

ORIGINAL ARTICLE

Asthma, atopy and exhaled nitric oxide in a cohort of 6-yr-old New Zealand children

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Abstract

Background: Exhaled nitric oxide has been promoted as a non-invasive measure of airway inflammation, with clinical utility for the diagnosis and management of asthma.

Aim: We studied associations between exhaled nitric oxide, asthma and atopy in a variety of clinically relevant phenotypes in a cohort of 6-yr-old children.

Method: Asthma was defined using standard questionnaire criteria, atopy was measured using skin prick tests (SPT) and specific IgE to common allergens, and exhaled nitric oxide was measured using a chemiluminescence analyser according to American and European Thoracic Society criteria.

Results: Exhaled nitric oxide was strongly related to atopy and in particular to sensitization to house dust mites. Children with non-allergic asthma had no increase in exhaled nitric oxide compared with non-asthmatic children. Compared with children who never wheezed both late onset and persistent, wheezing was associated with increased FE_{NO}, while early transient wheezing was not. Elevated levels of exhaled nitric oxide amongst children with allergic asthma were almost entirely explained by their levels of specific IgE to aeroallergens, predominantly *D pteronyssinus*.

Conclusion: Airway inflammation as measured by exhaled nitric oxide in young New Zealand children is related to their level of specific IgE to aeroallergens. This has implications for the utility of nitric oxide as a diagnostic and management tool in childhood asthma and for the importance of specific IgE as a marker of asthma severity.

Airway inflammation is considered to be a central pathological characteristic of asthma. The degree of airway inflammation is positively correlated with asthma symptom severity (1), and reducing airway inflammation is the cornerstone of asthma treatment, most commonly with inhaled corticosteroids (ICS). New Zealand has long been considered to have a higher prevalence and severity of asthma than many other developed countries in both adults (2) and children (3), with high rates of morbidity (4) and previously high rates of mortality (5).

Airway inflammation can be assessed by bronchial biopsy, bronchoalveolar lavage and by the examination of induced sputum. These methods are not suitable for large population surveys and are not easily applied in children. Exhaled nitric oxide has been suggested as a possible non-invasive marker

of airway inflammation in children (6) and has been increasingly used to assess airway inflammation, in particular eosinophilic airway inflammation. Fractional exhaled nitric oxide (FE_{NO}) levels are associated with blood and airway eosinophilia (7), and elevated FE_{NO} has been described in adult subjects (8) and in children (9) with atopy without associated allergic disease.

Multiple overlapping wheezing phenotypes have been described in children, including transient wheezing, non-atopic wheeze and IgE-associated wheeze (10). The aim of this study was to examine FE_{NO} levels in a birth cohort of children at age 6–7 yrs and assess associations with a variety of clinically relevant respiratory and atopic phenotypes, defined by SPT and by specific IgE to common allergens.

Methods

Participants

A full description of the recruitment and assembly of the New Zealand Asthma and Allergy Cohort Study (NZAACS) has previously been published (11). Briefly, mothers were recruited through a random sample of midwives in Wellington and Christchurch in New Zealand between 1997 and 2001. Ethics committee approval was obtained from the Wellington and Canterbury Regional Ethics Committees, and informed consent was obtained from each mother.

Questionnaires were administered by study nurses at recruitment, 3, 15, 24, 36, 48 and 60 months of age, by home visit at 3 and 15 months and subsequently by telephone. Outcome measures were collected using identical questions at 15, 24, 36, 48 and 60 months covering the period since birth or the previous visit. Between the ages of 6 and 7 yrs, families and children were invited to attend a clinical facility in Wellington or Christchurch for a further more detailed questionnaire and a variety of clinical assessments. These included measures of FE_{NO} , skin prick tests (SPT) to common food and environmental allergens and a blood sample for total and specific IgE (asIgE).

Measurement of exhaled nitric oxide

Fractional exhaled nitric oxide was measured online using an Aerocrine NIOX chemiluminescence analyser at a flow rate of 50 ml/s. The lower and upper limits of detection were 2 and 200 p.p.b., respectively. The device was operated and calibrated in accordance with the manufacturer's instructions, and FE_{NO} was measured according to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (12). Just prior to the nitric oxide measurement, mothers or caregivers were asked whether the child currently had a cold or chest infection and whether the child had undertaken any exercise in the last hour.

Skin prick testing

Allergen sensitivity was measured to the following allergens: *D. pteronyssinus*, cat, dog, horse, cockroach mix (American and German), rye grass, olive tree, *Aspergillus fumigatus*, *Alternaria tenuis*, egg white, peanut, cow's milk (Dome/Hollister-Stier, Spokane, WA, USA), together with a positive (histamine 10%) and negative control.

Allergens were applied to the volar aspects of both forearms with a wooden applicator. Vertical punctures were made using individual stainless steel lancets (Dome/Hollister-Stier). The histamine positive control was measured at 10 min, and all other wheals measured at 15 min with wheals traced using a fine-tip pen. The wheals were transferred to a permanent paper record using Micropore™ (3M, St. Paul, Minnesota, USA) tape. Mean wheal diameter was measured using both the longest and orthogonal diameters, recorded to the nearest 0.5 mm. A positive reaction was defined as a mean wheal diameter of 3 mm or greater to any allergen, with atopy by SPT defined as one or more positive reactions.

Training ensured that the coefficients of variation in histamine wheal size for each investigator was <0.2 . Interobserver variability in histamine wheal size and atopy prevalence was monitored throughout the study and addressed with retraining where disparate.

Wheezing, asthma and family history definitions

The mother or caregiver was asked about the child's wheezing at each visit. Wheezing was classified by onset and persistence (13) into early transient, one or more episodes of wheezing between birth and 3 yrs but no wheezing between 3 and 6 yrs; late onset, no wheezing between birth and 3 yrs but wheezing between 3 and 6 yrs; and persistent wheeze, wheezing between birth and 3 yrs and between 3 and 6 yrs. Current asthma was defined as a previous doctor's diagnosis of asthma at any time and current wheezing or current inhaler use (current refers to the last 12 months). A family history of allergic disease was defined as a parental report of either parent having a history of asthma, hay fever or eczema.

IgE measurements

Total and specific IgE was measured with a third-generation chemiluminescent enzyme immunoassay (IMMULITE 2000 Siemens Medical, Deerfield, IL, USA). The same 12 allergens used for skin prick testing were included in the specific IgE measurements. Atopy was defined as any asIgE ≥ 0.35 kU/l.

Statistical analysis

All analyses were undertaken using SAS version 9.1 (SAS Institute, Cary, NC, USA). FE_{NO} and asIgE values were not normally distributed, and values were $\log_{10}$ -transformed for the analyses. FE_{NO} is presented as geometric mean values, with *t*-tests used to assess the significance of differences between groups. Chi-squared tests were used to test for trend. Geometric mean ratios were derived using the GLM procedure in SAS after adjustment for reported colds present on the day (yes vs. no) and height (as a continuous variable). This analysis was further stratified by the atopic status of the child. IgE response to specific allergen groups was selectively added to the models as explanatory variables, and groups compared by analysis of covariance.

Results

Of the 1105 infants recruited at birth, 1064 (96.3%) children with questionnaire data available were seen at 3 months. At 15 months, 1011 (91.4%) had questionnaire data available. At age 6 yrs, of the 1011 eligible participants, six families withdrew. Of the remaining 1005, 920 were able to be contacted. A total of 846 were able to attend a clinical laboratory facility in Wellington or Christchurch, the remaining 74 were living overseas or outside the two study centres and agreed to complete a questionnaire by mail or e mail.

Of the 846 who attended the laboratory, 17 children did not attempt any nitric oxide measurement for a variety of

Table 1 Geometric mean FE_{NO} in p.p.b. with 95% confidence intervals, by demographic and pre-test conditions

	n/N	FE _{NO}
All children	681	9.1 (8.6–9.7)
Men	350/681	9.5 (8.7–10.3)
Women	331/681	8.8 (8.1–9.5)
Māori	94/681	9.2 (7.9–10.8)
Pacific people	26/681	12.9 (9.7–17.2)
European	534/681	8.9 (8.3–9.5)
Other	27/681	10.1 (7.0–14.5)
No current cold	513/680	8.7 (8.1–9.3)*
Current cold	167/680	10.6 (9.5–11.8)
Height		1.01 (1.00–1.02)**
Exercise within an hour of test	22/680	8.8 (6.4–12.1)
No exercise within an hour of test	658/680	9.1 (8.6–9.7)
Family history	570/673	9.3 (8.7–9.9)
No family history	103/673	8.1 (7.0–9.4)
Wellington	341/681	9.1 (8.4–9.9)
Christchurch	340/681	9.1 (8.4–9.9)

Family history = either parent with a history of eczema, hayfever or asthma. (Numbers do not add to the total N because of missing paternal data). Height = increase in FE_{NO} (p.p.b.) per unit increase (cms) in height. *p < 0.01; **p < 0.05.

reasons including the occasional breakdown of the analyser, and 148 children could not achieve reproducible values. The remaining 681 (62% of the original birth cohort) children were able to produce reproducible exhalations with at least two plateaus that agreed within 10% or 1 p.p.b.

There were no significant differences in geometric mean FE_{NO} by gender or ethnicity or by study site. Neither exercise within an hour of the test nor family history of asthma or allergic disease had any effect on mean FE_{NO}. Children with current colds had higher levels of FE_{NO} than children without; height was also positively but weakly associated with FE_{NO} (Table 1). Adjustment for height and colds had minimal effects on FE_{NO}. As neither reported colds nor height was associated with current asthma or atopy, Tables 2 and 3 show unadjusted FE_{NO} values.

Asthma, wheezing and FE_{NO}

Children with a history of any wheezing from birth to 6 yrs had higher levels of FE_{NO} at 6 yrs than those that had not wheezed. Early transient wheeze was not associated with FE_{NO} at 6 yrs, while those children with persistent wheeze and late onset wheeze had elevated levels of FE_{NO} at 6 yrs. Both current wheezing and current asthma were associated with elevated mean levels of FE_{NO}. Amongst children with asthma, those currently taking ICS had higher levels of FE_{NO} than children not currently taking them (Table 2).

Atopy, current asthma and FE_{NO}

Geometric mean levels of exhaled nitric oxide were associated with atopy at 6 yrs as measured by a positive SPT (Table 3).

Table 2 Geometric mean FE_{NO} in p.p.b. with 95% confidence intervals amongst children with wheezing and asthma

	n/N	FE _{NO}
No wheezing birth to 6 yrs	229/680	7.8 (7.2–8.4)**
Any wheezing birth to 6 yrs	451/680	9.8 (9.1–10.6)
Never wheezed (reference)	218/664	7.8 (7.2–8.5)
Early transient	162/664	7.3 (6.6–8.1)
Late onset	221/664	12.0 (9.8–14.6)**
Persistent	63/664	11.6 (10.3–13.0)**
Current wheeze (12 m prior)	172/680	15.0 (13.1–17.1)**
No current wheeze (12 m prior)	508/680	7.7 (7.3–8.1)
Current asthma	161/680	14.5 (12.5–16.7)**
No current asthma	519/680	7.9 (7.4–8.3)
Amongst children with current asthma		
Current ICS	83/161	17.8 (14.7–21.5)*
No current ICS	78/161	11.6 (9.3–14.4)

*p = < 0.01; **p = < 0.0001. Early transient wheezing ≥1 episode wheeze 0–3 yrs but none between 3 and 6 yrs. Late onset (No wheezing 0–3 yrs and ≥1 episode between 3 and 6 yrs). Persistent ≥1 episode wheeze 0–3 yrs and ≥1 episode between 3 and 6 yrs. Current asthma = A doctor's diagnosis of asthma at any time + wheezing and or asthma medication in the last year. Numbers do not add to total N because of missing data at one or more data points between birth and 6 yrs. ICS, inhaled corticosteroids.

Mean FE_{NO} was almost twice as high in atopic children who had a positive SPT to HDM (House Dust Mite) compared with atopic children negative to HDM. Both the number of positive wheals and the cumulative size of wheals were associated with increasing levels of FE_{NO}, and there was a positive trend for higher levels of FE_{NO} by quartiles of total serum IgE. For children with current asthma who were not atopic, FE_{NO} was not significantly different from those without asthma and atopy (Table 3). Amongst the 89 children with current asthma and atopy, 73 (82%) were positive for HDM and their mean FE_{NO} was higher (24.2 p.p.b.) than atopic asthmatic children without a positive HDM reaction (14.4 p.p.b.; p = 0.021).

FE_{NO}, specific IgE and asthma

Figure 1 shows the positive relationship between log FE_{NO} and log sum specific IgE to aeroallergens for children with and without current asthma who have a specific IgE to any aeroallergen (see Table 4 for definition of aeroallergens) ≥0.35 kU/l.

We have explored this relationship further, by modelling the effects of specific IgE on the relationship between FE_{NO} and current asthma stratified by atopy defined by SPT and by specific IgE. All models are adjusted for height and for reported current colds. In addition, log specific IgE for a variety of specific IgE combinations has then been added to the model (Table 4). Because ICS reduce FE_{NO}, we have further explored these relationships amongst atopic children who are not currently taking ICS. In these models, the elevated FE_{NO} amongst current asthmatics is almost entirely explained by the level of asIgE to aeroallergens and *D pteronyssinus* in particular, but not by asIgE to food allergens.

	n/N	FE _{NO} (p.p.b. and 95%CI)	p
No atopy @ 6 yrs	444/624	7.1 (6.8–7.5)	<0.0001
Atopic @ 6 yrs	180/624	17.7 (15.7–20.0)	
No atopy	444/624	7.1 (6.8–7.5)	Ref
Atopic (inc HDM)	135/624	20.4 (17.7–23.5)	<0.0001
Atopic (exc HDM)	45/624	11.5 (9.4–14.2)	<0.0001
Not atopic	444/624	7.1 (6.8–7.5)	<0.0001*
Atopy pos wheals(1–2)	142/624	16.8 (14.6–19.2)	
Atopy pos wheals(3+)	38/624	22.3 (16.7–20.0)	
Total wheal size 0	391/624	7.0 (6.6–7.4)	<0.0001*
0–3 mm	41/624	7.4 (6.1–8.9)	
3–6 mm	74/624	14.2 (11.7–17.2)	
6–12 mm	69/624	17.3 (14.3–21.0)	
>12 mm	49/624	22.7 (18.2–28.4)	
Quartiles total IgE (kU/L)			
0.5–14.5	128/537	6.9 (6.3–7.6)	<0.001*
14.5–52.75	133/537	6.9 (6.4–7.5)	
52.75–182	140/537	9.4 (8.3–10.7)	
≥182	136/537	16.2 (14.1–18.6)	
No atopy and no current asthma	385/623	7.0 (6.7–7.3)	Ref
Current asthma without atopy	59/623	7.9 (6.5–9.7)	0.23
Current asthma with atopy	89/623	22.1 (18.6–26.1)	<0.0001
Atopy without current asthma	90/623	14.1 (11.9–16.6)	<0.0001

*p for trend <0.0001. Otherwise p-values are based on comparison with reference value (t-test).

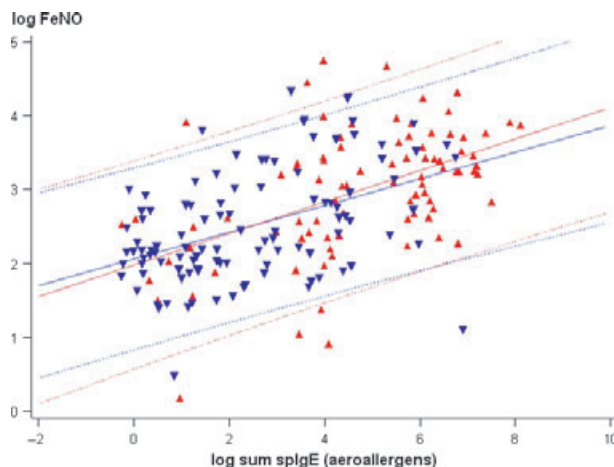


Figure 1 Relationship between FE_{NO} and log sum specific IgE to aeroallergens for children with asthma ▲ ($r = 0.50$, $p < 0.0001$) and without asthma ▼ ($r = 0.44$, $p < 0.0001$) with regression lines and 95% confidence bands amongst children with a positive specific IgE (≥ 0.35 kU/l) to any aeroallergen.

Discussion

In this birth cohort of 6-yr-old children, we have shown that FE_{NO} was increased in children who report current upper respiratory tract symptoms and in taller children. FE_{NO} was significantly increased in children who report current wheezing or have current asthma, but the increase in children with current asthma is confined to those children who are also

atopic. In keeping with these observations, FE_{NO} was elevated in children with late onset and persistent wheeze where atopy is more frequent, but not in children who only have early transient wheezing. Higher levels of FE_{NO} were found with increasing numbers of positive SPT and with increasing total wheal size.

FE_{NO} was elevated in children who were atopic regardless of current asthma and was significantly higher in those atopic children sensitized to house dust mites compared with atopic children not sensitized to house dust mites. This confirms the central role of house dust mite allergen as the major cause of airway inflammation amongst atopic children in New Zealand. This strong relationship between HDM sensitivity and elevated FE_{NO} has been observed in adults and children (8, 14).

The widespread use of SPT for atopy in community surveys and in the clinical setting leads to children being considered as atopic or non-atopic. Our results refute this and suggest that in terms of airway inflammation as measured by FE_{NO}, the 'degree' of atopy as measured by specific IgE is crucial and largely determines an individual child's level of exhaled nitric oxide. Similarly, the level of specific IgE is strongly associated with a diagnosis of asthma, as shown previously by Simpson et al. (15) for the risk of current wheeze. In our 6-yr-old New Zealand children, this is true for aeroallergens when combined and specifically for *D. pteronyssinus* alone, but not for food allergens. This holds true for all asthmatic children and when asthmatic children currently taking ICS are excluded.

In 2003, Franklin et al. (16) showed that FE_{NO} was elevated in children with a combination of atopy and airway

Table 3 Geometric mean FE_{NO} in p.p.b. and 95% confidence intervals by atopy, total IgE and atopic asthma

Table 4 Adjusted geometric mean ratio of FE_{NO} and 95% confidence intervals by current asthma amongst atopic and non-atopic children

Study sample or subsample	Model variables*	n	Adjusted geometric mean ratio and 95% CI	p
All children	Height, cold	679	1.81 (1.59–2.06)	0.010
Non-atopic children (SPT –ve)	Height, cold	443	1.09 (0.94–1.26)	0.26
Atopic children (SPT +ve)	Height, cold	179	1.56 (1.23–1.98)	0.010
Atopic children (any asIgE ≥0.35 kU/l)	Height, cold	250	1.75 (1.43–2.15)	<0.001
	Height, cold, log sum all asIgE	241	0.98 (0.80–1.21)	0.86
Atopic children (any asIgE ≥0.35 kU/l)	Height, cold, log asIgE DPt	247	1.08 (0.90–1.29)	0.43
	Height, cold, log sum asIgE AA	241	1.01 (0.82–1.24)	0.95
	Height, cold, log sum asIgE FOOD	241	1.76 (1.41–2.19)	<0.001
Atopic children (any asIgE ≥0.35 kU/l)	Height, cold, log sum all asIgE	178	0.85 (0.64–1.12)	0.25
(excluding children currently taking ICS)	Height, cold, log asIgE D Pt	184	0.98 (0.76–1.26)	0.43
	Height, cold, log sum asIgE AA	178	0.89 (0.67–1.17)	0.40
	Height, cold, log sum asIgE FOOD	178	1.36 (0.98–1.89)	0.07

Height and the log sum of specific IgE were entered as continuous variables, reported recent cold as a dichotomous variable. Aeroallergens (AA) included *D Pteronyssinus* (DPt), rye grass, *Alternaria*, *Aspergillus*, cat, dog, horse, cockroach and olive. Food allergens (FOOD) include milk, egg and peanut. ICS, inhaled corticosteroids; SPT, skin prick tests.

hyper-responsiveness (AHR) but not with physician-diagnosed asthma or reported wheeze in the last 12 months. The association with FE_{NO} did not hold for atopy or AHR alone, and the authors suggested that an elevated FE_{NO} was the result of a mechanism linking AHR and atopy. Our results are compatible with such a mechanism and suggest that the link is with specific IgE to aeroallergens. In support of this, Nogalo et al. (17) recently showed in mite sensitive non-asthmatic subjects a positive relationship between specific IgE to mite and AHR. We did not measure AHR in our children and cannot therefore confirm these associations directly. However, it is entirely plausible that the level of specific IgE determines the lung's inflammatory response to allergen exposure as measured by FE_{NO}. In the New Zealand context, this is very largely because of the universal and high level of exposure to house dust mite allergen found in infant bedding (18) and in New Zealand homes (19). In other environments, the allergen profile will be different and it remains to be determined whether sensitivity to house dust mite allergens results in greater airway inflammation than other common aeroallergens.

We have included children with asthma who were currently prescribed ICS that are known to reduce FE_{NO}. Removing children taking ICS does not alter the relationships between FE_{NO} and specific IgE.

Based on the results of a number of studies that have shown that an elevated FE_{NO} is strongly associated with atopy (8, 20) and our findings that this elevation reflects the 'degree' of atopy as measured by specific IgE, regardless of asthma, FE_{NO} is unlikely to be useful in the diagnosis of asthma in children unless levels of specific IgE are considered. Malmberg and colleagues recently published height-adjusted reference values for FE_{NO} in healthy non-atopic children (21) and suggested that FE_{NO} data should not be interpreted in children without knowing their atopic status; our present study would support this approach. Specific IgE levels vary with season for seasonal allergens (22) and to

some extent for perennial allergens (23); they also decrease with allergen avoidance (24) and with inhaled corticosteroid therapy (25), and they have also been shown to correlate with asthma severity (26).

The role of FE_{NO} in the management of established atopic asthma may be of more clinical value and in particular in the titration of ICS treatment in adults (27). However, the value of FE_{NO} in guiding ICS treatment in adults has recently been challenged (28) Buchvald et al. (29) have reported heterogeneity in the response to ICS treatment in children with some children whose asthma was well controlled on high doses of ICS continuing to maintain a high level of FE_{NO}. Similarly, Wilson et al. (30) failed to demonstrate a relationship between asthma symptoms and FE_{NO} in children. Our study suggests that these children are likely to have particularly high levels of specific IgE that may drive their inflammatory lung response, as measured by FE_{NO}, to inhaled allergen. In such children, trying to reduce FE_{NO} with increasing doses of ICS would not necessarily improve their asthma management.

In conclusion, in a population sample of 6-yr-old children, we have shown that FE_{NO} levels are closely related to levels of specific IgE to aeroallergens amongst allergic children and that FE_{NO} is not elevated in non-allergic asthmatic children. The main driver of airway inflammation, as measured by FE_{NO}, in these children is their level of specific IgE to aeroallergens and to *D pteronyssinus* in particular and their continuing high level of exposure to this allergen in the New Zealand domestic environment.

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