important role. Sensitization to certain groups of

indoor allergens, namely pet dander and house dust mites, has recently been shown to possess a much greater ability to increase FeNO levels in asthmatic children than other groups of allergens [13]. However, it is worth noting that the same may be true for other allergic diseases in children [14], probably due to specific features of the main proteins in both allergen groups [14,15].

The aim of our study was to assess risk factors of increased FeNO in asthmatic children with no animal at home. Therefore, we evaluated the relationship between FeNO, allergy profile, seasonal allergen exposure, forced expiratory volume in one second (FEV<sub>1</sub>) and asthma severity in a sample of school children with allergist-diagnosed asthma.

## Material and methods

### Study design

It was a retrospective, cross-sectional study with a primary goal to evaluate the environmental predictors of exhaled nitric oxide in children with allergic and nonallergic asthma, with or without AR. We evaluated data from medical documentation of 316 children (age 6–18) who attended our Allergic Outpatient Clinic from January 2008 to March 2009. Three physicians (allergists) assessed the participants. The diagnosis of asthma and allergic rhinitis were universally established by the doctors according to standard definitions of both diseases in the latest guidelines [1,16,17]. Medical documentation of the patients was analyzed with special attention to an allergic rhinitis diagnosis, the results of FeNO, allergen sensitization, and animal presence at home. Asthma control was established according to the newest guidelines of Global Initiative for Asthma (GINA) [1]. We analyzed the mean doses of inhaled glucocorticosteroids and the management approach based on control [1], which were assessed throughout the period of three months preceding the measurements of FeNO and spirometry. GINA report underlines that the patient's current level of asthma control and current treatment determine the selection of pharmacologic treatment [1]. For instance, if asthma is not controlled on the current treatment regimen, the treatment should be stepped up until control is achieved [1]. Pharmacological treatment steps, according to GINA for children older than 5, adolescents and adults are as follows [1]:

- Step 1: As-needed reliever medication.
- Step 2: Reliever medication plus a single controller.
- Step 3: Reliever medication plus one or two controllers.
- Step 4: Reliever medication plus two or more controllers.
- Step 5: Reliever medication plus additional controller options.

In our Allergic Outpatient Clinic, FeNO was measured prior to a forced flow-volume curve measurement on the same day (in the morning, between 9 a.m. and 11 a.m.) in all patients. The study was approved by the Medical Ethical Committee of the Medical University of Lodz. All parents or guardians of the patients gave their oral consent for the evaluation of data from medical documentation of their children.

### Inclusion criteria

School children with allergic and nonallergic asthma, with or without allergic rhinitis, reporting not having kept a cat at home ever.

### Exclusion criteria

Exclusion criteria included a diagnosis of a specific respiratory disease, such as cystic fibrosis, primary ciliary dyskinesia,

interstitial lung disease, pneumonia; tuberculosis and/or current upper airway infection; other clinically significant pulmonary, hematological, hepatic, gastrointestinal, renal, endocrine, neurological, cardiovascular, and/or psychiatric diseases or malignancies that could influence the results of the study; obesity; inability to obtain an appropriate FeNO and spirometry measurement.

### Allergen sensitization

In our outpatient clinic, using skin prick tests (SPT), we tested the subjects' reaction to the following most common inhalant and food allergens: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria*, *Cladosporium*, cat dander, dog dander, mixed grass pollen (Yorkshire fog, Cocksfoot, Rye-grass, Timothy, Blue grass (Kentucky), Meadow fescue), rye, birch, hazel, ribwort, alder, motherwort, feather. Positive (histamine chloride, 10 mg/mL) and negative (glycerol) controls (extracts from Nexter-Allergopharma, Reinbek, Germany) were also used. A positive SPT reaction was defined as a mean wheal diameter of 3 mm in excess of the negative control. Atopy was defined as a positive skin test response to any of the 18 allergens tested.

### Allergen exposure during the time of FeNO measurement

Seasonal allergen exposure from January 2008 to March 2009 during the time of FeNO measurement was assessed using pollen calendars from the internet service of Polish Bibliography of Clinical Palynology [18], valid for the period from January 2007 till July 2009 in Lodz region. Seasonal allergen exposure was defined as allergen exposure causing rapid and reproducible onset and offset of symptoms of allergic disease that appear only during certain times of the year. The exposure was based on the individual patients' allergy profiles and referred to as being "positive" when a patient had FeNO measured during the seasonal exposure to the allergens he/she was sensitized to, and described as being "negative" when a patient was out of the seasonal exposure time during FeNO measurement.

### Nitric oxide measurement

The NO measurements were performed according to the European Respiratory Society/American Thoracic Society (ERS/ATS) recommendations [19] 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 0.1–500 ppb. Environmental NO was measured before and after each test and never exceeded 5 ppb. All subjects were studied in the 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<sub>2</sub>O) 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 three successive, reproducible recordings was retained for statistical analysis.

### Fev<sub>1</sub>

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

**Table 1**  
Baseline characteristics of patients.

|                                              | N              | %    |
|----------------------------------------------|----------------|------|
| Age [years] mean $\pm$ SD                    | 10.2 $\pm$ 4.1 |      |
| Male gender                                  | 230            | 72.3 |
| Asthma treatment according GINA <sup>1</sup> |                |      |
| Level 1                                      | 42             | 13.3 |
| Level 2                                      | 42             | 13.3 |
| Level 3                                      | 98             | 31.0 |
| Level 4                                      | 134            | 42.4 |
| Allergic rhinitis                            |                |      |
| Absence                                      | 120            | 38.0 |
| Presence                                     | 196            | 62.0 |
| Number of sensitizing allergen               |                |      |
| None                                         | 77             | 24.4 |
| One                                          | 99             | 31.3 |
| Two                                          | 73             | 23.1 |
| Three                                        | 51             | 16.1 |
| Four                                         | 16             | 5.1  |
| Sensitization to grass pollen                | 154            | 48.7 |
| Sensitization to tree pollen                 | 93             | 29.4 |
| Sensitization to house dust mite             | 141            | 44.6 |
| Sensitization to molds                       | 74             | 23.4 |
| Sensitization to cat                         | 65             | 20.6 |
| Seasonal allergen exposure                   | 164            | 51.9 |

study of healthy controls provided by the manufacturer of the lung function test equipment [20,21]. Flow-volume curves were performed according to the American Thoracic Society standards [22]. The highest of three 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.

### Statistics

The study was designed to achieve at least 90% power (for 300 patients completing the study) in detecting at least 4 ppb difference in FeNO between atopic and non-atopic patients. Calculations were performed for a Mann–Whitney test based on the assumption that the variability and distribution of our primary outcome was similar to that observed in a previous study [23]. The associations of a range of clinical parameters (see Table 1) with FeNO level were assessed by Mann–Whitney or Kruskal–Wallis tests. The influence of the potential confounding variables such as age or the coexistence of allergic rhinitis on the relation between cat allergy and FeNO levels was analyzed by linear regression in a multivariate model (Table 3). Associations between more severe asthma (step 4 vs. other steps according to GINA treatment steps), defined as a dependent variable, and the allergy profile (independent variables) were assessed by logistic regression analysis in a univariate (Table 4) and multivariate model, which allows to point out the best predictor of a dependent variable. All statistical analyzes were performed using Statistical Package for the Social Sciences (SPSS) 11.5.  $P < 0.05$  was used as a definition of statistical significance.

### Results

Data obtained from three hundred and sixteen patients were included in the analysis. Baseline characteristics of the patients are shown in Table 1. Sixty-five patients (20.6%) were allergic to cat dander.

Sensitization to cat dander was associated with the highest median value of FeNO concentration compared to other allergens in our patients (Table 2 and Fig. 1), and the co-existing sensitization did not affect FeNO level (Fig. 1). These results were consistent in all patients independently of seasonal allergen exposure. The

number of sensitizing allergens had no influence on median FeNO concentration in our patients (Table 2 and Fig. 1). The median levels of FeNO increased linearly with the patient's age (Table 2 and Fig. 1). In asthmatics with AR, the levels of FeNO were increased significantly compared to asthmatics without AR (Table 2 and Fig. 1). The above findings suggested the influence of the potential confounding variables such as age or the coexistence of allergic rhinitis on relation between cat allergy and FeNO levels (Table 2 and Fig. 1). The potential confounding was assessed in a multivariate model of linear regression analyzes, with FeNO as a dependent variable and cat allergy, age and AR coexistence as independent variables. The results (Table 3) showed that cat allergy was the independent and the strongest predictor of FeNO level in our population.

In the second step, we aimed to assess the impact of cat allergy on asthma severity. Since allergic rhinitis coexistence determined more severe asthma in our patients (data not shown) we conducted the analyzes in two subgroups, with and without AR. The univariate model of logistic regression analysis showed that in patients without allergic rhinitis sensitization to cat allergen compared to other allergy was associated with more severe asthma (step 4 vs. Other steps according to GINA treatment steps) (Fig. 1, Table 4). Moreover, cat dander sensitization was the only independent factor associated with more severe asthma in a multivariate model.

Cat dander allergy increased the risk of having more severe asthma 9 times in patients without AR (OR 9.14). The above relation was not observed in patients with AR. We did not observe any correlation between the allergy profile and FEV<sub>1</sub> among the patients in neither subgroup nor in the general population.

### Discussion

Our study was conducted in the population of children with asthma, sensitized to cat dander, who were reporting not having

**Table 2**  
FeNO level by study variables.

|                                              | FeNO         |                | P level |
|----------------------------------------------|--------------|----------------|---------|
|                                              | Median value | Quartile range |         |
| Age < lower quartile                         | 14.3         | 9.8–20.2       | <.001   |
| Lower quartile–median                        | 15.9         | 11.5–25.7      |         |
| Median–higher quartile                       | 23.1         | 14.9–39.1      |         |
| >Higher quartile                             | 27.6         | 18.2–58.6      |         |
| Female gender                                | 19.9         | 13.3–42.4      | .219    |
| Male gender                                  | 18.9         | 12.9–29.5      |         |
| Asthma treatment according GINA <sup>1</sup> |              |                | .055    |
| Level 1                                      | 21.4         | 14.8–37.2      |         |
| Level 2                                      | 20.7         | 13.8–36.5      |         |
| Level 3                                      | 16.9         | 11.6–28.0      |         |
| Level 4                                      | 19.8         | 13.7–37.6      |         |
| Allergic rhinitis                            |              |                | <.001   |
| Absence                                      | 16.3         | 11.0–23.6      |         |
| Presence                                     | 20.8         | 14.8–38.9      |         |
| Number of sensitizing allergens              |              |                | .066    |
| None                                         | 16.9         | 11.6–23.2      |         |
| One                                          | 19.1         | 12.8–31.9      |         |
| Two                                          | 22.3         | 16.0–44.9      |         |
| Three                                        | 20.2         | 13.5–40.7      |         |
| Four                                         | 21.1         | 15.7–36.7      |         |
| Sensitization to grass pollen                | 19.5         | 13.2–38.7      | .410    |
| Sensitization to tree pollen                 | 21.1         | 13.7–42.4      |         |
| Sensitization to house dust mite             | 22.5         | 14.6–40.7      | .001    |
| Sensitization to molds                       | 19.9         | 13.6–32.7      |         |
| Sensitization to cat                         | 28.4         | 18.9–57.2      | <.001   |
| Seasonal allergen exposure no                | 17.3         | 11.7–24.3      |         |
| Yes                                          | 22.5         | 14.8–41.6      | <.001   |

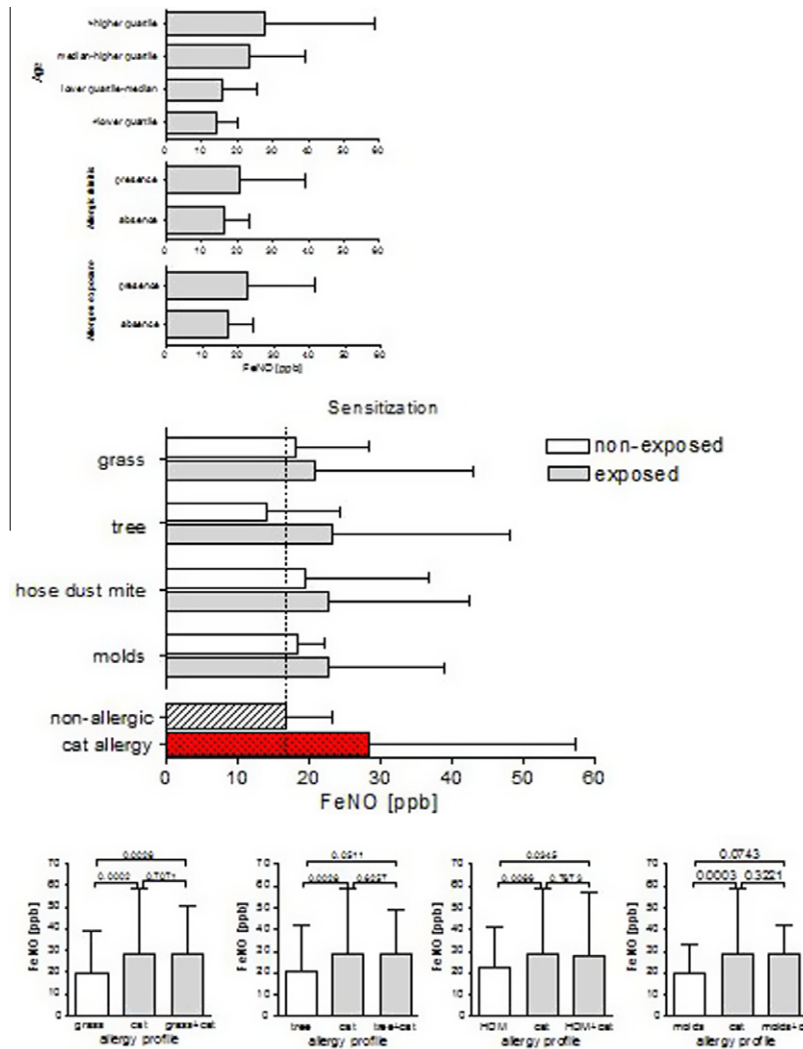


Fig. 1. FeNO level in relation to age, presence of allergic rhinitis, allergen exposure and sensitization profile.

Table 3

Association between FeNO level (dependent variable) and cat allergy, age, allergic rhinitis coexistence (independent variables) in multivariate model of linear regression analysis. Data are presented with risk ratios with 95% confidence intervals (RR  $\pm$  95%CI).

| Coefficient          | RR <sup>a</sup> | –95%CI | +95%CI | P     |
|----------------------|-----------------|--------|--------|-------|
| Sensitization to cat | 19.1            | 10.5   | 27.7   | <.001 |
| Allergic rhinitis    | 2.40            | –4.74  | 9.54   | 0.509 |
| Age                  | 2.33            | 1.49   | 3.18   | <.001 |

<sup>a</sup> Dependent variable: FeNO level.

any cat at home ever. We were the first to demonstrate that in those children sensitization to cat dander was associated with a higher level of FeNO concentration than in case of sensitization to other allergens. There was no influence of other allergens sensitization on FeNO level in children allergic to cat dander. We observed that in the patients without allergic rhinitis sensitization to cat allergen was associated with more severe asthma, compared to other sensitization. This phenomenon had not been described in any previous study. There are numerous studies that are partly in line with our results; however, only one study showed the correlation of increased FeNO with asthma severity, but it was performed in adults sensitized and exposed to cat dander [11,12,24–29].

The estimated number of cats in Poland is close to six millions [30]. Therefore, residential exposure to cat allergens, even in cat-free homes, may also be of considerable importance as far as causing airway sensitization and/or triggering asthma exacerbation in already sensitized patients is concerned [31]. In our study, we included pediatric patients who had never kept a cat at home, but we are aware that the absence of a cat at home does not equal the absence of residential exposure to cat allergen. The main cat dander protein (*Fel d 1*) is able to induce specific IgE production in a high percentage of cat-sensitized patients (about 85–95%) [15]. *Fel d 1* is characterized by great heat stability and might preferentially induce interleukin 5 (IL-5) rather than interferon-gamma (IFN-gamma). As a result, it could play a very important role in respiratory allergy pathogenesis [15]. Cat dander particles can ‘stick’ to all available surfaces, entailing their consequent distribution in all indoor environments recognized as theoretically ‘cat-free’ (houses, schools, means of transport and different public buildings) [31–35]. Exposure to even small amounts of cat allergen might increase the degree of non-specific bronchial hyperreactivity [36]. The factors we analyzed, such as sensitizations and lung function, were the subject of a large number of studies [11–14,37–39]. Saito et al., in agreement with our results, found a significant positive correlation between FeNO and sensitization to cat dander,

**Table 4**  
Association between the highest intensity of anti-asthma treatment allergy profile in univariate logistic regression analysis. Analyses were conducted in two different subgroups: in children without (left panel) and with (right panel) allergic rhinitis (AR). Data are presented with odds ratios with 95% confidence intervals (OR  $\pm$  95%CI).

| Coefficient              | Children without AR |        |        |               | Children with AR |        |        |               |
|--------------------------|---------------------|--------|--------|---------------|------------------|--------|--------|---------------|
|                          | OR <sup>a</sup>     | –95%CI | +95%CI | P             | OR <sup>b</sup>  | –95%CI | +95%CI | P             |
| <i>Sensitization:</i>    |                     |        |        |               |                  |        |        |               |
| Grass                    | 1.70                | 0.79   | 3.66   | 0.1782        | 1.20             | 0.67   | 2.14   | 0.5431        |
| Tree                     | 1.78                | 0.71   | 4.44   | 0.2201        | 1.50             | 0.83   | 2.71   | 0.1802        |
| Molds                    | 0.65                | 0.25   | 1.68   | 0.3698        | 1.67             | 0.88   | 3.16   | 0.1176        |
| House dust mite          | 2.14                | 0.99   | 4.63   | <b>0.0535</b> | 1.64             | 0.92   | 2.91   | 0.0940        |
| Cat                      | 9.14                | 1.95   | 42.94  | <b>0.0050</b> | 0.91             | 0.48   | 1.75   | 0.7869        |
| <i>Co-sensitization:</i> |                     |        |        |               |                  |        |        |               |
| Grass + cat              | 5.57                | 1.13   | 27.43  | <b>0.0349</b> | 0.87             | 0.43   | 1.74   | 0.6858        |
| Tree + cat               | 9.68                | 1.15   | 81.35  | <b>0.0366</b> | 0.83             | 0.36   | 1.92   | 0.6673        |
| Molds + cat              | 1.88                | 0.30   | 11.69  | 0.4973        | 3.54             | 1.18   | 10.62  | <b>0.0242</b> |
| House dust mite + cat    | 9.68                | 1.15   | 81.35  | <b>0.0366</b> | 1.47             | 0.70   | 3.06   | 0.3047        |

Bold: statistical significance.

<sup>a</sup> Dependent variable: asthma treatment according step IV (GINA) in patients without AR.

<sup>b</sup> Dependent variable: asthma treatment according step IV (GINA) in AR patients.

house dust mites and cedar and a negative correlation between FeNO and FEV<sub>1</sub> in schoolchildren with asthma [13]. Janson et al. showed that sensitization to cat dander was a more important determinant of exhaled NO than sensitization to pollen and discovered higher FeNO levels in cat-sensitized children having a cat or other furred pets at home, compared to cat-sensitized children without pets [37]. This stands in contradiction to the results of our study. The above described special features of cat dander and the fact that it is virtually impossible to avoid being exposed to cat antigens, also in public places or even in cat-free homes, could explain that discrepancy between Janson's study and ours. One study, performed in adults, also showed that sensitization to cat allergen was related to the highest increase in specific NO parameters including FeNO [38]. The concentration of dust mite and cat allergen revealed a positive correlation with FeNO in the study by Raja et al. performed in asthmatic children [39]. A study by Kovesi et al., performed in a large group of schoolchildren, showed that FeNO concentrations appear to reflect an allergic condition, including allergic asthma, reported allergies and eczema, rather than asthma per se [23]. The results of the study by Spanier et al. showed that FeNO levels are responsive to common environmental triggers like allergen sensitization and exposure to cat dander and house dust mites, as well as a therapy for asthma in children [12]. We also found that the levels of FeNO were strongly associated with sensitization to mainly indoor allergens, like cat dander. Moreover, our study revealed that most of our pediatric patients with both asthma and allergic rhinitis (62% of our patients) had significantly more increased FeNO levels as compared to their solely asthmatic counterparts. Thus, we confirmed the results of the previous studies and showed that in asthmatic children with concomitance of allergic rhinitis there is a possibility of a more severe allergic disease. There were only few previous studies showing that triggers such as asthma and AR, together with sensitization to indoor allergens, increase the level of FeNO even when the symptoms of asthma are absent [25,40]. Finally, we observed that in patients without allergic rhinitis, sensitization to cat allergen, compared to other perennial allergy, was associated with more severe asthma.

The main limitation of our study is the retrospective design, which did not allow us to measure allergen levels at homes. We gathered data from the patients' medical documentation, which could have partly influenced the accuracy of our results. Since all patients in our study were on controller medications, this could also affect the results. The same could be true for a relatively wide range of the study subjects' age (from 6 to 18).

## Conclusions

The most important finding of our study was the strongest and independent influence of cat allergy on FeNO levels in patients with no cats in their homes. Our results suggest that children with cat allergy may not be exposed to cat dander at home. They also underline the correlation between more severe asthma and sensitization to cat dander in patients without allergic rhinitis. The question still remains whether such high levels of FeNO as those observed in our study can be a predictor of more frequent asthma exacerbations in children sensitized to cat dander. Therefore, a prospective follow-up observation of the subjects seems to be of great importance. We must be aware that in countries like Poland, with the population of six million cats, cat allergy diagnostic studies should be performed routinely in children with asthma. In children with difficult-to-treat asthma, cat dander allergy should be taken into account even without cat presence at home. Cat allergen avoidance activities, due to high prevalence of cats in the community, seem impossible to be performed. It appears that in case of perennial, difficult-to-treat asthmatic children suffering from multiple allergy- including sensitization to cat dander- specific immunotherapy with cat dander should be considered.

## Conflict of Interest statement

There is no conflict of interest.

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## Study was registered on

[www.ClinicalTrials.gov](http://www.ClinicalTrials.gov), NCT00815984.

## References

- [1] From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2009. [Cited 2010 Feb 5]. Available from: <<http://www.ginasthma.org/Guidelineitem.asp?i1=2&i2=1&intid=1561>>.
- [2] C. Lane, D. Knight, S. Burgess, P. Franklin, F. Horak, J. Legg, A. Moeller, S. Stick, Epithelial inducible nitric oxide synthase activity is the major determinant of nitric oxide concentration in exhaled breath, *Thorax* 59 (2004) 757–760.
- [3] Q. Hamid, D.R. Springall, V. Riveros-Moreno, P. Chanez, P. Howarth, A. Redington, J. Bousquet, P. Godard, S. Holgate, J. Polak, Induction of nitric oxide synthase in asthma, *Lancet* 342 (8886–8887) (1993) 1510–1513.

- [4] A.E. Redington, Q.H. Meng, D.R. Springall, T.J. Evans, C. Créminon, J. Maclouf, S.T. Holgate, P.H. Howarth, J.M. Polak, Increased expression of inducible nitric oxide synthase and cyclo-oxygenase-2 in the airway epithelium of asthmatic subjects and regulation by corticosteroid treatment, *Thorax* 56 (2001) 351–357.
- [5] F.H. Guo, S.A. Comhair, S. Zheng, R.A. Dweik, N.T. Eissa, M.J. Thomassen, W. Calhoun, S.C. Erzurum, Molecular mechanisms of increased nitric oxide (NO) in asthma: evidence for transcriptional and post-translational regulation of NO synthesis, *J. Immunol.* 164 (2000) 5970–5980.
- [6] M. Barreto, M.P. Villa, F. Monti, Z. Bohmerova, S. Martella, M. Montesano, M.T. Darder, R. Ronchetti, Additive effect of eosinophilia and atopy on exhaled nitric oxide levels in children with or without a history of respiratory symptoms, *Allergy Immunol.* 16 (2005) 52–58.
- [7] R.C. Strunk, S.J. Szeffler, B.R. Phillips, R.S. Zeiger, V.M. Chinchilli, G. Larsen, K. Hodgdon, W. Morgan, C.A. Sorkness, R.F. Jr, Lemanske; Childhood Asthma Research and Education Network of the National Heart, Lung, and Blood Institute: Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children, *J. Allergy Clin. Immunol.* 112 (2003) 883–892.
- [8] M. Ramser, J. Hammer, A. Amacher, D. Trachsel, The value of exhaled nitric oxide in predicting bronchial hyperresponsiveness in children, *J. Asthma* 45 (2008) 191–195.
- [9] M.M. del Giudice, F.P. Brunese, G.L. Piacentini, M. Pedullà, C. Capristo, F. Decimo, A.F. Capristo, Fractional exhaled nitric oxide (FENO), lung function and airway hyperresponsiveness in naïve atopic asthmatic children, *J. Asthma* 41 (2004) 759–765.
- [10] M. Silvestri, F. Sabatini, R. Sale, A.C. Defilippi, L. Fregonese, E. Battistini, M.G. Biraghi, G.A. Rossi, Correlations between exhaled nitric oxide levels, blood eosinophilia, and airway obstruction reversibility in childhood asthma are detectable only in atopic individuals, *Pediatr. Pulmonol.* 35 (2003) 358–363.
- [11] A.J. Spanier, R. Hornung, M. Lierl, B.P. Lanphear, Environmental exposures and exhaled nitric oxide in children with asthma, *J. Pediatr.* 149 (2006) 220–226.
- [12] A.J. Spanier, R.W. Hornung, R.S. Kahn, M.B. Lierl, B.P. Lanphear, Seasonal variation and environmental predictors of exhaled nitric oxide in children with asthma, *Pediatr. Pulmonol.* 43 (2008) 576–583.
- [13] J. Saito, K. Inoue, A. Sugawara, M. Yoshikawa, K. Watanabe, T. Ishida, Y. Ohtsuka, M. Munakata, Exhaled nitric oxide as a marker of airway inflammation for an epidemiologic study in schoolchildren, *J. Allergy Clin. Immunol.* 114 (2004) 512–516.
- [14] D. Hervás, J.M. Milán, J. Garde, Differences in exhaled nitric oxide in atopic children, *Allergol. Immunopathol. (Madr)* 36 (2008) 331–335.
- [15] G. Liccardi, G. D'Amato, M. Russo, G.W. Canonica, L. D'Amato, M. De Martino, G. Passalacqua, Focus on cat allergen (*Fel d 1*): immunological and aerodynamic characteristics, modality of airway sensitization and avoidance strategies, *Int. Arch. Allergy Immunol.* 132 (2003) 1–12.
- [16] C. Bachert, P. van Cauwenberge, N. Khaltaev, World Health Organization, Allergic rhinitis and its impact on asthma. In collaboration with the World Health Organization. Executive summary of the workshop report. 7–10 Dec 1999 Geneva, Switzerland, *Allergy* 57 (2002) 841–855.
- [17] J. Bousquet, N. Khaltaev, A.A. Cruz, J. Denburg, W.J. Fokkens, A. Togias, T. Zuberbier, C.E. Baena-Cagnani, G.W. Canonica, C. Van Weel, I. Agache, N. Ait-Khaled, C. Bachert, M.S. Blaiss, S. Bonini, L.P. Boulet, P.J. Bousquet, P. Camargos, K.H. Carlsen, Y. Chen, A. Custovic, R. Dahl, P. Demoly, H. Douagui, S.R. Durham, R.G. Van Wijck, O. Kalayci, M.A. Kaliner, Y.Y. Kim, M.L. Kowalski, P. Kuna, L.T. Le, C. Lemiere, J. Li, R.F. Lockey, S. Mavale-Manuel, E.O. Meltzer, Y. Mohammad, J. Mullol, R. Naclerio, R.E. O'Hehir, K. Ohta, S. Ouedraogo, S. Palkonen, N. Papadopoulos, G. Passalacqua, P. Pawankar, T.A. Popov, K.F. Rabe, J. Rosado-Pinto, G.K. Scadding, F.E. Simons, E. Toskala, E. Valovirta, P. Van Cauwenberge, D.Y. Wang, M. Wickman, B.P. Yawn, A. Yorgancioglu, O.M. Yusuf, H. Zar, I. Annesi-Maesano, E.D. Bateman, A. Ben Kheder, D.A. Boakye, J. Bouchard, P. Burney, W.W. Busse, M. Chan-Yeung, N.H. Chavannes, A. Chuchalin, W.K. Dolen, R. Emuzyte, L. Grouse, M. Humbert, C. Jackson, S.L. Johnston, P.K. Keith, J.P. Kemp, J.M. Klossek, D. Larenas-Linnemann, B. Lipworth, J.L. Malo, G.D. Marshall, C. Naspi, Nekam K, B. Niggemann, E. Nizankowska-Mogilnicka, Y. Okamoto, M.P. Orru, P. Potter, D. Price, S.W. Stoloff, O. Vandenplas, G. Viegi, D. Williams, World Health Organization/GA(2)LEN; AllerGen. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen), *Allergy* 63 (Suppl. 86) (2008) 8–160.
- [18] Polish Bibliography of Clinical Palynology. Warsaw, Poland. 2010 [cited 2010 February] Available from <<http://www.alergen.info.pl/kalendarz/region2.html>>.
- [19] Recommendations for standardized procedures for the on-line, off-line measurement of exhaled lower respiratory nitric oxide, nasal nitric oxide in adults, children-1999, This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999, *Am. J. Respir. Crit. Care Med.* 160 (1999) 2104–2117.
- [20] Ph.H. Quanjer Standardisation of lung function tests. 1993 Update. Report Working Party for the European Community for Steel and Coal, *Eur. Respir. J.* 6 (Suppl. 16) (1993).
- [21] Ph.H. Quanjer, P. Helms, J. Bjure, C.I. Gaultier, Standardisation of lung function tests in pediatrics, *Eur. Respir. J.* 25 (Suppl. 4) (1989).
- [22] M.R. Miller, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, R. Crapo, P. Enright, C.P. van der Grinten, P. Gustafsson, R. Jensen, D.C. Johnson, N. MacIntyre, R. McKay, D. Navajas, O.F. Pedersen, R. Pellegrino, G. Viegi, J. Wanger, ATS/ERS Task Force et al.: Standardisation of spirometry, *Eur. Respir. J.* 26 (2005) 319–338.
- [23] T. Kovesi, R. Dales, Exhaled nitric oxide and respiratory symptoms in a community sample of school aged children, *Pediatric Pulmonol.* 43 (2008) 1198–2005.
- [24] A. Lindfors, H. van Hage-Hamsten, M. Rietz, S.L. Wickman, Nordvall: Influence of interaction of environmental risk factors and sensitization in young asthmatic children, *J. Allergy Clin. Immunol.* 104 (1999) 755–762.
- [25] A.H. Henriksen, T.L. Holmen, L. Bjermer, Sensitization and exposure to pet allergens in asthmatics versus non-asthmatics with allergic rhinitis, *Respir. Med.* 95 (2001) 122–129.
- [26] M. Barreto, M.P. Villa, S. Martella, F. Ronchetti, M.T. Darder, C. Falasca, F. Massa, Ronchetti, Exhaled nitric oxide in asthmatic and non-asthmatic children: influence of type of allergen sensitization and exposure to tobacco smoke, *Pediatr. Allergy Immunol.* 12 (2001) 247–256.
- [27] A.M. Warner, B. Björkstén, A.K. Munir, C. Moller, C. Schou, N.I. Kjellman, Childhood asthma and exposure to indoor allergens: low mite levels are associated with sensitivity, *Pediatr. Allergy Immunol.* 7 (1996) 61–67.
- [28] R. Sporik, S.P. Squillace, J.M. Ingram, G. Rakes, R.W. Honsinger, T.A. Platts-Mills, Mite, cat, and cockroach exposure, allergen sensitization, and asthma in children: a case-control study of the three schools, *Thorax* 54 (1999) 675–680.
- [29] S.J. Langley, S. Goldthorpe, M. Craven, J. Morris, A. Woodcock, A. Custovic, Exposure and sensitization to indoor allergens: association with lung function, bronchial reactivity, and exhaled nitric oxide measures in asthma, *J. Allergy Clin. Immunol.* 112 (2003) 362–368.
- [30] From the: Humanizacja zwierząt towarzyszących – ile Polacy wydają na pielęgnację psów i kotów. Rynek Zoologiczny Raport 4/2008. Available from: <[http://www.rynekzoologiczny.pl/nr/raport/humanizacja\\_zwierzat\\_towarzyszacych\\_ile.html](http://www.rynekzoologiczny.pl/nr/raport/humanizacja_zwierzat_towarzyszacych_ile.html)>.
- [31] C. Almqvist, P.H. Larsson, A.C. Egmar, M. Hedrén, P. Malmberg, M. Wickman, School as a risk environment for children allergic to cats and a site for transfer of cat allergen to homes, *J. Allergy Clin. Immunol.* 103 (1999) 1012–1017.
- [32] A.K. Munir, R. Einarsson, C. Schou, S.K. Dreborg, Allergens in school dust. I. The amount of the major cat (*Fel d 1*) and dog (*Can f 1*) allergens in dust from Swedish schools is high enough to probably cause perennial symptoms in most children with asthma who are sensitized to cat and dog, *J. Allergy Clin. Immunol.* 91 (1993) 1067–1074.
- [33] S.D. De Lucca, T.J. O'meara, E.R. Tovey, Exposure to mite and cat allergens on a range of clothing items at home and the transfer of cat allergen in the workplace, *J. Allergy Clin. Immunol.* 106 (2000) 874–879.
- [34] G. D'Amato, G. Liccardi, M. Russo, D. Barber, M. D'Amato, J. Carreira, Clothing is a carrier of cat allergens, *J. Allergy Clin. Immunol.* 99 (1997) 577–578.
- [35] J.S. Neal, L.G. Arlian, M.S. Morgan, Relationship among house-dust mites, *Der 1*, *Fel d 1*, and *Can f 1* on clothing and automobile seats with respect to densities in houses, *Ann. Allergy Asthma Immunol.* 88 (2002) 410–415.
- [36] E. Ihre, O. Zetterström, Increase in non-specific bronchial responsiveness after repeated inhalation of low doses of allergen, *Clin. Exp. Allergy* 23 (1993) 298–305.
- [37] C. Janson, P. Kalm-Stephens, T. Foucard, D. Norbäck, K. Alving, S.L. Nordvall, Exhaled nitric oxide levels in school children in relation to IgE sensitisation and window pane condensation, *Respir. Med.* 99 (2005) 1015–1021.
- [38] A. Malinowski, C. Janson, T. Holmkvist, D. Norbäck, P. Meriläinen, M. Högman, IgE sensitisation in relation to flow-independent nitric oxide exchange parameters, *Respir. Res.* 7 (2006) 92.
- [39] S. Raja, Y. Xu, A.R. Ferro, P.A. Jaques, P.K. Hopke, Resuspension of indoor aeroallergens and relationship to lung inflammation in asthmatic children, *Environ. Int.* 36 (2010) 8–14.
- [40] M.J. Alvarez, J.M. Olaguibel, B.E. García, A. Rodríguez, A.I. Tabar, E. Urbiola, Airway inflammation in asthma and perennial allergic rhinitis. Relationship with nonspecific bronchial responsiveness and maximal airway narrowing, *Allergy* 55 (2000) 355–362.