

ORIGINAL ARTICLE

Exhaled NO is a poor marker of asthma control in children with a reported use of asthma medication: a pharmacy-based study

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Abstract

Background: A high fraction of nitric oxide in exhaled breath (FeNO) has been suggested to be a marker of ongoing airway inflammation and poorly controlled disease in asthma. The usefulness of FeNO to monitor asthma control is still debated today.

Aim: To assess the validity of FeNO as a marker of asthma control in children with reported use of asthma medication.

Methods: Fraction of nitric oxide in exhaled breath was measured in 601 children (aged 4–12 yr) with reported use of asthma medication in the past 6 months and in 63 healthy non-asthmatic children (aged 5–12). Asthma control was assessed by the Asthma Control Questionnaire (ACQ). A receiver-operator characteristics (ROC) curve was generated to assess the accuracy of FeNO as a marker for asthma control. Logistic regression analysis was used to study whether clinical, healthcare, medication, and environmental factors are associated with high FeNO levels (> 25 ppb).

Results: Fraction of nitric oxide in exhaled breath had a poor accuracy to discriminate well-controlled from not well-controlled asthma [area under the ROC curve: 0.56 (95% CI: 0.52–0.61, $p = 0.008$)]. In addition, high FeNO (> 25 ppb) was associated with lower medication adherence rates (OR: 0.4; 95% CI: 0.3–0.6), fewer antibiotic courses in the past year (OR: 0.6; 95% CI: 0.4–0.9), fewer leukotriene antagonists use in the past year (OR: 0.4; 95% CI: 0.2–0.9), and fewer visits to a (pulmonary) pediatrician (OR: 0.6; 95% CI: 0.4–0.9). Children living in a non-urban environment had more often high FeNO levels (OR: 1.7; 95% CI: 1.1–2.6).

Conclusion: High FeNO is a poor marker of asthma control in children with reported use of asthma medication. Various other factors, including medication adherence and medication use, are associated with increased FeNO levels.

Over the last decade, a high fraction of nitric oxide in exhaled breath (FeNO) has emerged as a promising non-invasive marker of airway inflammation in asthma (1, 2). A high FeNO has been reported as a surrogate marker for eosinophilic airway inflammation and may reflect uncontrolled asthma and predict asthma exacerbations (1).

The production of nitric oxide (NO) from L-arginine is catalyzed by nitric oxide synthases (NOS). There are three known isoforms of NOS; two forms of constitutive NOS (neuronal NOS and endothelial NOS) and a form of inducible NOS. All three forms are expressed in airway epithelium. Inducible NOS can be upregulated by a wide range

of inflammatory cytokines, and it is thought that this isoform is predominantly responsible for the elevated FeNO levels observed in atopic asthma (2). Apart from inflammatory cytokines, various other factors like age, atopy, viruses, lipopolysaccharide (LPS), oxidative stress, and corticosteroid use are thought to influence the production of NO (1).

The clinical usefulness of FeNO to monitor asthma control is still debated. Studies assessing the relation between clinical symptoms, pulmonary function, and FeNO have provided inconsistent results (3–7). Furthermore, a meta-analysis showed that the adjustment of asthma treatment based on FeNO levels does not improve asthma outcomes (8).

Most studies focussing on FeNO in asthmatic children have recruited patients through secondary or tertiary healthcare services. Therefore, these patients may represent a subpopulation of patients with more severe disease compared with the total population of individuals with asthma symptoms that has generally mild-to-moderate severe disease. In this study, we assessed the specificity and sensitivity of high FeNO as a marker for not well-controlled asthma in a large population of children with a reported use of asthma medication, recruited through community pharmacies (primary care setting). In addition, we investigated whether other factors including medication adherence, healthcare usage, and living environment are associated with high FeNO levels in this population.

Methods

Study setting and study population

Our study population consists of children (aged 4–12 yr) who participate in the PACMAN (Pharmacogenetics of Asthma medication in Children: Medication with Anti-inflammatory effects) cohort study. The ongoing PACMAN cohort study has been initiated in April 2009 and has currently enrolled 744 children. Details of the study protocol have been described elsewhere (9). Data were collected with the help of pharmacists belonging to the Utrecht Pharmacy Practice Network for Education and Research (UPPER), and the work was conducted in compliance with the requirements of the UPPER institutional review board of the Department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University. Briefly, children who are regular users of asthma medication [$\geq$ three prescriptions (Anatomical Therapeutic Chemical code R03) within the last 2 yr and $\geq$ one prescription in the last 6 months] were recruited through community pharmacies in the Netherlands (9). Children and their parents (or guardian) were invited for a visit to their own pharmacy. During this visit, the child's FeNO was measured and the caregivers filled in a questionnaire including questions on their child's respiratory symptoms, asthma diagnosis, medication use, adherence, caregivers' beliefs about medicines, environmental and socio-demographic factors. The questionnaire included the Asthma Control Questionnaire (ACQ) (10) and the Medication Adherence Report Scale (MARS) (11). Upon inclusion of a child in the PACMAN cohort study, a letter was sent to the child's treating general practitioner to inquire whether the participant has been diagnosed with

asthma. Written informed consent was provided by the parents or guardian. For this study, we restricted our study population to children who were able to perform the FeNO expiration maneuver correctly and whose parents/guardian completed the questionnaire ($n = 601$). The PACMAN cohort study has been approved by the Medical Ethics Committee of the University Medical Centre Utrecht.

Fraction of nitric oxide in exhaled breath

A single-breath on-line measurement of FeNO was carried out with a hand-held electrochemical analyzer (NIOX Mino; Aerocrine, Solna, Sweden) with an expiration time of 6 s (12). To investigate correlations between FeNO and other factors (e.g., asthma control, age, gender), FeNO values below 5 ppb (the detection limit of the NIOX Mino) were set on 2.5 ppb. Sensitivity analyses were performed whereby the FeNO values below 5 ppb were set on 0 and 5 ppb, respectively, which did not influence our results. To assess factors related to high FeNO levels in children with reported use of asthma medication, FeNO was dichotomized using a cutoff value of 25 ppb based on the work of Buchvald et al. (13) who showed that FeNO values in healthy children and adolescents are below 25 ppb. In addition, sensitivity analyses were performed to test the robustness of our findings using cutoff values of 17.5 ppb, based on the results of our receiver-operator characteristics (ROC) analysis (Appendix I) and 35 ppb, the threshold value advised for children aged < 12 yr by the manufactory guidelines of NIOX Mino and the American Thoracic Society (14) (Appendix II).

Asthma control

Asthma control was assessed using the 6-item version of the ACQ (symptoms plus rescue medication use) (15). An ACQ score < 0.75 was considered 'well-controlled asthma', a score ≥ 0.75 was considered 'not well-controlled asthma' (10).

Definition of main factors studied

Factors that could be associated with high FeNO (> 25 ppb) were divided into five categories; (i) child characteristics, (ii) clinical characteristics, (iii) medication use in the past year, (iv) healthcare use in the past year, and (v) environmental factors.

1 Child characteristics: age and gender.

2 Clinical characteristics: atopy was defined as a parental-reported history of eczema, allergic rhinitis, and/or food allergy. Current use of inhaled corticosteroids (ICS) was defined as parental-reported use of ICS in the week previous to the study visit.

3 Medication use in the past year: ICS use, oral corticosteroids (OCS), short-acting beta agonists (SABA), long-acting beta agonists (LABA), leukotriene antagonists (LTRA), or antibiotic courses (AB) in the past year. Parental-reported compliance to medication was assessed using the MARS, comprising five questions on medication use behavior (16), for example questions on forgetting to take a dose, altering the dose, deciding to miss a dose, taking less medication as

instructed by the physician and stop taking medication for a while. The MARS was dichotomized using a cutoff point for the sum-score, ≥ 21 being considered as high adherence. A previous study by Menckeberg et al. (11) showed that the MARS is a satisfactory screening tool for non-adherence among adult ICS users irrespective of the chosen cutoff point, and that it correlates well with pharmacy dispensing data.

4 Healthcare use in the past year: general practitioner visit and specialist physician visit for respiratory complaints (pediatric pulmonologist or pediatrician visit).

5 Environmental factors: pet exposure, passive smoking, and current living environment (non-urban/urban).

Healthy controls

To compare FeNO values of our population of children with reported use of asthma medication with healthy controls, we studied healthy non-asthmatic children (aged 5–12 yr) who participated in the previous cross-sectional Utrecht Pulmonary Function Reference Data Study (The Utrecht dataset) (17). The Utrecht dataset is a cross-sectional study of the department of Paediatric Pulmonology of the University Medical Center Utrecht that was designed to generate new reference values for spirometry, lung volumes, airway resistance, CO diffusion, and interrupter resistance (R_{int}). Healthy non-asthmatic children were recruited at ten randomly selected primary and secondary schools between January 2004 and April 2009 by experienced pediatric pulmonary function technicians (17). Data collection included FeNO measurement with NIOX Mino. For this study, we restricted our study population to children without a history of asthma, who did not show symptoms of wheezing in the last 12 months and did not use asthma medication. Written informed consent was obtained from the parents. The Utrecht dataset study has been approved by the Medical Ethics Committee of the University Medical Centre Utrecht.

Statistical analyses

Differences in characteristics between the population of children with reported use of asthma medication and the control group and between children with a high or normal FeNO were tested using chi-square test, independent sample *t*-test, or Mann–Whitney *U*-test when appropriate. Correlations between FeNO and other factors (e.g., age, asthma control) were tested using Spearman rank tests. A ROC curve was generated to assess the value of FeNO to discriminate well-controlled asthma ($ACQ < 0.75$) from not well-controlled asthma ($ACQ \geq 0.75$). In a ROC curve, the true-positive rate is plotted against the false-positive rate for different cutoff values of a diagnostic test. The area under the ROC curve (AUC) is a measure for test accuracy; an AUC of 0.5 reflects poor predictive accuracy – no better than flipping a coin – while AUC of 1.00 implies excellent predictive accuracy. To study factors associated with high FeNO in children with reported use of asthma medication, logistic regression analyses were used, resulting in the estimation of (adjusted) odds ratios (OR) and 95 percent confidence intervals (95% CI).

Only variables associated with high FeNO values ($p < 0.10$) in the univariate analyses were included in the multivariate analysis. Potential confounding factors were included in the multivariate model if they induced a 10% change or more in the crude regression coefficient for the determinant of interest (18). Age, gender, current ICS use, OC use in the past year, SABA use in the past year, LABA use in the past year, LTRA use in the past year, asthma control, therapy adherence, pet exposure, passive smoking, parents' level of education and atopy were considered potential confounding factors. All analyses were performed using SPSS version 16 for Windows (SPSS, Chicago, IL, USA).

Results

Characteristics of children with reported use of asthma medication

From April 2009 until August 2011, 744 children were included in the PACMAN cohort study. Details on the response rate have been described elsewhere (19). Of the included children, 137 (18.4%) were unable to perform the FeNO expiratory maneuver and 6 (0.8%) caregivers did not fill in the questionnaire. Therefore, 601 children (80.8%) were included for analyses. Additional data were made available by the general practitioners for 267 of these children (44.4%). General characteristics of the children with reported use of asthma medication are listed in Table 1. The majority of patients were boys (62.9%) and the mean age of the population was 9.0 yr. Of these children; 88.5% reported to have used ICS in the past year and 54.4% to have used ICS less than a week previous to the study visit. In addition, 56.2% could be classified as 'well-controlled' based on the ACQ score. Of the children from whom additional data were provided by the general practitioners, 76.0% had a doctor's diagnosis of asthma. FeNO was > 25 ppb in 161 children (26.8%).

Characteristics of children with reported use of asthma medication who were unable to perform the FeNO measurement

Children who were not able to perform the FeNO measurement were significantly younger than children who were able to perform the measurement (mean age 5.7, respectively 9.0 yr) ($p < 0.0001$) and were less likely to use ICS in the past year (81.0%, 87.6%, respectively) ($p = 0.041$), whereas gender and SABA use did not significantly differ between both groups.

Fraction of nitric oxide in exhaled breath in children with reported use of asthma medication compared to a healthy population

A FeNO value was available for 63 healthy non-asthmatic children (age: 5–12). Compared to the children with reported use of asthma medication, the healthy population consisted of fewer males (46.0% vs. 62.9%, $p = 0.009$) and was older (mean age: 11.1 yr vs. 9.0 yr, $p < 0.001$). FeNO was significantly higher in the children with a reported use of asthma

Table 1 General characteristics of the children with a reported use of asthma medication

	Children with a reported use of asthma medication (n = 601)
General characteristics	
Gender, male %	62.9
Age (yr), mean (s.d.)	9.0 (2.2)
Clinical characteristics	
Doctor-diagnosed asthma, %	76.0 (203/267)*
Well-controlled asthma†	58.0 (338/583)*
Atopy‡	80.1 (463/578)*
Current inhaled corticosteroids (ICS) users§	54.4
Medication use in past year	
Short-acting beta agonists, %	84.4
Long-acting beta agonists, %	25.0
ICS, %	88.5
Oral corticosteroids, %	6.3
Leukotriene antagonists, %	9.3
Antibiotic course, %	32.0 (191/596)*
Environmental factors	
Passive smoking	12.9 (77/597)*
Pet exposure	41.3 (247/598)*
Urban living environment	65.1 (388/596)*

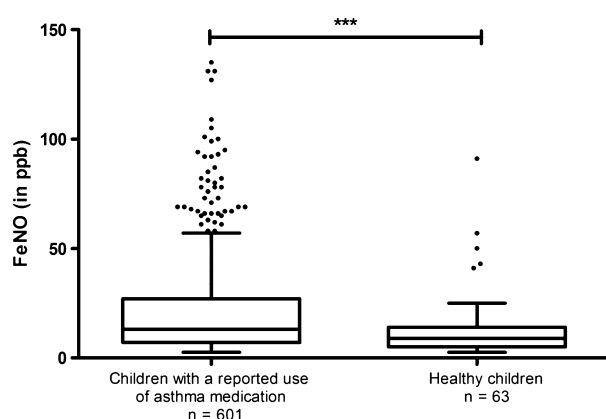
*Data not available for the total population (number of children/total number of children with available data).

†Asthma Control Questionnaire score <0.75.

‡Parental-reported history of eczema, hay fever, or food allergy.

§Use of ICS less than a week previous to the study visit.

medication (median: 13.0 ppb, IQR: 7.0–27.0 ppb) compared with the healthy children (median: 9.0 ppb, IQR: 5.0–14.0 ppb) ($p < 0.0001$) (Fig. 1). Of the children with a reported use of asthma medication, 6.0% had a FeNO value below 5 ppb, compared with 12.7% of the healthy children.

**Fig 1** Fractional exhaled nitric oxide (FeNO) values in children with a reported use of asthma medication compared to healthy children (no history of asthma or asthma medication use). Box and whisker plots show median, 25th and 75th percentiles, minimum, maximum values and outliers. *** $p < 0.0001$.

The influence of a doctor's diagnosis of asthma on FeNO in children with a reported use of asthma medication

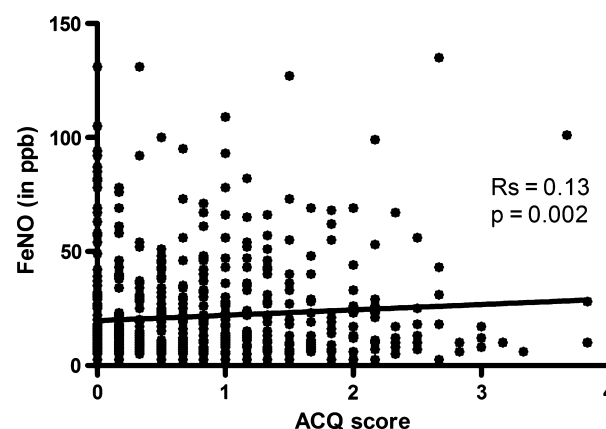
Within the group of children with reported use of asthma medication, those with a doctor's diagnosis of asthma had a slightly higher FeNO level (median: 13.0 ppb, IQR: 8.0–27.0) compared with the children without a doctor's diagnosis of asthma (median: 9.0 ppb, IQR: 7.0–18.8), yet the difference was not statistically significant ($p = 0.10$).

The influence of age and gender on FeNO in healthy children and children with a reported use of asthma medication

Fraction of nitric oxide in exhaled breath levels did not significantly differ between boys and girls with reported use of asthma medication (median: 13.0 ppb, IQR: 7.0–26.0 ppb vs. 12.5 ppb, IQR: 7.0–27.3, respectively, $p = 0.51$), nor between healthy boys and girls (median: 9.0 ppb, IQR: 5.0–15.5 ppb vs. 8.0 ppb, IQR: 5.0–10.3, $p = 0.22$). We did observe a weak correlation between FeNO and age in the group with reported asthma medication use ($R_s = 0.30$, $p < 0.0001$), but the correlation was borderline significant in the healthy population ($R_s = 0.25$, $p = 0.05$).

Fraction of nitric oxide in exhaled breath as a marker of asthma control

There was a very weak correlation between FeNO levels and total ACQ score ($R_s = 0.13$, $p = 0.002$) (Fig. 2). In a sensitivity analysis in children with a doctor's diagnosis of asthma, the R_s value for the correlation between FeNO and ACQ score was comparable; however, it did not remain statistically significant in this smaller population ($R_s = 0.11$, $p = 0.14$). To assess the predictive accuracy of FeNO as a marker for not well-controlled asthma ($ACQ \geq 0.75$), a ROC curve was generated (Fig. 3). FeNO showed to be a poor marker of not well-controlled asthma, the AUC being 0.56

**Fig 2** Correlation between fractional exhaled nitric oxide (FeNO) concentrations and Asthma Control Questionnaire score (ACQ) in children using asthma medication. FeNO levels are shown as parts per billion (ppb). R_s : Spearmans Rank correlation coefficient.

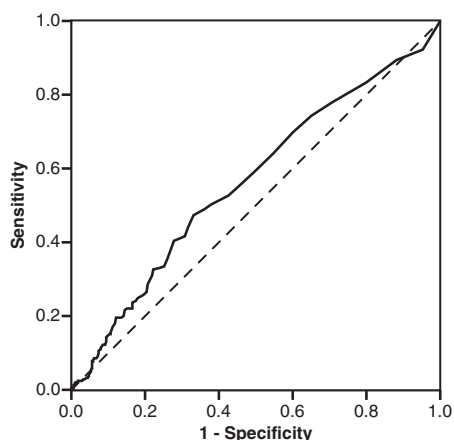


Fig 3 Receiver-operator characteristic (ROC) curve indicating the sensitivity and specificity of fraction of nitric oxide in exhaled breath (FeNO) for predicting not well-controlled asthma (ACQ ≥ 0.75) in our population of children using asthma medication. Area under the curve (AUC): 0.56, 95% CI: 0.52–0.61, $p = 0.008$.

(95% CI: 0.52–0.61, $p = 0.008$). The highest sensitivity and specificity were observed with a cutoff value of 17.5 ppb (47.3%, 66.9%, respectively). A sensitivity analysis in children with confirmed doctor's diagnosis of asthma showed that the accuracy of FeNO as a marker of not well-controlled asthma did not improve when children without an asthma diagnosis were excluded from the analysis (AUC: 0.54; 95% CI: 0.46–0.62, $p = 0.33$).

Factors associated with high FeNO in children with reported use of asthma medication

In the multivariate analysis, age, asthma control, therapy adherence, LTRA, and antibiotic use in the past year were significantly associated with high FeNO values (Table 2). Patients with high FeNO levels were less well controlled (OR: 0.6; 95% CI 0.4–1.0), less adherent (OR: 0.4; 95% CI: 0.3–0.6) and used fewer courses of antibiotic (OR: 0.6; 95% CI: 0.4–0.9) in the past year. High FeNO levels were also associated with fewer LTRA use (OR: 0.4; 95% CI 0.2–0.9) in the past year, but this association did not remain significant in the sensitivity analysis using a cutoff value of 17.5 ppb (Appendix I). In addition, a non-urban living environment was significantly associated with FeNO levels >25 ppb ($p < 0.05$), though the association between non-urban living environment and high FeNO (>35 ppb) was just above the threshold of significance in the sensitivity analysis ($p = 0.05$) (Appendix II). Furthermore, we found a significant negative association between high FeNO levels (>25 ppb) and visits to a pediatrician or pediatric pulmonologist in the past year (OR: 0.6; 95% CI 0.4–0.9); this association did not remain significant when we set the FeNO threshold to 35 ppb (Appendix II). There was no significant independent association between high FeNO levels (>25 ppb) and current use of ICS, though we did observe a significant negative association in the sensitivity analyses (Appendices I and II).

Table 2 Factors associated with a high fraction of nitric oxide in exhaled breath (> 25 ppb)

	Univariate analysis OR (95% CI)	Multivariate analysis Adjusted OR (95% CI)
General characteristics		
Male gender	1.1 (0.8–1.6)	
Age	1.3 (1.2–1.4)*	1.3 (1.2–1.4)*
Clinical characteristics		
Well-controlled asthma†	0.6 (0.4–0.9)*	0.6 (0.4–1.0)*
Atopy‡	1.1 (0.7–1.8)	
Current use of inhaled corticosteroids (ICS)§	0.7 (0.5–0.9)*	0.7 (0.4–1.1)
Good adherence¶	0.4 (0.2–0.5)*	0.4 (0.3–0.6)*
Medication use in the past year		
ICS use	0.7 (0.4–1.1)	
Oral corticosteroids use	0.8 (0.4–1.8)	
Short-acting beta agonists use	1.2 (0.7–1.9)	
Long-acting beta agonists use	0.8 (0.5–1.3)	
Leukotriene antagonists use	0.3 (0.1–0.7)*	0.4 (0.2–0.9)*
Antibiotic use	0.5 (0.3–0.8)*	0.6 (0.4–0.9)*
Healthcare use in the past year		
General practitioner visit	1.0 (0.7–1.5)	
Specialist physician visit**	0.5 (0.3–0.7)*	0.6 (0.4–0.9)*
Emergency Room (ER) visit	0.3 (0.1–1.1)	
Environmental factors		
Pet exposure	1.1 (0.8–1.6)	
Passive smoking	1.1 (0.7–1.9)	
Non-urban environment	1.8 (1.2–2.6)*	1.7 (1.1–2.6)*

Factors that caused a 10% change or more in the regression coefficient were included in the multivariable model.

* $p < 0.05$.

†Asthma Control Questionnaire-score <0.75 .

‡History of eczema, hay fever, or food allergy.

§Use of ICS less than a week previous to the study visit.

¶Medication Adherence Report Scale-score ≥ 21 .

**Pediatric pulmonologist or pediatrician visit.

Discussion

To the best of our knowledge, this is the first study assessing the relationship between FeNO and asthma control in a large pharmacy-based cohort of children with reported use of asthma medication. We found a weak positive correlation between FeNO and not well-controlled disease. Our results are in line with previous (smaller) studies (4, 5). Rosias et al. (4) found no correlation between FeNO and ACQ in 23 children with mild-to-moderate asthma (age: 6–16 yr). Mahut et al. (5) made similar observations in 200 asthmatic patients (children and adults) who were followed for 12 wks.

High levels of FeNO are thought to be a surrogate marker of ongoing eosinophilic inflammation. Clinical symptoms of asthma and underlying eosinophil airway inflammation seem to overlap only partially (20). This is also supported by the poor accuracy of FeNO in our study to discriminate well-controlled children from not well-controlled children.

Nevertheless, Robroeks et al. (21) have reported that FeNO was a good marker of asthma control in 114 asthmatic children (age: 5–16 yr), especially when combined with levels of 8-isoprostane, IFN- γ and IL-4 in exhaled breath condensate (EBC) (sensitivity = 82%; specificity = 80%). Specificity and sensitivity of FeNO solely for asthma control have not been reported previously, and the additional predictive value of the inflammatory markers in EBC remains unclear. Because asthma is a complex disease characterized by a wide variety of distinct phenotypes, measuring a combination of inflammatory markers, such as markers in EBC and FeNO, might give a more complete view of disease status than focusing on a single marker. However, the measurement of inflammatory markers in EBC is still in its experimental phase, and many methodological issues (e.g., standardization of EBC collection, the establishment of reference values for individual markers in EBC) need to be addressed before this technique can enter clinical practice (22). More research in large populations is necessary to assess the clinical validity of such an approach.

We found that several factors were independently associated with high FeNO values, including age, poor therapy adherence, antibiotic use, and LTRA use. The (weak) positive correlation between age and FeNO has also been observed in healthy children (13) and is probably related to the developmental increase in total pulmonary surface and thereby increased production capacity of NO.

The other associations we identified are less clear, especially because the direct relationship between FeNO and airway inflammation remains to be elucidated. Generally, the concept has evolved that high levels of FeNO are indicative of more uncontrolled eosinophilic airway inflammation and that high levels of FeNO in steroid-treated children might be caused by low medication compliance, ongoing allergen exposure and infections, imminent exacerbations, inadequate ICS dosing, or steroid resistance (1). Our finding that children with high FeNO are more often non-compliant to therapy fits within this concept. A lack of compliance to corticosteroids may contribute to persistent eosinophilic airway inflammation and, thereby, increased FeNO levels. Children with asthmatic symptoms and high FeNO in our study used less LTRA, less AB and visited their pediatrician or pediatric pulmonologist less often than their counterparts with normal FeNO levels. This is less compatible with the hypothesis that high FeNO could be an indicator of a more severe asthma phenotype. However, it may be supporting the observation that tailoring treatment on FeNO is not effective in decreasing asthma exacerbations (8).

An alternative view is that high FeNO might be a sign of a steroid-responsive asthma phenotype and that inter-individual differences in FeNO levels might represent distinct underlying inflammatory patterns. A recent study by Wang et al. (23) identified four different inflammatory patterns in stable asthmatic children based on sputum analyses: paucigranulocytic (49.0%), eosinophilic (28.6%), neutrophilic (20.4%), and mixed granulocytic (2.0%). Considering that eosinophils and neutrophils differ with regard to corticosteroids sensitivity (24), patients with eosinophilic asthma might respond better to corticosteroids than patients with non-

eosinophilic asthma. FeNO correlates positively with the presence of eosinophils in sputum (25) and may therefore reflect a more corticosteroid-responsive phenotype. This idea is supported by various prospective clinical studies that reported a better therapy response in asthmatic patients with elevated baseline FeNO levels (26).

It is tempting to speculate that fewer LTRA and antibiotic use and fewer visits to a pediatrician or pediatric pulmonologist that were associated with high FeNO levels in our cohort might indirectly be a sign of a more steroid-responsive (eosinophilic) asthma phenotype. Clinical practice shows that an antibiotic course is regularly prescribed in the case of asthma exacerbations (27), and therefore, fewer courses of antibiotics might indirectly reflect fewer asthma exacerbations. Furthermore, children with high FeNO were less likely to use LTRA. The pediatric asthma guidelines of the Dutch Pediatric Society advice to prescribe LTRA when treatment with corticosteroids and SABA is not successful to obtain asthma control (28). This also suggests that high FeNO might be indirectly associated with a better response to steroid treatment. LTRA have also been approved for the treatment of allergic rhinitis, but the prevalence of parental-reported rhinitis did not significantly differ between the children with or without reported use of LTRA (51.8% vs. 43.9%, respectively, $p = 0.18$). Lastly, there was a negative trend between FeNO and visits to pediatrician or pediatric pulmonologist in the past year, which also fits in the concept of high FeNO as a marker of a steroid-responsive inflammation. However, we could not assess therapy response in this cross-sectional study, and a prospective design is needed to further investigate the relationship between levels of exhaled NO, underlying inflammatory patterns and response to corticosteroids in children.

Another remarkable finding of our study was the positive trend between FeNO and a non-urban living environment. Epidemiological studies showed that children living on farms have a lower risk in developing asthma, atopic dermatitis, or allergic sensitization (29). Because atopy has reported to be closely related to FeNO, one would expect lower FeNO values in children living in non-urban environments. However, in our study, we did not find an association between FeNO and parental-reported atopic disease. To the best of our knowledge, few studies addressed the influence of urbanization on FeNO. A study by Malmberg et al. (30) found a trend between growing up in the countryside and lower FeNO levels in Finnish and Russian populations, but the countryside in those countries might be incomparable to the countryside in the densely populated Netherlands. It is unlikely that ambient NO has contaminated our results because it was removed during the FeNO measurement through the use of a NO filter (31). The socio-economic status of a child is often determined by assessing the parent's educational level and the household income. We did not have information on the latter parameter, but we did assess whether the parent's educational level confounded the association between living environment and high levels of FeNO, which was not the case. More research is needed to assess the influence of living environment on exhaled NO.

Potential limitations of our study include the relatively low response rate of the general practitioners: we are unaware of a

possible doctor's diagnosis of asthma for 56% of our participants. Therefore, the percentage of asthma diagnosis might not be representative for our total study population. In addition, the ACQ used in this study has been validated and should therefore officially be assessed, in asthmatic children. Not all the children in our study with reported use of asthma medication have an official diagnosis of asthma. However, sensitivity analyses showed that the correlation between FeNO and ACQ, nor the accuracy of FeNO as a marker for not well-controlled asthma, improved when we excluded children without a confirmed doctor's diagnosis. Another limitation of our study is that we cannot exclude that confounding by disease severity might have influenced our results. We did correct for OCS use in the past year, but lacked information on the number of hospital admissions or severity of previous exacerbations. Children with more severe disease might have been more compliant to therapy and have used healthcare more frequently, which may have confounded the identified associations.

Furthermore, symptoms and adherence were based on reporting by caregivers, which could also be a possible flaw. Parents and caregivers might over- or underestimate the health status and/or compliance of their child. But this resembles daily clinical practice, where asthma management in young children is mainly based on parental reporting. Atopy was also based on parental reporting in the questionnaire, and measurements of specific immunoglobulin E levels might have been more accurate to establish the atopic status of the child. Furthermore, other comorbidities such as chronic rhinosinusitis can also increase FeNO and might have confounded the association between FeNO and asthma control. In this study, we found that 6% of the children with a reported use of asthma medication and 12.7% of the healthy children had a FeNO below 5 ppb. Very low FeNO levels have also been observed in primary ciliary dyskinesia or cystic fibrosis patients, however; these diseases have a low prevalence and are unlikely to account for all the cases of low FeNO we found.

A major strength of our study was the availability of information on asthma symptoms and FeNO measurements in a large population of pediatric patients included in the primary care setting. This has the advantage that the PACMAN cohort focuses on a very broad range of asthmatics and not only on children in a specialized care group, as most asthmatic children are also treated within the primary care setting.

In summary, this study does not support a role for FeNO to assess current asthma control in every day practice. FeNO appears a poor marker of asthma control in children with

reported use of asthma medication. Symptoms may overlap only partly with underlying airway inflammation, and our study shows that various other clinical healthcare and environmental factors influence FeNO.

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Conflict of interest

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Appendix I

Factors associated with a high fraction of nitric oxide in exhaled breath (> 17.5 ppb)

	Univariate analysis OR (95% CI)	Multivariate analysis Adjusted OR (95% CI)
General characteristics		
Male gender	1.1 (0.8–1.5)	
Age	1.3 (1.2–1.4)*	1.3 (1.2–1.4)*
Clinical characteristics		
Asthma control†	0.6 (0.4–0.8)*	0.6 (0.4–0.8)*
Atopy‡	1.1 (0.7–1.7)	
Current use of inhaled corticosteroids (ICS)§	0.6 (0.5–0.9)*	0.7 (0.4–1.0)*
Good adherence¶	0.4 (0.3–0.6)*	0.4 (0.3–0.6)*
Medication use in the past year		
ICS use	0.8 (0.5–1.4)	
Oral corticosteroids use	1.3 (0.7–2.5)	
Short-acting beta agonists use	1.4 (0.9–2.2)	
Long-acting beta agonists use	0.9 (0.6–1.3)	
Leukotriene antagonists use	0.5 (0.3–0.9)*	0.5 (0.3–1.1)
Antibiotic use	0.6 (0.4–0.8)*	0.6 (0.4–1.0)*

Appendix I (Continued)

	Univariate analysis OR (95% CI)	Multivariate analysis Adjusted OR (95% CI)
Healthcare use in the past year		
General practitioner visit	0.9 (0.7–1.3)	
Specialist physician visit**	0.5 (0.4–0.8)*	0.6 (0.4–0.9)*
ER visit	0.4 (0.2–1.0)*	0.4 (0.1–1.0)*
Environmental factors		
Pet exposure	1.0 (0.7–1.4)	
Passive smoking	1.4 (0.8–2.2)	
Non-urban environment	1.8 (1.3–2.5)*	1.8 (1.2–2.7)*

Factors that caused a 10% change or more in the regression coefficient were included in the multivariable model.

* $p < 0.05$

†Asthma Control Questionnaire-score < 0.75 .

‡History of eczema, hay fever, or food allergy.

§Use of ICS less than a week previous to the study visit.

¶Medication Adherence Report Scale-score ≥ 21 .

**Pediatric pulmonologist or pediatrician visit.

Appendix II

Factors associated with a high fraction of nitric oxide in exhaled breath (> 35 ppb)

	Univariate analysis OR (95% CI)	Multivariate analysis Adjusted OR (95% CI)
General characteristics		
Male gender	1.0 (0.7–1.5)	
Age	1.3 (1.2–1.4)*	1.3 (1.2–1.4)*
Clinical characteristics		
Asthma control†	0.6 (0.4–0.9)*	0.5 (0.4–0.8)*
Atopy‡	1.6 (0.9–2.9)	
Current use of inhaled corticosteroids (ICS)§	0.5 (0.4–0.8)*	0.6 (0.4–1.0)*
Good adherence¶	0.4 (0.2–0.6)*	0.4 (0.2–0.6)*
Medication use in the past year		
ICS use	0.7 (0.4–1.3)	
Oral corticosteroids use	1.5 (0.7–3.2)	
Short-acting beta agonists use	1.4 (0.8–2.7)	
Long-acting beta agonists use	1.2 (0.7–1.9)	
Leukotriene antagonists use	0.2 (0.0–0.6)*	0.2 (0.0–0.8)*
Antibiotic use	0.5 (0.3–0.8)*	0.5 (0.3–0.9)*
Healthcare use in the past year		
General practitioner visit	1.0 (0.6–1.5)	
Specialist physician visit**	0.5 (0.3–0.8)*	0.6 (0.3–1.1)
ER visit	0.5 (0.2–1.7)	
Environmental factors		
Pet exposure	1.0 (0.7–1.5)	
Passive smoking	1.6 (0.9–2.9)	
Non-urban environment	1.6 (1.0–2.4)*	1.6 (1.0–2.5)

Factors that caused a 10% change or more in the regression coefficient were included in the multivariable model.

* $p < 0.05$.

†Asthma Control Questionnaire-score < 0.75 .

‡History of eczema, hay fever, or food allergy.

§Use of ICS less than a week previous to the study visit.

¶Medication Adherence Report Scale-score ≥ 21 .

**Pediatric pulmonologist or pediatrician visit.