Eur Respir J 2005; 25: 455–461
DOI: 10.1183/09031936.05.00079604

Exhaled nitric oxide in 4-year-old children: relationship with asthma and atopy

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ABSTRACT: Airway inflammation is an early feature of asthma. Early detection and anti-inflammatory treatment may have important therapeutic impact. Exhaled nitric oxide is a noninvasive marker of airway inflammation. The current study investigated the association between exhaled nitric oxide and asthma, wheezing phenotypes, atopy and blood eosinophilia in a large group of 4-yr-old children from the general population.

All children participated in the Prevention and Incidence of Asthma and Mite Allergy study, a birth cohort study of high-risk (atopic mother) and low-risk children in the Netherlands. Nitric oxide levels were successfully determined in 429 children.

Although there was overlap in the distribution of values of children with and without asthma or atopy, mean values were higher in children with atopy or doctor's diagnosed asthma (geometric mean (ppb) 9.4 and 10.0, respectively) as compared to those without (7.7 and 7.9). Values were highest in atopic symptomatic children. Values were not associated with wheezing phenotype or blood eosinophilia.

This study is one of the few large-scale epidemiological studies among 4-yr-old children from the general population showing that children with symptoms of asthma and atopy have higher levels of exhaled nitric oxide than those without.

KEYWORDS: Allergy, asthma, cohort studies, nitric oxide, pre-school child

There is evidence that airway inflammation may precede the onset of asthma, suggesting that asymptomatic young children may already suffer from chronic airway inflammation [1]. As chronic airway inflammation may induce airway remodelling and reduced lung function, early detection and anti-inflammatory treatment might have an important therapeutic impact [2].

Airway inflammation can be detected by several methods, such as bronchial biopsy, bronchoalveolar lavage and induced sputum. However, due to their invasive character or low practical applicability, these methods are not suitable for use in young children or in large study populations.

A method that can be applied in children is the measurement of the nitric oxide fraction in exhaled air ($F_{E,NO}$). This noninvasive method is simple and suitable even for young children [3]. Measurements can be performed online, with direct exhalation into the NO analyser, or offline. With the offline method, exhaled breath is collected in an NO impermeable balloon, which allows storage for several hours [4]. Thus it is not necessary to have an NO analyser present during collection of exhaled air, which makes the method suitable for use in epidemiological field studies. Furthermore, in older subjects, the technique has been shown to yield similar results as obtained with the ''gold standard'' [3].

Previous studies have shown that $F_{E,NO}$ is elevated in adults and school-aged children with asthma and atopy [5–7], with highest levels in atopic asthmatics [8, 9]. In atopic asthmatics, higher $F_{E,NO}$ levels are associated with higher blood and airway eosinophil numbers [10, 11], indicating that $F_{E,NO}$ reflects eosinophilic airway inflammation. Recently, elevated $F_{E,NO}$ levels have also been demonstrated in pre-school children with recurrent wheeze and doctor's diagnosed asthma [12–14].

Until now, most studies have been conducted in children with doctor's diagnosed asthma or children referred to hospital because of severe respiratory symptoms. Large scale epidemiological studies of $F_{E,NO}$ in young children from the general population are scarce [15, 16], and are not yet available for pre-school children.

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Received:
July 01 2004
Accepted after revision:
November 16 2004

SUPPORT STATEMENT
This work was supported by the Netherlands Organisation for Health Research and Development, the Netherlands Organisation for Scientific Research, the Netherlands Asthma Fund, the Netherlands Ministry of Spatial Planning, Housing and the Environment and the Netherlands Ministry of Health, Welfare and Sport.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

The Prevention and Incidence of Asthma and Mite Allergy study, a prospective birth cohort study, offers the opportunity to study $F_{E,NO}$ values in a large group of pre-school children recruited from the general population. The aim of the current study was to investigate the association between $F_{E,NO}$ and asthma, wheezing phenotypes, atopy and peripheral blood eosinophilia in a large group of 4-yr-old children from the general population.

METHODS

Study design

The Prevention and Incidence of Asthma and Mite Allergy study involved 4,146 children. Children were recruited through pre-natal clinics in three regions of the Netherlands (western, middle and northern areas). During pregnancy, their mothers completed a validated screening questionnaire on asthma and inhalant allergies [17], from which their atopic status was determined. Based on the atopy of the mother, children were labelled as high-risk (atopic mother) and low-risk (nonatopic mother).

Data on demographic factors, respiratory symptoms and risk factors for asthma were collected by yearly questionnaires. At age 4 yrs, all high-risk children (n=1173) and a random sample of the low-risk children (n=635) were invited for a medical examination, including measurement of $F_{E,NO}$. A detailed description of the study design is given elsewhere [18]. The study protocol was approved by the medical ethics committees of the participating institutes. All parents gave written informed consent.

Study subjects

From the 1,808 children invited, medical examinations were performed in 1,279. In 344 of 1,279 children, it was not possible to obtain $F_{E,NO}$ values, mostly because NO analysers were not available for the total medical examination period in the middle and northern part of the Netherlands. Thus, an attempt to measure $F_{E,NO}$ was made in 935 children. Exhaled air was successfully collected in 659 children. From these 659 children, 230 children were excluded from statistical analysis because of missing questionnaires (n=22), medication use in the 12 h before measurement (n=22), high ambient NO (>20 ppb) during measurement (n=114), technical problems during analysis of NO balloons (n=62) or large differences (>10 ppb) between duplicate NO measurements (n=10). Finally, the data of 429 children were available for statistical analysis. From these 429 children, 35 used inhaled corticosteroids at aged 4 yrs. A detailed flow chart of the study population is given in figure 1.

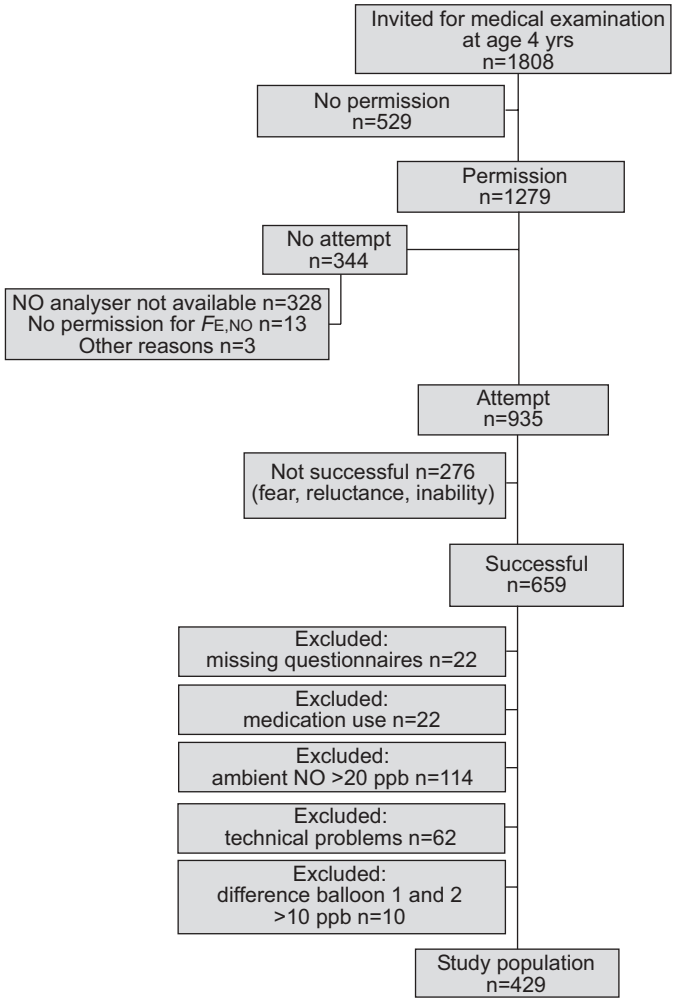

FIGURE 1. Flow chart of the number of children progressing through the medical examination at age 4 yrs. $F_{E,NO}$: nitric oxide fraction in exhaled air.

$F_{E,NO}$ measurement

$F_{E,NO}$ was measured offline by the balloon method, according to recent European Respiratory Society/American Thoracic Society guidelines [19]. In short, children were asked to take a deep breath through a charcoal NO scrubber, and to exhale immediately into a collection device employing dynamic flow restriction, using a two-way valve. Exhalation flow was kept constant at 50 $mL \cdot s^{-1}$ over a pressure range of 5–20 cm H_2O. Mouth pressure was monitored during measurement using a manometer. After discarding dead space air for 3–4 s, exhaled air was collected in a NO impermeable 150 mL Mylar balloon (Jurjen de Vries BV, Leeuwarden, the Netherlands) [3]. For every child, duplicate exhaled air samples and a sample of ambient air were collected. Balloons were sealed, stored and analysed within 8 h with a chemiluminescence analyser. In the western and middle areas of the Netherlands, a Sievers NOA 280 analyser (Boulder, CO, USA; sensitivity 0.5 ppb, detection range 0.5–500.000 ppb, sample flow 200 $mL \cdot min^{-1}$, sampling rate $4 \cdot s^{-1}$ (middle), $20 \cdot s^{-1}$ (western)), was used. In the northern area, an Ecophysics CLD 700 AL analyser (Ecophysics, Basel, Switzerland; sensitivity 1 ppb, detection range 0–50.000 ppb, sample flow rate 700 $mL \cdot min^{-1}$, data sampling rate $0.5 \cdot s^{-1}$) was used. A check for systematic differences between the different NO analysers was performed by measuring balloons filled with calibration gas on all three analysers on the same day. This analysis revealed a good agreement, with no significant systematic differences between the analysers.

Asthma and wheezing phenotypes

Information about doctor's diagnosed asthma was collected by the following question: ''Did a doctor ever diagnose asthma in your child?''. Symptoms of wheeze were assessed by International Study of Asthma and Allergies in Childhood core questions [20]. Based on the longitudinal questionnaire

data on wheeze symptoms in the first 4 yrs of life, children were divided into four wheezing phenotypes [21]: ''never wheeze'', ''early transient wheeze'' (at least one episode of wheeze in the first 3 yrs of life), ''late-onset wheeze'' (at least one episode of wheeze in the 4th yr of life) and ''persistent wheeze'' (at least one episode of wheeze in the first 3 yrs of life, and at least one episode of wheeze in the 4th yr of life). In the analyses, late-onset and persistent wheezing phenotypes were combined into one category.

Specific IgE and blood eosinophils

Total and specific immunoglobulin (Ig)E to inhalant allergens (house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, grass (*Dactylis glomerata*), birch (*Betula verrucosa*) and mould (*Alternaria alternata*)) were determined by Radio Allergo Sorbent Test. Atopy was defined as specific IgE concentration >0.35 IU·mL^{-1} for at least one inhalant allergen. The numbers of eosinophil granulocytes in peripheral blood were determined by automated differential cell count after peroxidase staining. Blood eosinophilia was arbitrarily defined as eosinophil counts >400 per μL [22]. From the 429 children for whom reliable $F_{E,NO}$ values were obtained, specific IgE and eosinophil counts were available for 247 and 234 children, respectively. The remaining children either refused or venepuncture failed. As eosinophil count followed a right-skewed distribution, data were $\log_{10}$-transformed.

Statistical analysis

Because $F_{E,NO}$ values followed a right-skewed distribution, data were $\log_{10}$-transformed. Crude and adjusted geometric mean $F_{E,NO}$ values were estimated by (multiple) linear regression analysis, with $\log_{10}$ ($F_{E,NO}$) as the dependent variable and asthma, wheezing phenotype, atopy or eosinophilia as independent variables. Due to the stratified study design, all analyses were performed for the total study population as well as separately for high-risk and low-risk children. Recent symptoms of cold might influence $F_{E,NO}$ and might be associated with asthma, therefore, these were considered as a potential confounder. To adjust for possible differences between study regions, region was taken into account. Atopy of the mother, sex, education of the mother, exposure to environmental tobacco smoke, exposure to pets, contact with other children and lower respiratory tract infections were also considered as potential confounders. The effect of inhaled corticosteroids was studied separately. To study the association of $F_{E,NO}$ with eosinophil count without dividing the latter into categories, Pearson correlation coefficients were calculated. In the analyses, p-values <0.05 were considered to be statistically significant.

RESULTS

Selection of the study population

In total, 1,279 of the 1,808 children who were invited participated in the medical examination at 4 yrs of age. Children who did not participate (n=529) more often had a mother with low education (32% *versus* 20%). Due to the relatively high percentage of children with missing questionnaires at 4 yrs of age (29% (154/529) *versus* 3% for the children who did participate), it was difficult to compare the percentages of children with respiratory symptoms between those who did and did not participate. However, with respect to respiratory symptoms at the ages of 1–3 yrs, there seemed to be no major differences between the two groups.

There were no differences in the percentage of young males, children with an atopic mother, children with low educated mothers, asthma, wheeze, atopy or blood eosinophilia between the children who participated in the medical examination, but were not included in the final study population (n=850), and the final study population (n=429). Within the study population, there were no differences between the children from whom blood samples were or were not available.

General characteristics of the study population

The study population consisted of 274 high-risk and 155 low-risk children (table 1). High-risk children were more frequently living in the western area and less often in the middle area of the Netherlands and they less often had siblings than low-risk children. The percentage of low educated mothers was similar for high-risk and low-risk children. As compared with low-risk children, high-risk children more frequently reported doctor's diagnosed asthma (11% *versus* 6%; p=0.08) and more frequently had specific IgE to inhalant allergens (25% *versus* 14%; p=0.06) at the age of 4 yrs. These differences were of borderline statistical significance. The percentages of children with late-onset/persistent wheeze or peripheral blood eosinophilia were not different for high- and low-risk children (15% *versus* 14% and 30% *versus* 27%, respectively).

TABLE 1 General characteristics of the study population

	High-risk children	Low-risk children
Subjects n	274	155
Sex % males	52	52
Age yrs	4.06±0.22#	4.14±0.21
Height m	1.06±0.04	1.06±0.04
Weight kg	18±3	18±2
Study region %		
West	51#	40
Middle	30#	44
North	19	16
Education of the mother %		
Low	21	23
Middle	36¶	46
High	42#	31
Exposure to ETS at 4 yrs %	22	26
Exposure to pets at 4 yrs %	47	48
Contact with siblings at 4 yrs %	78#	87
Contact with other children (no siblings) at 4 yrs %	94	91
LRTI at 4 yrs %	12	10
Symptoms of cold 2 weeks before NO+ %	45	43
Use of inhaled corticosteroids at 4 yrs %	9	7

Data are presented as mean±SD or %, unless otherwise stated. ETS: environmental tobacco smoke; LRTI: lower respiratory tract infections (bronchitis, pneumonia, pertussis). #: $p<0.05$ as compared with low-risk children; ¶: $p<0.10$ as compared with low-risk children; +: fever, cough, wheeze, shortness of breath, tightness of chest, coughing up phlegm.

$F_{E,NO}$ values in the total study population

The overall geometric mean $F_{E,NO}$ value (95% confidence interval (CI)) was 8.0 ppb (7.7–8.3) (n=429). In the children who did not have doctor's diagnosed asthma, never wheezed, did not have specific IgE to inhalant allergens or blood eosinophilia and did not use inhaled corticosteroids at 4 yrs of age (asymptomatic, nonatopic group), the geometric mean $F_{E,NO}$ value (95% CI) was 7.9 ppb (7.1–8.8) (n=64). $F_{E,NO}$ values were slightly higher in children with doctor's diagnosed asthma and in children with specific IgE to at least one inhalant allergen as compared with those without (table 2). Mean $F_{E,NO}$ values were not associated with wheezing phenotype or peripheral blood eosinophilia. $F_{E,NO}$ values were highest in children with both elevated specific IgE and doctor's diagnosed asthma, late-onset/persistent wheeze or peripheral blood eosinophilia. Because results were similar for the combinations of IgE with asthma, wheezing phenotype or eosinophilia, only the combination of IgE with asthma is shown in table 2. There were no major differences between the results of the crude and adjusted analyses. Further adjustment for potential confounders did not alter the results of the analyses. There were also no major differences between the analyses in which children using inhaled corticosteroids at 4 yrs of age were included or excluded. When analyses were repeated for children for whom ambient NO levels were <10 ppb at the time of measurement, similar results were obtained.

The correlation between $F_{E,NO}$ and eosinophil count (children using inhaled corticosteroids excluded) was 0.12 (r (Pearson), p=0.07). The correlation was stronger for children with specific IgE (r=0.34, p=0.03) as compared with those without (r=0.05, p=0.52).

$F_{E,NO}$ values in high-risk and low-risk children

In high-risk and low-risk children respectively, the overall geometric mean $F_{E,NO}$ values (95% CI) were 8.0 ppb (7.6–8.5) and 7.9 (7.4–8.4). As was seen in the total study population, $F_{E,NO}$ values were slightly higher in children with doctor's diagnosed asthma and in children with specific IgE to at least one inhalant allergen as compared with those without (table 3, fig. 2). In high-risk children, $F_{E,NO}$ values were highest in children with both elevated specific IgE and doctor's diagnosed asthma, late-onset/persistent wheeze or blood eosinophilia. In low-risk children, high $F_{E,NO}$ values were especially observed in children with elevated specific IgE, independent of asthma, wheezing phenotype or blood eosinophilia. However, the analyses of the combinations of specific IgE and asthma, wheezing phenotype or peripheral blood eosinophilia were limited by the low numbers of symptomatic children.

The correlation between $F_{E,NO}$ and eosinophil count was 0.09 (r Pearson, p=0.29) for high-risk children and 0.20 (r Pearson, p=0.08) for low-risk children. As was seen in the total study population, correlations were stronger for children with specific IgE (high-risk: r (Pearson)=0.29, p=0.13; low-risk: r=0.64, p=0.047) as compared with those without (high-risk: r=0.03, p=0.77; low-risk: r=0.09, p=0.48) (children using inhaled corticosteroids excluded).

DISCUSSION

Mean $F_{E,NO}$ values were higher in 4-yr-old children with specific IgE to inhalant allergens and in children with doctor's diagnosed asthma as compared with those without. $F_{E,NO}$ values were highest in children with both specific IgE and

TABLE 2 Crude and adjusted geometric mean nitric oxide levels in exhaled air ($F_{E,NO}$; ppb) for doctor's diagnosed asthma, wheezing phenotypes, specific immunoglobulin (Ig)E and peripheral blood eosinophilia in the total study population

	Include inhaled corticosteroids				Exclude inhaled corticosteroids			
	n	Crude	Adjusted (95% CI)#		n	Crude	Adjusted (95% CI)#	
Doctor's diagnosed asthma -	390	7.9	7.9	(7.6–8.2)	376	7.9	7.9	(7.5–8.2)
Doctor's diagnosed asthma +	39	8.9¶	8.8	(7.7–10.0)	18	10.1§	10.0§	(8.3–12.1)
Never wheeze	225	7.9	8.0	(7.5–8.4)	221	7.9	7.9	(7.5–8.4)
Early transient wheeze	123	8.1	8.1	(7.5–8.7)	114	8.1	8.0	(7.4–8.7)
Late/persistent wheeze	63	8.2	8.2	(7.4–9.1)	42	8.5	8.6	(7.5–9.7)
Specific IgE -	194	7.8	7.8	(7.3–8.2)	179	7.7	7.7	(7.2–8.1)
Specific IgE +	53	9.3f	9.5f	(8.5–10.6)	47	9.1f	9.4f	(8.4–10.5)
Eosinophilia -	166	8.0	8.0	(7.6–8.5)	153	7.9	7.9	(7.4–8.4)
Eosinophilia +	68	8.7	8.6	(7.8–9.5)	62	8.5	8.4	(7.6–9.3)
Asthma - IgE -	178	7.7	7.7	(7.3–8.2)	172	7.7	7.6	(7.2–8.1)
Asthma - IgE +	45	8.9##	9.0¶¶	(8.0–10.2)	44	8.7##	8.9¶¶	(8.0–10.1)
Asthma + IgE -	16	8.7	8.3	(6.8–10.1)	7	9.1	8.5	(6.4–11.5)
Asthma + IgE +	8	12.4¶¶	12.8¶¶	(9.6–17.0)	3	17.7¶¶	19.0¶¶	(12.2–29.8)

#: Geometric mean $F_{E,NO}$ value (95% confidence interval (CI)) (ppb), adjusted for atopy of the mother, study region and symptoms of cold in the two weeks before $F_{E,NO}$ measurement; -: negative; +: positive; ¶: p<0.10 as compared with geometric mean $F_{E,NO}$ in children without doctor's diagnosed asthma; §: p<0.05 as compared with geometric mean $F_{E,NO}$ in children without doctor's diagnosed asthma; f: p<0.05 as compared with geometric mean $F_{E,NO}$ in children without specific IgE; ##: p<0.10 as compared with geometric mean $F_{E,NO}$ in children without asthma and without specific IgE; ¶¶: p<0.05 as compared with geometric mean $F_{E,NO}$ in children without asthma and without specific IgE.

TABLE 3 Crude and adjusted geometric mean nitric oxide levels in exhaled air ($F_{E,NO}$; ppb) for high and low-risk children

	High-risk children				Low-risk children			
	n[#]	Crude	Adjusted (95% CI)[¶]		n[#]	Crude	Adjusted (95% CI)[¶]	
Doctor's diagnosed asthma -	235	7.8	7.8	(7.4–8.3)	141	7.9	7.9	(7.4–8.4)
Doctor's diagnosed asthma +	15	10.2[§]	10.2[§]	(8.3–12.6)	3	10.0	9.4	(5.9–14.8)
Never wheeze	132	7.9	7.8	(7.3–8.4)	89	8.0	8.1	(7.4–8.8)
Early transient wheeze	75	8.2	8.2	(7.4–9.0)	39	7.9	7.8	(6.9–8.9)
Late/persistent wheeze	30	8.5	8.6	(7.4–10.1)	12	8.4	8.3	(6.6–10.4)
Specific IgE -	113	7.7	7.6	(7.1–8.3)	66	7.8	7.8	(7.1–8.5)
Specific IgE +	37	8.8	9.0[ƒ]	(7.9–10.4)	10	10.5[ƒ]	10.7[ƒ]	(8.4–13.5)
Eosinophilia -	98	7.7	7.7	(7.1–8.4)	55	8.2	8.3	(7.5–9.1)
Eosinophilia +	40	8.3	8.3	(7.3–9.5)	22	8.8	8.5	(7.3–10.0)
Asthma - IgE -	108	7.6	7.6	(7.0–8.2)	64	7.8	7.8	(7.1–8.5)
Asthma - IgE +	34	8.3	8.4	(7.3–9.7)	10	10.5[##]	10.7[##]	(8.4–13.5)
Asthma + IgE -	5	9.3	8.8	(6.2–12.6)	2	8.7	7.6	(4.5–12.8)
Asthma + IgE +	3	17.7[##]	19.0[##]	(12.0–30.2)	0			

[#]: Children using inhaled corticosteroids were excluded from analysis; [¶]: geometric mean $F_{E,NO}$ value (95% confidence interval (CI)) (ppb), adjusted for study region and symptoms of cold in the 2 weeks before $F_{E,NO}$ measurement; -: negative; +: positive; [§]: $p<0.05$ as compared with geometric mean $F_{E,NO}$ in children without doctor's diagnosed asthma; [ƒ]: $p<0.05$ as compared with geometric mean $F_{E,NO}$ in children without specific immunoglobulin (Ig)E; [##]: $p<0.05$ as compared with geometric mean $F_{E,NO}$ in children without asthma and without specific IgE.

doctor's diagnosed asthma, late-onset/persistent wheeze or peripheral blood eosinophilia.

Despite a careful study design, some methodological considerations should be taken into account. Selection bias may have occurred if the association between $F_{E,NO}$ and asthma or atopy was different in the children who participated *versus* those who did not participate in the medical examination. The last group more often had a mother with low education. However, in the data from the current study, the association between $F_{E,NO}$ and asthma or atopy was not different for children with low and high-educated mothers. Therefore, selection bias due to this aspect seems unlikely.

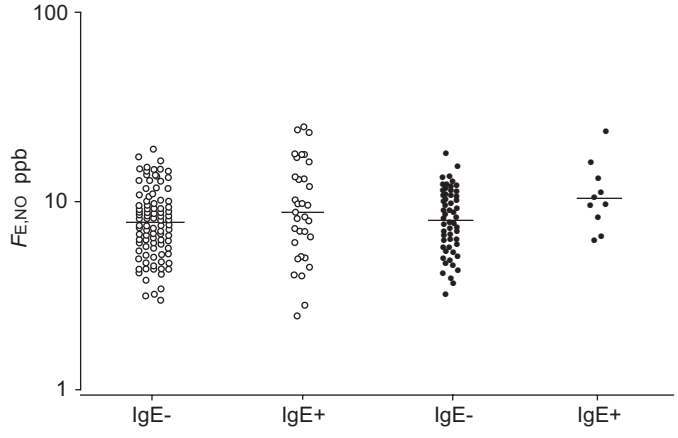

FIGURE 2. Distribution of nitric oxide levels in exhaled air ($F_{E,NO}$) in high-risk (○) and low-risk (●) children with (+) and without (-) elevated specific immunoglobulin (Ig)E to inhalant allergens. Children using inhaled corticosteroids were excluded. Bars represent geometric means.

Selection bias may also have occurred if the association between $F_{E,NO}$ and asthma or atopy was different for the children from whom reliable $F_{E,NO}$ values or blood samples were not obtained *versus* those from whom $F_{E,NO}$ values and blood samples were obtained. There were several reasons why reliable $F_{E,NO}$ values were not obtained for the children, such as the absence of NO analysers, fear or reluctance of the child, inability to comply with the test procedure or technical problems during $F_{E,NO}$ measurement. The reasons for the absence of blood samples for the children were that children either refused or venepuncture failed. As all of the above mentioned reasons were unrelated to the health characteristics of the child, they are unlikely to selectively introduce bias to the association between $F_{E,NO}$ and asthma or atopy.

Another potential problem is misclassification bias. Much effort was taken to obtain valid $F_{E,NO}$ values. The use of a dynamic flow restrictor, which created a positive mouth pressure, prevented contamination of $F_{E,NO}$ with nasal NO [3]. Also, because high ambient NO levels are known to influence $F_{E,NO}$ measurement [19], children were asked to inhale through a NO scrubber before exhalation, and $F_{E,NO}$ values that were measured when ambient NO values were >20 ppb were excluded from analysis. Symptoms and risk factors for asthma were determined independently of $F_{E,NO}$ measurement, thus, it seems unlikely that differential misclassification has occurred in these variables.

In the current study with pre-school children, $F_{E,NO}$ levels were elevated in atopic children and in children with doctor's diagnosed asthma, which is in accordance with the results of previous studies in school-aged children [5–7, 9, 16, 23]. $F_{E,NO}$ values were highest in atopic asthmatic children. This interaction between asthma and atopy was also observed in

other studies investigating the association between asthma and atopy in school-aged children and adults [8, 9, 15, 23].

Elevated $F_{E,NO}$ levels were not detectable in children with late-onset or persistent wheeze at 4 yrs of age. This may be because persistent wheeze is mainly an indication of airway obstruction, which has been shown not to correlate with $F_{E,NO}$ in previous studies with older subjects [10, 24]. When $F_{E,NO}$ levels were studied in atopic and nonatopic wheezers separately, children who were both atopic and had late-onset or persistent wheeze had higher $F_{E,NO}$ values than children with late-onset or persistent wheeze without atopy. This finding is in accordance with the hypothesis that in nonatopic wheezers, wheeze is a nonspecific symptom that can be due to asthma, but more frequently to relatively small airway size that causes wheeze during viral infections only [25], while in atopic wheezers, wheeze might be related to asthma.

In high-risk children, $F_{E,NO}$ levels seemed only elevated in atopic symptomatic children. This is in accordance with studies in older asthmatics [8, 9, 15, 23], and suggests that even at this young age airway inflammation is present in atopic children with respiratory symptoms. In low-risk children, elevated $F_{E,NO}$ in atopic children appeared to occur independently of respiratory symptoms. A possible explanation is poorer symptom recognition in the children of nonatopic mothers, causing symptomatic children to be classified as asymptomatic. However, it might also indicate that elevated $F_{E,NO}$ levels are more strongly associated with atopy than with respiratory symptoms [16].

The overall correlation between $F_{E,NO}$ and peripheral blood eosinophils was weak. The correlation was stronger in atopic children as compared with nonatopic children. This confirms previous studies in atopic asthmatic children [10, 11]. However, the results of other studies vary. Some studies showed a weak or moderate correlation between $F_{E,NO}$ and eosinophils in blood [26], sputum [27], bronchoalveolar lavage [11] or bronchial biopsy [28], though others did not [29]. Clearly, $F_{E,NO}$ as a new marker of airway inflammation in asthma behaves differently from other disease activity markers. The interpretation of $F_{E,NO}$ requires further investigation.

In conclusion, this study is one of the few large-scale epidemiological studies among 4-yr-old children from the general population showing that children with symptoms of asthma and atopy have higher levels of nitric oxide in exhaled air than those without. Nitric oxide levels were highest in atopic asthmatic children. However, mean differences between symptomatic and asymptomatic children were relatively small and there was a large overlap in the distribution of the nitric oxide levels of individual children with and without asthma or atopy. This makes it difficult to identify individual children at high risk of asthma based on their levels of nitric oxide in exhaled air. Further follow-up of these children is in progress, which will inform about the predictive value of nitric oxide levels in exhaled air alone or in combination with other characteristics.

ACKNOWLEDGEMENTS

The authors would like to thank all the children and their parents for their cooperation. They also thank all field workers (H. Oosterloo, W. Winters, M. Routledge, M. Bolling, K. Corver, M. Seesink, M. Oldenwening, I. Oosting, S. de Wind, M. Siekmans, R. Beelen, R. van Strien, J. Spithoven, M. Giovannangelo), the data manager (A. Vos) and the laboratory personnel (J. de Vrieze, B. Verlaan, W. Holland, Y. Wallbrink, L. de la Fonteyne, B. Nagarajah, S. Lever, A. Kroon, E. van der Wiel-Kooy, E. van Duyn-van de Water) for their efforts.

J.C. de Jongste has acted as a scientific advisor for Aerocrine, Sweden (manufacturer of NO analysers) and has lectured several times on request of Aerocrine. Payments for these services went directly to Sophia's Children's Hospital. Sophia's Children's Hospital/Sophia BV of Erasmus University has a reference center agreement with Aerocrine, Sweden. This has until now not resulted in any payments.

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