

The relationship between exhaled nitric oxide and allergic sensitization in a random sample of school children

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Summary

Background Exhaled nitric oxide (NO) has been proposed as novel a non-invasive marker of airway inflammation.

Objective The level of exhaled NO was determined in a random sample of school children (7–12 years old) with the aim of investigating the relationship between exhaled NO and sensitization to common allergens.

Results In the 450 children tested by skin prick tests (SPT), the prevalence of sensitization was 29.5% (overall), 21.9% (sensitization to indoor allergens), and 15.0% (sensitization to outdoor allergens). Regression analysis showed that levels of exhaled nitric oxide were closely associated with various measures of sensitization to aeroallergens. Sensitization to indoor allergens was associated with higher levels of exhaled NO (eNO) than sensitization to outdoor allergens when assessed by IgE but not when assessed by SPT. Children with reported wheeze in the past 12 months had much stronger associations between sensitization and eNO than children without wheeze.

Conclusion We conclude that allergic sensitization is strongly associated with increased levels of exhaled NO, especially in children with wheeze.

Keywords allergic sensitization, exhaled NO, nitric oxide

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Introduction

During the last decades the prevalence of allergic disorders has gradually increased. Environmental as well as lifestyle factors are believed to be responsible for this increase. Barnes and Liew [1] suggested that activation of the nitric oxide (NO) synthase system is the initiating step of allergic disease via inhibition of Th1 cells by NO. As such, exhaled NO (eNO) may serve as a marker of airway inflammation before the onset of clinical symptoms.

In established allergic asthma, pulmonary NO production is increased by pro-inflammatory mediators via the induction of NO synthase [2]. Indeed, relatively high values of eNO have been observed in subjects with asthma [3–9]. Bronchial hyper-responsiveness is a known characteristic of asthma but its relation with eNO is less clear. Bronchial hyper-responsiveness was shown to be positively correlated with eNO in a random sample of young adults [10] but not in asthmatic subjects [11, 12]. In addition, a significant positive correlation between eosinophilic

count in sputum and eNO has been observed [13–15], showing that eNO accompanies eosinophilic inflammation.

Level of eNO is also increased in subjects with perennial and seasonal rhinitis [10, 16–25] which show a fall in eNO upon avoidance of allergen exposure [17, 26, 27]. Together these observations suggest that eNO is an appropriate non-invasive marker of the inflammatory component of allergic airway disease [28, 29]. The aim of the study was to investigate the relationship between sensitization to common allergens and eNO in a random sample of school children (7–12 years old).

Materials and methods

Study subjects

The study sample consisted of a convenience sample of 450 children (7–12 years old) out of a total sample of 2504 children living in different urban areas. Children were recruited from seven public schools situated near motorways. Permission was obtained to assess skin prick testing (SPT) and vena puncture. The Medical Ethics Committee of the Wageningen University approved the study protocol, and parents gave written consent to participate in the study. No specific inclusion or exclusion criteria were applied.

The SPTs were performed according to the standardized International Study of Asthma and Allergies in Childhood

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(ISAAC) phase II protocol [30]. The ISAAC panel of six aero-allergens was used: *Dermatophagoides farinae*, *D. pteronyssinus*, cat, *Alternaria tenuis*, mixed tree pollen (*Betula verrucosa*, *Alnus glutinosa*, *Corylus avellana*) and mixed grass pollen (*Dactylis glomerata*, *Lolium perennae*, *Festuca pratensis*, *Poa pratensis*, *Phleum pratense*, *Avena eliator*). In addition, dog allergen was used. Standardized allergen extracts (all but *Alternaria*) and positive and negative controls (saline and histamine 10 mg/mL) were provided by ALK (Copenhagen, Denmark). The test was performed on the volar side of the left forearm using ALK lancets. After 15 min the outside contour of the weal was measured and the mean of the longest diameter and the length of the perpendicular line through its middle was calculated. Children with a reaction (>0 mm) to the negative control or no reaction to histamine were excluded from further analysis. A mean weal diameter of 3 mm or more is regarded as a positive reaction according to the position paper on skin tests of the European Academy of Allergy and Clinical Immunology [31].

Specific IgE

After centrifugation, serum specimens were frozen and stored at -20°C until further analysis for specific IgE [radioallergosorbent test (RAST) method] against *D. pteronyssinus*, cat, moulds (*A. tenuis*, *Penicillium notatum*, *Cladosporium herbarum*), mixed trees (*B. verrucosa*, *C. avellana*, *A. incana*, *Quercus alba*, *Salix caprea*), mixed grasses (*P. pratense*, *L. perennae*, *Anthoxanthum odouratum*, *Secale cereale*, *Holcus lanatus*) and dog. All the analyses were performed at the same laboratory (Pharmacia CAP systems, Pharmacia & Upjohn Diagnostics, Woerden, the Netherlands). Specific IgE is considered elevated if its value is 0.35 kU/L or more. Atopy is defined as an elevated specific IgE for ≥ 1 allergen and/or SPT-reactivity for ≥ 1 allergen. To investigate whether results were different when using a different criterion, we also defined atopy as having two or more positive SPTs. The outdoor allergen panel consisted of mixed grasses, mixed trees allergens and *Alternaria*. The indoor allergen panel consisted of cat, dog and house dust mite (HDM) allergens.

Sampling of exhaled air and assay of eNO

Exhaled air of the children was sampled in balloons using a sampling device equipped with a 'pre-balloon' of 500 mL to exclude dead space volume from the sample, as previously described [32]. In short, children exhaled air at 20 cm water back pressure into 500 mL NO-impermeable aluminium foil bags (Mylar balloon, ABC ballonnen, Zeist, the Netherlands). After exhalation into the 'pre-balloon' at a high speed of 6000 mL/min, exhaled air was collected at a low flow rate of 500 mL/min to obtain relatively high eNO concentrations. Although this method is different from European Respiratory Society (ERS) and American Thoracic Society (ATS) recommended methods [33, 34], we have shown previously that it correlates well with on-line measurements made in the laboratory which have followed ERS and ATS recommendations [35].

Outdoor air was sampled by an air sampling pump (Dupont de Nemours & Company Inc., model P-200, Wilmington, DE, USA) in aluminium foil air bags and ambient temperature was measured continuously. A rapid response chemiluminescence analyser (Sievers 280B, Boulder, CO, USA) with a response time <200 ms was used to determine the concentration of

eNO. The analyser sample flow rate was kept constant at 200 mL/min. eNO-concentration was measured within 4 h after sampling. In the aluminium foil bags the NO concentration remains constant for at least 48 h.

Questionnaire

The questionnaire consisted of the 'core questionnaire module' and the 'additional respiratory questions module' of the ISAAC questionnaire [30]. Additional questions were asked about the child's home, family composition, education and smoking habits of the residents.

Statistical analysis

Associations between eNO and sensitization to common allergens were analysed by mixed model analysis of variance to allow for potential dependence of observations of children attending the same school, using the SAS proc MIXED. eNO values were ln-transformed before analysis to normalize residuals. The 95% confidence intervals (95% CI) refer to back-transformed ratios from the analysis which was in the log-scale. The analyses were adjusted for the following potential confounders: age, gender, gas cooking, unvented water heater, environmental tobacco smoke exposure and having a cold during sampling.

Results

Table 1 shows that the children in the study sample were not different from the larger total population from which they were drawn. The prevalence rates of sensitization to common allergens in the children either tested via specific serum IgE or SPT are depicted in Table 2. The prevalence of sensitization to common inhalant allergens in the children tested via IgE or SPT was 32.3% and 29.5%, respectively. The highest prevalence rates were observed for sensitization to mite and grass pollen.

The geometric mean level of eNO was about 1.5 times higher in children sensitized to indoor allergens as compared to non-sensitized children ($P < 0.05$), whereas geometric mean eNO level of children sensitized to outdoor allergens was somewhat

Table 1. Characteristics of the study population and the total sample

Variable	Study population	Total sample
<i>n</i>	450	4626
Age (year)	10.0	9.5
% boys	50.0	48.4
Itchy rash last year*	19.0	15.5
Wheeze last year	10.0	9.0
Dry cough last year	21.9	22.9
Runny nose last year	25.6	24.7
Conjunctivitis last year	10.9	8.8
Phlegm last year	11.3	10.1
Eczema ever	29.1	29.8
Asthma ever	9.0	7.9
Hayfever ever	8.2	7.5
DD bronchitis last year	8.4	7.0
DD indoor allergy	11.8	9.3
DD outdoor allergy	6.8	6.5

*Prevalence rates of reported complaints are given as percentage. DD: doctor-diagnosed.

Table 2. Prevalence of sensitization in study population

Variable	SPT (n = 319)	IgE (n = 229)
Grass	14.4	20.5
Trees	3.8	9.6
Cat	8.8	7.9
Dog	4.1	10.0
<i>D. pteronyssinus</i>	17.2	–
<i>D. farinae</i>	14.1	–
Mite	–	21.8
Alternaria	1.9	2.6
Overall	29.5	32.3
Indoor allergens	21.9	23.1
Outdoor allergens	15.0	21.8
Number of positive SPT or positive IgE = 0	70.5	67.7
Number of positive SPT or positive IgE = 1	14.1	13.1
Number of positive SPT or positive IgE = 2	5.6	8.3
Number of positive SPT or positive IgE = 3	4.4	5.7
Number of positive SPT or positive IgE = 4	2.5	1.3
Number of positive SPT or positive IgE = 5	1.6	3.1
Number of positive SPT or positive IgE = 6	0.9	0.9
Number of positive SPT = 7	0.3	

Number of positive SPT or positive IgE refers to number of positive reactions in the skin prick reaction (range 0–7), and specific IgE in RAST (range 0–6), respectively.

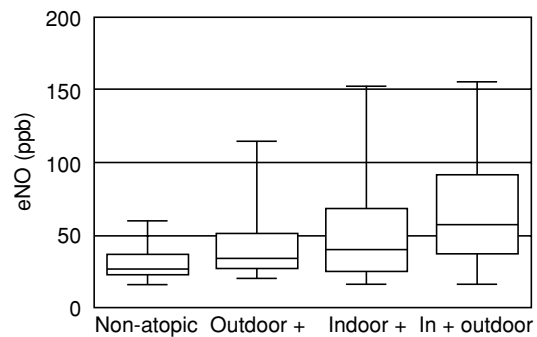
Table 3. Adjusted relative increase in eNO for children sensitized to indoor and outdoor common allergens

Variable	Relative increase	95% CI
Positive SPT to indoor allergens > 0	1.55	1.36–1.77
Positive SPT to indoor allergens > 1	2.02	1.66–2.47
Positive SPT to outdoor allergens > 0	1.48	1.27–1.72
Positive SPT to outdoor allergens > 1	2.00	1.46–2.76
Increased IgE to indoor allergens	1.65	1.43–1.90
Increased IgE to outdoor allergens	1.38	1.19–1.61
Increase in eNO per unit increase in total number of positive reactions in SPT	1.19	1.14–1.24
Increase in eNO per unit increase in total number of positive specific IgE	1.20	1.15–1.25

Adjusted for age, gender, gas cooking, unvented water heater, environmental tobacco smoke exposure and having a cold during exhaled air sampling.

less elevated (cf. Table 3 and Fig. 1). The multivariate analysis showed that eNO was associated with various measures of sensitization to aeroallergens as assessed via IgE or SPT. We investigated whether the associations were stronger when using two or more positive SPTs as criteria and we found that for indoor allergens, the relative increase in eNO then was 2.02 (95% CI 1.66–2.47), and for outdoor allergens 2.00 (95% CI 1.46–2.76), which indeed is greater than the relative increases found when one or more positive SPTs was used as the criteria.

It appears that the specific sensitizations to all indoor and outdoor allergens are significantly associated ($P < 0.05$) with an increase in the level of eNO. Sensitization to indoor allergens is associated with higher levels of eNO than sensitization to outdoor allergens but only when assessed by IgE. In addition, Table 3 shows that eNO gradually increases with the number of positive responses, i.e. number of positive SPTs or height of specific IgE-titres.

**Fig. 1.** Box-and-whisker plots of eNO in non-atopic children and children sensitized to indoor and outdoor allergens as assessed by SPT. In + outdoor: sensitized to both indoor and outdoor allergens. Mean eNO of non-atopic lower as compared to SPT-positive groups ($P < 0.05$). Box represents 25th, 50th and 75th percentile of distribution; whiskers represent 5th and 95th percentile, respectively.**Table 4.** Adjusted relative increase in eNO for children sensitized to indoor and outdoor common allergens – children with and without wheeze in the past 12 months

Variable	Relative increase	95% CI
No wheeze (n = 208 for SPT, 290 for IgE)		
Positive SPT to indoor allergens	1.36	1.19–1.57
Positive SPT to outdoor allergens	1.24	1.06–1.46
Increased IgE to indoor allergens	1.47	1.26–1.70
Increased IgE to outdoor allergens	1.27	1.08–1.48
Wheeze (n = 29 for SPT, 21 for IgE)		
Positive SPT to indoor allergens	2.99	1.89–4.71
Positive SPT to outdoor allergens	2.89	1.85–4.50
Increased IgE to indoor allergens	3.44	1.80–6.55
Increased IgE to outdoor allergens	1.56	0.72–3.38

Adjusted for age, gender, gas cooking, unvented water heater, environmental tobacco smoke exposure and having a cold during exhaled air sampling.

Table 4 shows that after stratification for wheeze in the last 12 months, the association between sensitization and eNO was much stronger in the children with wheeze than in the children without wheeze. Nevertheless, associations were significant in the non-wheezers as well.

Additional analyses showed that there was no association between current pet ownership and eNO (results not shown). We also investigated whether children using inhaled corticosteroids had lower eNO than children not on medication. Even after adjustment in all categories of wheezers for 'wheeze in the last 12 months' and 'ever having received a diagnosis of asthma', there was no significant effect (results not shown).

Discussion

This study shows that, in a random sample of school children, the eNO level was positively associated with various measures of sensitization to common inhalant allergens. The prevalence of sensitization to common allergens was similar to the prevalence found in community studies by von Mutius et al. [36–39].

Relationships between eNO and allergic sensitization have been reported previously [10, 16–25], and eNO was shown to be

associated with the number of positive SPTs [12, 40] and wheal size [6, 12, 24].

The present results from a cross-sectional study in a random population of school children extend these observations and show that the level of eNO was positively associated with SPT-positivity, specific serum IgE and the number of positive SPT reactions. The analysis showed that the relative increase in eNO was larger in children sensitized to indoor allergens as compared to children sensitized to outdoor allergens when assessed by IgE. This observation suggests that continuous exposure to indoor allergens, like HDM, evokes a strong inflammatory response due to permanent and repetitive activation by allergens. This is in agreement with observations that eNO is higher in children sensitized to multiple allergens [40], and allergic subjects have higher eNO upon exposure to allergens [17, 19, 26, 27]. We could not demonstrate that eNO was higher in children who had pets in the home in this study. It is well known that pets are removed from some households when a child develops allergic airway disease, so there may have been a selection effect preventing us from seeing higher eNO at continued pet exposure. The lower concentration of eNO in children sensitized to outdoor allergens may also be explained by the relatively large size of the pollen grains (30–60 µm) which prevent their entry in the lower distal airways. Such a difference in eNO responsiveness was also recently reported by Leuppi et al. [25].

The origin of the elevated eNO concentrations in sensitized subjects is not known but probably results from a subclinical eosinophilic inflammatory process throughout the respiratory tract. Indeed, histopathological studies have demonstrated the presence of airway inflammation in atopic subjects without signs of clinical asthma [41].

The reported positive association between allergic asthma and eNO is predominantly based on studies performed in selected samples of patients [3–9]. The present random sample includes non-symptomatic children, and shows that allergic sensitization as such is clearly associated with increased eNO.

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