Eur Respir J 2013; 41: 189–194
DOI: 10.1183/09031936.00019811

Factors associated with elevated exhaled nitric oxide fraction in infants with recurrent respiratory symptoms

Anne Kotaniemi-Syrjänen, L. Pekka Malmberg, Kristiina Malmström, Anna S. Pelkonen and Mika J. Mäkelä

ABSTRACT: Exhaled nitric oxide fraction (*F*eNO) has been proposed as a noninvasive marker of eosinophilic bronchial inflammation in active asthma, and supposed to reflect responsiveness to corticosteroid therapy. There are several factors influencing *F*eNO, and its role in early childhood respiratory disorders needs to be established.

Between 2004 and 2008, 444 children aged <3 yrs with recurrent lower respiratory tract symptoms were referred to a tertiary centre for further investigation. 136 full-term, steroid-free, infection-free infants, median age of 16.4 months (range 4.0–26.7 months), successfully underwent measurement of *F*eNO, lung function tests, and a dosimetric methacholine challenge test.

The median level of *F*eNO was 19.3 ppb (interquartile range 12.3–26.9 ppb). Elevated *F*eNO (⩾27 ppb, the highest quartile) was associated with maternal history of asthma (adjusted OR 3.2, 95% CI 1.3–8.1; p=0.012), and increased airway responsiveness (the provocative dose of methacholine causing a 40% fall in maximal expiratory flow at functional residual capacity ⩽0.30 mg) (adjusted OR 4.1, 95% CI 1.4–12.7; p=0.012). Atopy, blood eosinophilia and lung function were not associated with elevated *F*eNO.

In conclusion, maternal history of asthma, and increased airway responsiveness are associated with elevated *F*eNO in infants with recurrent lower respiratory tract symptoms.

KEYWORDS: Atopy, exhaled nitric oxide fraction, increased airway responsiveness, lung function tests, recurrent lower respiratory tract symptoms, wheezing

AFFILIATIONS
Dept of Allergology, Helsinki University Central Hospital, Helsinki, Finland.

CORRESPONDENCE
L. Pekka Malmberg
Skin and Allergy Hospital
P.O. Box 160
FIN-00029 HUS
Finland
E-mail: pekka.malmberg@hus.fi

Received:
Feb 03 2011
Accepted after revision:
May 31 2012
First published online:
June 27 2012

Exhaled nitric oxide fraction (*F*eNO) is a widely used noninvasive biomarker of active asthma in adults and school children [1]. Elevated *F*eNO has been proposed as a marker of eosinophilic inflammation in bronchial mucosa, supposed to imply a favourable response to corticosteroid therapy [1]. In addition, increased airway responsiveness has been related to elevated levels of *F*eNO [2–4].

At pre-school age, children with probable asthma present with elevated *F*eNO [5]. However, the role of *F*eNO in respiratory disorders of early childhood has not been established: wheezing illnesses in infancy are usually not associated with eosinophilic bronchial inflammation [6, 7], and although increased airway responsiveness may play a role in recurrent lower respiratory tract symptoms of early childhood [8], studies on the relationship of *F*eNO and increased airway responsiveness in infants are scarce [9]. In addition, there are several characteristics influencing *F*eNO values: *i.e.* methods used [10], the height of the child [2], the presence of atopic eczema [11, 12], skin-prick test reactivity [2, 3, 7, 12, 13], acute respiratory symptoms [14–16], and tobacco smoke exposure [16–18].

The aims of the present study were to evaluate the relationship between *F*eNO and increased airway responsiveness, and to clarify whether there are any associations between elevated *F*eNO and anthropometrics and exposure to risk factors for respiratory morbidity in infants with recurrent lower respiratory tract symptoms. The initial findings for this study were presented, in part, at the European Respiratory Society Annual Congress in 2011 [19].

Earn CME accreditation by answering questions about this article. You will find these at the back of the printed copy of this issue or online at www.erj.ersjournals.com/misc/cmeinfo.xhtml

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

METHODS

Study subjects

Between August 18, 2004 and November 26, 2008, a total of 444 children aged <3 yrs were referred to the Dept of Allergology at the Helsinki University Central Hospital (Helsinki, Finland) for investigation of recurrent lower respiratory tract symptoms (including wheeze, dry or productive cough, and/or shortness of breath). Of these children, 187 successfully underwent (in a respective order) measurement of F_{ENO}, lung function testing by whole body plethysmography and the rapid thoracic compression technique, and the dosimetric methacholine challenge test. The data of the remaining 257 cases were excluded because of missing (n=220) or technically unacceptable (n=37) measurements of F_{ENO}, lung function, and/or airway responsiveness. In addition, pre-term (gestational age <36 weeks) infants, those on corticosteroid medication (during 1 month prior to testing), and those with any symptoms of acute respiratory infection (during 2 weeks prior to testing) were excluded, leaving 136 children for final analyses. Excluded children did not differ from included infants in any aspects of baseline characteristics. However, excluded children more often had abnormal results in baseline lung function measured by rapid thoracic compression technique (p=0.021). Although, there were no differences in F_{ENO} levels between included and excluded children (p=0.161).

All tests were performed as part of the recruitment phase of an intervention study on infant asthma, after informed consent was given from the parents to perform the tests. In addition, the parents were asked for further written consent to allow the use of the F_{ENO} measurements, lung function and methacholine-challenge test results, skin-prick test (SPT) results, and related clinical data for research purposes. The intervention study, including measurement of: F_{ENO}, performance of the baseline lung function tests, and the methacholine challenge test during the recruitment phase, was approved by the local ethics committee.

During the hospital visit, clinical data were collected with a standardised form designed for the intervention study by interviewing the parents, and by reviewing the medical records of the children. A small amount of blood, <1% of the circulating blood volume, was collected for analysis of peripheral blood eosinophil count.

Measurement of F_{ENO}

F_{ENO} was assessed with a modification of online single-breath measurement [20]. During sedation, the babies spontaneously breathed room air through a mask covering the nose and the mouth, and a pneumotach attached to the mask. Rapid thoracoabdominal compression technique was applied to generate a forced expiration starting from end-inspiration. The expired air was led to a chemiluminescence analyser (NIOX®; Aerocrine, Solna, Sweden) *via* a shutter mechanism of the pneumotach and a three-way valve, and a dynamic resistor that restricted the expiratory flow to 50 $mL \cdot s^{-1}$. By using a three-way valve, sampling of the exhaled air occurred between the mask and the shutter system, at immediate proximity to the mask. The effective dead space consisted mainly of the mask (10 mL). F_{ENO} was measured from the end-expiratory sample by using the plateau phase of the NO profile. Repeated exhalations were performed in order to obtain three reproducible F_{ENO} measurements (variation <10%, maximum of 5 ppb). The mean value of these measurements was recorded as well as the ambient NO (NO_{amb}) during the test. Only measurements with $NO_{amb} \leqslant 5$ ppb (83% of the measurements) were included in the analyses.

Lung function tests and the dosimetric methacholine challenge

Lung function tests and the dosimetric methacholine challenge were performed according to the protocols used in our hospital [8]. All infants were studied when they were free from signs of acute respiratory infection, and β2-agonists were withheld for 12 h prior to lung function and challenge tests. In brief, during lung function testing, the sedated infant was lying supine with the head supported in the midline and the neck slightly extended to minimise airway or glottic obstruction. All measurements were recorded and calculations performed with commercial paediatric pulmonary function equipment (MasterScreen™ BabyBody; Erich Jaeger GmbH, Würtzburg, Germany). Functional residual capacity (FRC) was measured by whole body plethysmography. The maximal partial expiratory flow volume (PEFV) was obtained using the rapid thoracic compression technique (*i.e.* tidal squeeze) by rapid inflation of a thoracoabdominal jacket at the beginning of expiration. Flow was measured at the infant's nose and mouth with a pneumotachometer attached to a face mask. The compression pressure was progressively increased until there was no further increase in forced expiratory flow at FRC ($V'_{max,FRC}$), and the mean $V'_{max,FRC}$ of three technically acceptable PEFV curves obtained at that compression pressure was recorded. The baseline lung function results were expressed as z-scores, which are equivalent to the number of standard deviations by which the measured value deviates from the height- and sex-corrected reference value.

For the dosimetric methacholine challenge, a calibrated nebuliser (Salter Labs 8900, Arvin, CA, USA) was connected to an automatic, inhalation-synchronised dosimeter (Spira Electro II; Spira Respiratory Care Center Ltd, Helsinki, Finland). The dosimeter was set to be triggered by an inhaled volume of 20 mL, after which a methacholine chloride dose of 50 μg was nebulised within 0.2 s in an air volume of 25 mL in each breath. By calculating the number of breaths with nebulised methacholine, a rapid dosage scheme with four non-cumulative dose steps was applied (0.1, 0.3, 0.9 and 1.8 mg), with $V'_{max,FRC}$ being recorded after each dose. At each phase, the applied compression pressure was the same as that achieved in the highest flows at baseline. The provocative dose of methacholine causing a 40% fall in $V'_{max,FRC}$ (PD_{40} $V'_{max,FRC}$) was determined from the dose–response curves. In cases where the maximal dose was reached and PD_{40} $V'_{max,FRC}$ could not be determined from the dose–response curves, for statistical purposes, PD_{40} $V'_{max,FRC}$ was defined as twice the highest dose of methacholine 3.60 mg.

During lung function measurements and the challenge test, oxygen saturation and heart rate were continuously monitored with a pulse oximeter. Following the challenge test, the children received inhaled salbutamol (0.6 mg; Ventoline Evohaler 0.1 mg per dose) *via* Nebunette (AstraZeneca Liquid production, Lund, Sweden) and the measurement of $V'_{max,FRC}$ was repeated 15 min after the salbutamol inhalation.

Skin-prick tests

Sensitisation to common food and/or inhalant allergens, including: egg white, cow's milk, wheat, soy bean, cod, shrimp,

peanut, birch pollen, timothy grass pollen, dog epithelial dander, cat epithelial dander, and house dust mite *Dermatophagoides pteronyssinus*, was tested by SPTs. A positive SPT was defined as a wheal with a diameter of ⩾3 mm against at least one of the tested allergens [21]. Physiologic saline was applied as a negative control.

Definitions

Food allergy was defined as a diagnosis confirmed by a positive food challenge. Atopic eczema was defined as a current diagnosis made by a paediatrician or a dermatologist. Atopy was defined as the presence of atopic eczema and/or a positive SPT. A parental history of asthma or allergy was defined as physician-diagnosed asthma or allergy, respectively, in either of the parents. A maternal history of asthma or allergy was defined as physician-diagnosed asthma or allergy, respectively, in the mother of the child. Blood eosinophilia was defined as eosinophils accounting for ⩾4% of the total white blood cells [22].

Lung function parameters that were regarded as abnormal had an FRC z-score of ⩾2 [8], and $V'_{max,FRC}$ z-score of ⩽ -2 [8]. F_{eNO} values of ⩾27 ppb, *i.e.* highest quartile, were considered elevated. PD_{40} $V'_{max,FRC}$ ⩽0.30 mg was considered as increased airway responsiveness to methacholine, PD_{40} $V'_{max,FRC}$ 0.31–0.90 mg as intermediate responsiveness to methacholine, and PD_{40} $V'_{max,FRC}$ ⩾0.91 mg as no/mild responsiveness to methacholine.

Statistics

To evaluate the statistical differences between the groups, Chi-squared test or Fisher's exact test (if the expected frequency for any cell was <5) were used to analyse categorical data, and Kruskal–Wallis test or Mann–Whitney U-test were applied to analyse continuous data. Correlations between continuous variables were determined by Spearman's rank correlation test. Logistic regression analysis was performed to calculate the adjusted odds ratios and related 95% confidence intervals in a multivariate setting, as follows: a dichotomous variable indicating elevated F_{eNO} was included in the analysis as a dependent variable, and explanatory (those with $p<0.05$ in univariate analyses) or confounding variables (selected by clinical importance, *i.e.* atopy and height) were included as covariates. All covariates were simultaneously added in the multivariate model. Two-tailed tests were used in all analyses. p-values <0.05 were considered statistically significant. The data were analysed using IBM SPSS 19.0 for Windows.

RESULTS

The median (range) age of the children studied was 16.4 months (4.0–26.7 months), and the median (range) height 80.7 cm (62.0–94.2 cm). None of the children had major congenital cardiac or other malformations.

The median (range) duration of recurrent lower respiratory tract symptoms was 7 months (2–26 months), and cough was most commonly (72%) reported as a main respiratory symptom. However, up to 105 (77%) of the children had experienced at least one episode of wheezing confirmed by a physician. A parental history of asthma or allergy was present in 107 (79%) children; in the majority (60%), this was a maternal history of asthma or allergy. 57 (42%) children had either atopic eczema, food allergy, and/or a positive SPT. Blood eosinophilia was present in 31 (23%) children.

The median F_{eNO} was 19.3 ppb (IQR 12.3–26.9 ppb). The median (range) FRC z-score was 0.7 (-2.2–4.8), and the median (range) $V'_{max,FRC}$ z-score -0.8(-3.8–1.5). FRC was regarded as abnormal in 27 (20%), and $V'_{max,FRC}$ in 23 (17%) children. Median coefficients of variation for F_{eNO}, FRC, and $V'_{max,FRC}$ were 7%, 3%, and 4%, respectively. During the methacholine provocation, the median changes $V'_{max,FRC}$ and oxygen saturation were -53% and -3%, respectively. 43 (32%) children were found to have increased airway responsiveness to methacholine (*i.e.* PD_{40} $V'_{max,FRC}$ ⩽0.30 mg).

When the relationships between F_{eNO} and baseline parameters were assessed, we found no correlation with age (r_s=0.114, p=0.188), height (r_s=0.148, p=0.087), or percentage of eosinophils in peripheral blood (r_s= -0.052, p=0.583) (fig. 1), and no association between elevated F_{eNO} (*i.e.* F_{eNO} of ⩾27 ppb) and blood eosinophilia (*i.e.* eosinophils ⩾4% in peripheral blood) (p=0.140). When associations between elevated F_{eNO} and baseline characteristics were evaluated, a significant association between elevated F_{eNO} and a maternal history of asthma was found (table 1). However, there were no other statistically significant associations between elevated F_{eNO} and any other baseline characteristics in univariate analyses.

When lung function and methacholine challenge data were evaluated with regard to F_{eNO}, we found no correlations between F_{eNO} and FRC (r_s= -0.121, p=0.163), or between F_{eNO} and $V'_{max,FRC}$ (r_s= -0.083, p=0.339). When analysed as a continuous variable, F_{eNO} was not associated with airway responsiveness to methacholine (fig. 2). However, there was an association between elevated F_{eNO} and increased airway responsiveness (fig. 3).

Finally, by performing multivariate logistic regression analysis, we were able to show that adjustment for the clinically most important confounding factors, *i.e.* atopy and height, did not

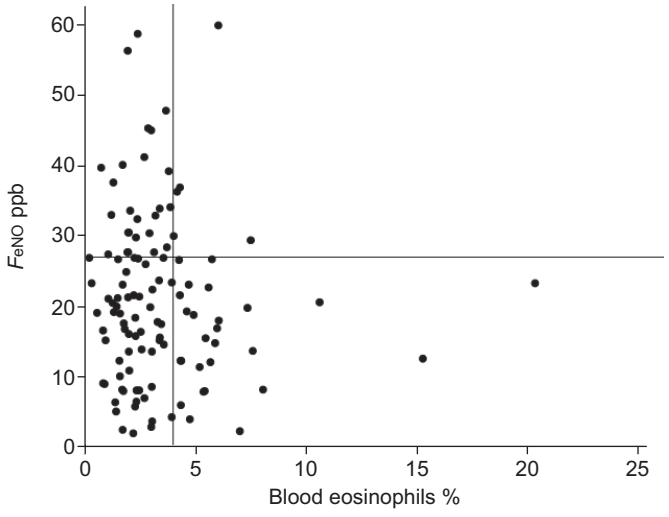

FIGURE 1. Exhaled nitric oxide fraction (F_{eNO}) in relation to the percentage of blood eosinophils of the total white blood cells. No correlation was found between F_{eNO} and the percentage of blood eosinophils (p=0.583). In addition, there was no association between elevated F_{eNO} (*i.e.* ⩾27 ppb, horizontal line) and blood eosinophilia (*i.e.* ⩾4%, vertical line) (p=0.140).

TABLE 1 Exhaled nitric oxide fraction (F_{ENO}) and baseline characteristics

Baseline characteristics	F_{ENO}		p-value#
	<27 ppb	⩾27 ppb	
Subjects	104	32	
Male/female	74 (71)/30 (29)	23 (72)/9 (28)	0.937
History of asthma			
Parental	40 (38)	16 (50)	0.246
Maternal	23 (22)	14 (44)	0.016
History of allergy			
Parental	77 (74)	25 (78)	0.641
Maternal	61 (59)	17 (53)	0.580
Exposure to ETS	34 (33)	9 (28)	0.604
Furry animals at home	31 (30)	10 (31)	0.876
Atopic eczema	28 (27)	4 (13)	0.093
Skin-prick test positive	27/102 (26)	7 (22)	0.602
Atopy	41 (39)	8 (25)	0.137
Food allergy	25 (24)	7 (22)	0.801
Parentally reported main respiratory symptom			
Dry cough	57 (55)	16 (50)	0.633
Productive cough	20 (19)	5 (16)	0.645
Wheeze	14 (13)	5 (16)	0.774
Shortness of breath	13 (13)	6 (19)	0.389
Physician-confirmed wheeze	79 (76)	26 (81)	0.533

Data are presented as n or n (%), unless otherwise stated. Results are from the univariate analyses. ETS: environmental tobacco smoke. #: analyses were performed using Chi-squared test or Fisher's exact test.

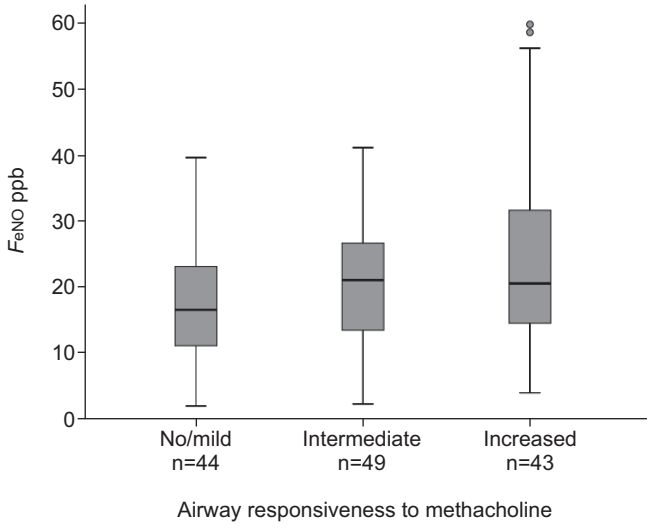

FIGURE 2. Exhaled nitric oxide fraction (F_{ENO}) and airway responsiveness to methacholine. The range of F_{ENO} was wide in all three levels of airway responsiveness, and no statistically significant associations were observed between F_{ENO} and airway responsiveness (p=0.165). The circles reprecent outliers.

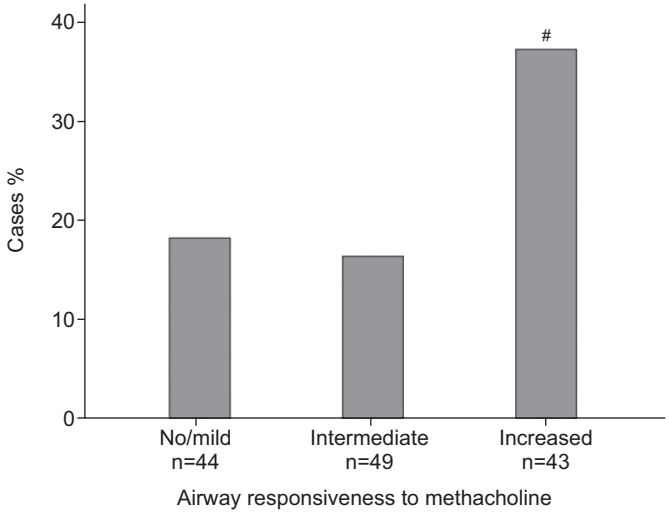

FIGURE 3. Cases of elevated exhaled nitric oxide fraction (F_{ENO}) (*i.e.* ⩾27 ppb) with regard to airway responsiveness. Increased airway responsiveness to methacholine (*i.e.* the provocative dose of methacholine causing a 40% fall in maximal expiratory flow at functional residual capacity (PD_{40} $V'_{max,FRC}$) ⩽0.30 mg) was associated with elevated F_{ENO} when compared with no/mild airway responsiveness to methacholine (*i.e.* PD_{40} $V'_{max,FRC}$ ⩾0.91 mg). #: p=0.047.

change the significant results obtained in the univariate analyses. Both the maternal history of asthma and increased airway responsiveness were found to be independently associated with elevated F_{ENO} (table 2).

DISCUSSION

In infants with recurrent lower respiratory tract symptoms, elevated F_{ENO} was associated with a maternal history of asthma, and with increased airway responsiveness to methacholine. In contrast, no associations were found between elevated F_{ENO} and age, height, sex, atopic manifestations, blood eosinophilia, paternal history of asthma or allergy, parentally reported respiratory symptoms, physician-confirmed wheeze, environmental exposures, or lung function.

TABLE 2 Baseline characteristics and factors with regard to elevated exhaled nitric oxide fraction (⩾27 ppb)

Parameter	OR (95% CI#)	p-value#
Maternal history of asthma	3.2 (1.3–8.1)	0.012
Atopy	0.4 (0.1–1.1)	0.064
Height	1.0 (1.0–1.1)	0.252
Airway responsiveness to methacholine		
Increased	4.1(1.4–12.7)	0.012
Intermediate	1.4 (0.4–4.7)	0.547
No/mild	1.0	

Results of the multivariate logistic regression analysis. OR: odds ratio; 95% CI: 95% confidence interval; #: adjusted for the maternal history of asthma, atopy, height, and airway responsiveness to methacholine.

In earlier studies, the relationship between increased airway responsiveness and F_{ENO} has been controversial in school children. As regards online F_{ENO} measurements, there are reports with no correlation between airway responsiveness and F_{ENO} values [23, 24], as well as reports on the association between F_{ENO} and increased airway responsiveness to methacholine [3], histamine [2], and acetylcholine chloride [4]. Studies on infants are scarce: in a study on the relationship of F_{ENO} and airway responsiveness in infants with eczema, there was a correlation between F_{ENO} and increased airway responsiveness to methacholine [9].

At pre-school age, F_{ENO} seemed superior to baseline respiratory function and bronchodilator responsiveness in identifying children with probable asthma in our earlier study [5]. In wheezy infants, anti-inflammatory therapy has been shown to reduce levels of F_{ENO} [25–27]. Whether F_{ENO} plays any role as a predictor of later childhood asthma in symptomatic infants is not known. In recently published studies, elevated F_{ENO} predicted decline in lung function in infants with recurrent wheezing [28], and risk of future wheezing both in healthy neonates [29] and wheezy infants [28], although no correlation was found between F_{ENO} and lung function measures at baseline [28, 29]. In line with recent findings, we could not find any association between baseline lung function and F_{ENO} levels. However, there was an association between increased airway responsiveness to methacholine and elevated F_{ENO} in infants with recurrent lower respiratory tract symptoms, regardless of atopy and height.

Parental asthma is a well-known risk factor for asthma in offspring [22], and reduced lung function has been found in children with prolonged breastfeeding with asthmatic and atopic mothers [30]. As well, the maternal history of asthma or atopy has been reported to modify F_{ENO} levels in certain selected cohorts of infants [9, 17], that is consistent with our finding on the association between elevated F_{ENO} and a maternal history of asthma. It has been speculated that the milk of asthmatic and/or atopic mothers may differ from the milk of nonasthmatic and/or nonatopic mothers with regard to immunologically active substances and affect the outcome [30]. However, the detailed mechanisms underlying the increased risk related to maternal asthma are still to be elucidated.

In adults and school children F_{ENO} has been proposed as a surrogate marker of eosinophilic airway inflammation [1], and there are reports on the association between elevated F_{ENO} and blood or sputum eosinophilia in school children [2, 3, 13, 31]. According to our previous findings, eosinophilic airway inflammation is rarely seen in infancy [6, 7]. However, during infancy the airways and lungs are in the process of growth and, among other factors, NO has various functions in the maturating lung [32]. In older subjects, changing expression of inducible NO synthase (NOS) is thought to explain exhaled NO variability, whereas in infancy, constitutional NO synthases may also contribute to exhaled NO concentrations [32]. In a recent article on normative data for exhaled NO in healthy infants [33], an upper limit for normal exhaled NO was determined as 26.1 ppb. In line with that, F_{ENO} level of $\geqslant$27 ppb was regarded elevated in symptomatic infants in the present study. However, we could not find a correlation between elevated F_{ENO} and blood eosinophilia, and we presume that in infants with recurrent lower respiratory tract symptoms, elevated F_{ENO} may rather reflect other aetiology than eosinophilic airway inflammation. In asthmatic subjects, certain viruses, *e.g.* rhinoviruses, have been found to increase production of exhaled NO by activating inducible NOS in the airways, and to induce airway hyperresponsiveness simultaneously [34]. Currently there are a lack of studies on the relationship of specific viral infections and F_{ENO} levels in infants. Nevertheless, it has been postulated that there might be a causal relationship between recurrent lower respiratory tract symptoms in infancy, development of increased airway responsiveness, and airways inflammation later in childhood [35].

The clinical usefulness of F_{ENO} in infants and older children has been hampered by several characteristics that have been pointed out to influence F_{ENO} values: *i.e.* used methods [10], height [2], presence of atopic eczema [11, 12], skin-test reactivity [2, 3, 7, 12, 13], acute respiratory symptoms [14–16], and tobacco smoke exposure [16–18]. In the present study, the used method for F_{ENO} measurement mimics the single breath online method used in older children and adults by standardising the between-subjects variation in tidal flow. However, the results are not directly comparable with those obtained by using the raised volume thoracoabdominal compression technique [10], due to different lung volumes at which the measurement occurs. The F_{ENO} levels may also be confounded with NO_{amb} [36], and in order to avoid that effect, cases with measurements of NO_{amb} $>$5 ppb were excluded from the analyses. To eliminate the effect of acute respiratory infection to F_{ENO} values, we excluded subjects with acute respiratory illness symptoms within the past 2 weeks from the final analyses. Because of avoiding performing methacholine challenge test in children with clinically evident bronchial obstruction, excluded children had more often abnormal results in baseline lung function measured by rapid thoracic compression technique. However, there were no differences in F_{ENO} levels between included and excluded children.

As the children in the study represented the child population that had been referred to a university hospital clinic because of the recurrent symptoms, there was no selection based on certain respiratory symptoms or severity of symptoms, rendering the study population heterogeneous. However, our goal was not to compare F_{ENO} in children representing different clinical entities, but to evaluate the association between F_{ENO} and airway responsiveness in symptomatic infants, and such a study design does not necessitate symptom-based selection. As the study subjects were selected by their recurrent lower respiratory tract symptoms, the result could have been different if the study subjects had been compared with non-selected healthy children. For ethical reasons, it was not possible to recruit healthy children for such extensive investigations requiring sedation, and consequently we needed to perform all analyses within the study group.

In conclusion, in infants with recurrent lower respiratory tract symptoms, elevated F_{ENO} values are related to increased airway responsiveness and to the maternal history of asthma. These findings may have implications in clinical practice when therapeutic measures are considered for infants with recurrent lower respiratory tract symptoms. In future, studies in symptomatic infants evaluating F_{ENO} as a predictor of later childhood asthma would clarify whether F_{ENO} will be suitable as a biomarker for monitoring early childhood wheezing illnesses.

SUPPORT STATEMENT

The study was supported by Helsinki University Central Hospital Research Funds, Sigrid Juselius Foundation, and Finnish Allergy Research Foundation.

STATEMENT OF INTEREST

A statement of interest for the study itself can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

ACKNOWLEDGEMENTS

We wish to thank registered nurses T. Rito and H. Punkari (both Dept of Allergology, Helsinki University Central Hospital, Helsinki, Finland) for their skill and care with the infants and their parents. The personnel of Spira Respiratory Care Center Ltd (Helsinki, Finland) is acknowledged for their help in the development of the inhalation-synchronised dosimetric equipment used in the methacholine challenge.

REFERENCES

1 Taylor DR, Pijnenburg MW, Smith AD, *et al.* Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006; 61: 817–827.
2 Franklin PJ, Turner SW, Le Souëf PN, *et al.* Exhaled nitric oxide and asthma: complex interactions between atopy, airway responsiveness, and symptoms in a community population of children. *Thorax* 2003; 58: 1048–1052.
3 Strunk RC, Szefler SJ, Phillips BR, *et al.* Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol* 2003; 112: 883–892.
4 Motomura C, Odajima H, Tezuka J, *et al.* Effect of age on relationship between exhaled nitric oxide and airway hyperresponsiveness in asthmatic children. *Chest* 2009; 136: 519–525.
5 Malmberg LP, Pelkonen AS, Haahtela T, *et al.* Exhaled nitric oxide rather than lung function distinguishes preschool children with probable asthma. *Thorax* 2003; 58: 494–499.
6 Saglani S, Malmström K, Pelkonen AS, *et al.* Airway remodelling and inflammation in symptomatic infants with reversible airflow obstruction. *Am J Respir Crit Care Med* 2005; 171: 722–727.
7 Malmberg LP, Malmström K, Kotaniemi-Syrjänen A, *et al.* Does tidal exhaled nitric oxide reflect mucosal airway inflammation in infants? *Thorax* 2010; 65: 1027.
8 Kotaniemi-Syrjänen A, Malmberg LP, Pelkonen AS, *et al.* Airway responsiveness: associated features in infants with recurrent respiratory symptoms. *Eur Respir J* 2007; 30: 1150–1157.
9 Tepper RS, Llapur CJ, Jones MH, *et al.* Expired nitric oxide and airway reactivity in infants at risk for asthma. *J Allergy Clin Immunol* 2008; 122: 760–765.
10 Franklin PJ, Turner SW, Mutch RC, *et al.* Comparison of single-breath and tidal breathing exhaled nitric oxide levels in infants. *Eur Respir J* 2004; 23: 369–372.
11 Dinakar C, Craff M, Laskowski D. Infants and toddlers without asthma with eczema have elevated exhaled nitric oxide levels. *J Allergy Clin Immunol* 2006; 117: 212–213.
12 Sonnappa S, Bastardo CM, Wade A, *et al.* Symptom-pattern phenotype and pulmonary function in preschool wheezers. *J Allergy Clin Immunol* 2010; 126: 519–526.
13 Thomas PS, Gibson PG, Wang H, *et al.* The relationship of exhaled nitric oxide to airway inflammation and responsiveness in children. *J Asthma* 2005; 42: 291–295.
14 Ratjen F, Kavuk I, Gärtig S, *et al.* Airway nitric oxide in infants with acute wheezy bronchitis. *Pediatr Allergy Immunol* 2000; 11: 230–235.
15 Franklin PJ, Turner SW, Hall GL, *et al.* Exhaled nitric oxide is reduced in infants with rhinorrhea. *Pediatr Pulmonol* 2005; 39: 117–119.
16 Gabriele C, Asgarali R, Jaddoe VW, *et al.* Smoke exposure, airway symptoms and exhaled nitric oxide in infants: the Generation R study. *Eur Respir J* 2008; 32: 307–313.
17 Frey U, Kuehni C, Roiha H, *et al.* Maternal atopic disease modifies effects of prenatal risk factors on exhaled nitric oxide in infants. *Am J Respir Crit Care Med* 2004; 170: 260–265.
18 Franklin PJ, Turner S, Mutch R, *et al.* Parental smoking increases exhaled nitric oxide in young children. *Eur Respir J* 2006; 28: 730–733.
19 Kotaniemi-Syrjänen A, Malmberg P, Malmström K, *et al.* Factors associated with elevated *F*eNO in infants with recurrent respiratory symptoms. *Eur Respir J* 2011; 38: Suppl. 55, 603s.
20 American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005; 171: 912–930.
21 Dreborg S, Frew A. Position paper: allergen standardization and skin tests. *Allergy* 1993; 48: Suppl. s14, 48–82.
22 Castro-Rodríguez JA, Holberg CJ, Wright AL, *et al.* A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med* 2000; 162: 1403–1406.
23 Silvestri M, Spallarossa D, Battistini E, *et al.* Dissociation between exhaled nitric oxide and hyperresponsiveness in children with mild intermittent asthma. *Thorax* 2000; 55: 484–488.
24 Miraglia del Giudice M, Brunese FP, Piacentini GL, *et al.* Fractional exhaled nitric oxide (*F*eNO), lung function and airway hyperresponsiveness in naïve atopic asthmatic children. *J Asthma* 2004; 41: 753–759.
25 Baraldi E, Dario C, Ongaro R, *et al.* Exhaled nitric oxide concentrations during treatment of wheezing exacerbation in infants and young children. *Am J Respir Crit Care Med* 1999; 159: 1284–1288.
26 Moeller A, Franklin P, Hall GL, *et al.* Inhaled fluticasone dipropionate decreases levels of nitric oxide in recurrently wheezy infants. *Pediatr Pulmonol* 2004; 38: 250–255.
27 Straub DA, Moeller A, Minocchieri, *et al.* The effect of montelukast on lung function and exhaled nitric oxide in infants with early childhood asthma. *Eur Respir J* 2005; 25: 289–294.
28 Debley JS, Stamey DC, Cochrane ES, *et al.* Exhaled nitric oxide, lung function, and exacerbations in wheezy infants and toddlers. *J Allergy Clin Immunol* 2010; 125: 1228–1234.
29 Chawes BLK, Buchvald F, Bischoff AL, *et al.* Elevated exhaled nitric oxide in high-risk neonates precedes transient early but not persistent wheeze. *Am J Respir Crit Care Med* 2010; 182: 138–142.
30 Guilbert TW, Stern DA, Morgan WJ, *et al.* Effect of breastfeeding on lung function in childhood and modulation by maternal asthma and atopy. *Am J Respir Crit Care Med* 2007; 176: 843–848.
31 Steerenberg PA, Janssen NAH, de Meer G, *et al.* Relationship between exhaled NO, respiratory symptoms, lung function, bronchial hyperresponsiveness., and blood eosinophilia in school children. *Thorax* 2003; 58: 242–245.
32 Singer F, Latzin P. Exhaled nitric oxide in infants: a marker of inflammation? *Eur Respir Monogr* 2010; 49: 56–70.
33 Fuchs O, Latzin P, Thamrin C, *et al.* Normative data for lung function and exhaled nitric oxide in unsedated healthy infants. *Eur Respir J* 2011; 37: 1208–1216.
34 de Gouw HWFM, Grünberg K, Schot R, *et al.* Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. *Eur Respir J* 1998; 11: 126–132.
35 Saglani S, Payne DN, Zhu J, *et al.* Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 2007; 176: 858–864.
36 Baraldi E, de Jongste JC. Measurement of exhaled nitric oxide in children, 2001. *Eur Respir J* 2002; 20: 223–237.