

Exhaled nitric oxide distinguishes between subgroups of preschool children with respiratory symptoms

Alexander Moeller, MD,^a Corinne Diefenbacher, MD,^a Andrea Lehmann, MD,^a Mascha Rochat, MD,^a Joanne Brooks-Wildhaber, MPH,^b Graham L. Hall, PhD,^c and Johannes H. Wildhaber, MD, PhD^a *Zurich and Davos, Switzerland, and Perth, Australia*

Background: Respiratory symptoms are common in early childhood. The clinical characterization of disease presentation and hence its likely disease progression has so far been proven difficult.

Objective: To investigate whether exhaled nitric oxide (NO) could be helpful to distinguish between subgroups of nonwheezy and wheezy young children less than 4 years of age.

Methods: Exhaled NO was measured in 391 children (age 3-47 months) with nonwheezy and wheezy respiratory symptoms. Children were divided into 3 groups: children with recurrent cough but no history of wheeze (group 1), with early recurrent wheeze and a loose index for the prediction of asthma at school age (group 2), and with frequent recurrent wheeze and a stringent index for the prediction of asthma at school age (group 3).

Results: Children from group 3 showed significantly higher median (interquartile range) fractional exhaled NO (FeNO) levels (11.7 [11.85]) than children from groups 1 (6.5 [5.5]; $P < .001$) and 2 (6.4 [6.5]; $P < .001$). No difference in FeNO levels was found between children from groups 1 and 2 ($P = .91$).

Conclusion: Wheezy young children less than 4 years of age with a stringent index for the prediction of asthma at school age have elevated levels of FeNO compared with children with recurrent wheeze and a loose index for the prediction of asthma at school age or children with recurrent cough.

(*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Exhaled nitric oxide, early childhood asthma, infants, young children

Respiratory symptoms, such as cough and wheeze, are common in young children with as many as 30% of children affected, mainly during a viral lower respiratory tract infection.^{1,2} The Tucson Children's Respiratory Study determined that risk factors

Abbreviations used

AR: Airway responsiveness
FeNO: Fractional exhaled nitric oxide
ICS: Inhaled corticosteroid
NO: Nitric oxide

such as not being breast-fed, sharing a room, having a young mother or a mother with low education, and being exposed to evaporative coolers or tobacco smoke all contribute to the incidence of lower respiratory tract infections.^{1,3-9}

The prime focus of the Tucson Children's Respiratory Study longitudinal study was to investigate factors that may be predictive of which children go on to develop chronic respiratory disease, mainly asthma, in later life. The study demonstrated that environmental, socioeconomic, genetic, physiological, and immunologic factors are predictive for the development of asthma in later life.³ These findings have recently been used to define a clinical algorithm to assist in predicting the development of asthma.^{1,10}

The addition of objective measures to this algorithm may improve our ability to define which child with respiratory symptoms is likely to develop asthma in later childhood. Reversible airway obstruction and bronchial hyperresponsiveness as well as airway inflammation are key pathophysiological mechanisms of asthma.¹¹ The assessment of respiratory function can be achieved noninvasively with routine standardized measurements. However, although advances have been made in the development of objective measurement of respiratory function (airway obstruction and bronchial hyperresponsiveness), these have yet to achieve widespread use in clinical practice.¹² Characterization of airway inflammation in patients suspected of having asthma would likely be of benefit. Some methods, such as bronchial biopsies and bronchoalveolar lavage for the assessment of airway inflammation, are too invasive for the use in small children, whereas the measurement of fractional exhaled nitric oxide (FeNO) has been proven to be noninvasive and suitable for the assessment of airway inflammation, even in infants.¹³ FeNO has been shown to be helpful in the assessment of asthma in older children, mainly in monitoring therapy, in the way that it measures compliance with treatment and in predicting exacerbations as well as the course of asthma.¹⁴⁻¹⁶ Airway inflammation is recognized as an early component of asthma and may even precede other physiological changes.¹⁷ Therefore, it could be hypothesized that differences in FeNO between subgroups of young children less than 4 years of age with nonwheezy and wheezy respiratory symptoms may be present. Hence, the objective measurement of FeNO in

From ^athe Swiss Pediatric Respiratory Research Group and Division of Respiratory Medicine, University Children's Hospital Zurich; ^bthe Alpine Children's Hospital, Davos; and ^cthe Department of Respiratory Medicine, Princess Margaret Hospital for Children and School of Pediatrics and Child Health, University of Western Australia.

Supported by an unrestricted grant by GlaxoSmithKline Switzerland.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication May 6, 2007; revised October 30, 2007; accepted for publication November 1, 2007.

Reprint requests: Alexander Moeller, MD, Division of Respiratory Medicine, University Children's Hospital, Steinwiesstrasse 75, CH-8032 Zurich, Switzerland. E-mail: alexander.moeller@kispi.unizh.ch.

0091-6749/\$34.00

© 2008 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2007.11.008

addition to the clinical characterization using an algorithm may improve the possibility to define disease presentation and to predict disease progression.

Therefore, the aim of our study was to investigate whether FeNO differentiates between subgroups of young children with nonwheezy or wheezy respiratory symptoms and to assess whether FeNO allows distinction between wheezy children categorized by the clinical algorithm into recurrently wheezy children with a loose index or a stringent index for the prediction of asthma at school age.

METHODS

Study population

This is a prospective cohort study with 391 young children less than 4 years of age consecutively undergoing assessment for respiratory symptoms at the University Children's Hospital Zürich, Switzerland, between 2000 and 2004. Exclusion criteria were a diagnosis of a specific respiratory disease such as cystic fibrosis, primary ciliary dyskinesia, interstitial lung disease, pneumonia, or tuberculosis and/or current upper airway infection or inability to obtain an appropriate FeNO measurement. The study was approved by the University Children's Hospital ethics committee. Written informed consent was obtained from all parents.

Procedures

Children were allocated into 3 diagnostic groups on the basis of history and clinical and laboratory findings. Group 1 consisted of children with chronic persistent or recurrent cough but no history of wheeze. Groups 2 and 3 included young children with a history of wheeze. Wheezy children were classified according to a previously published clinical index that defines the risk of asthma in young children with wheeze: group 2, loose index; group 3, stringent index¹⁰ (Table I).

Exhaled nitric oxide (NO) was measured according to American Thoracic Society/European Respiratory Society¹³ recommendations using an offline-reservoir technique that has been described previously.¹⁸ In brief, children were seated on the legs of the mother and breathed through a face mask that was gently placed on the child's face. The face mask was attached to a 2-way valve that allowed the inspiration of NO-free air from a reservoir to prevent contamination with ambient air. After 10 breaths of NO-free air, a reusable inert 750-mL collection bag (Quinton, Milwaukee, Wis) was attached to the expiratory side of the valve, and 5 breaths were collected. Nasal contamination was prevented by closing the velum by exhaling against 5 cm H₂O oral pressure, achieved by a resistor between the collection bag and the expiratory side of the valve. If the child was not collaborating (crying, irregular breathing, leak), the collection was repeated after several minutes. If more than 3 attempts did not allow FeNO collection as recommended, the child was not included in the study. The NO concentration in the bags was analyzed within 15 minutes of collection by using a fast-response chemoluminescence analyzer (CLD 77 AM; Eco Medics, Duernten, Switzerland) at a sampling flow of 100 mL/min.

Allergy testing was performed from a blood sample by RAST (Unicap; Pharmacia, Duebendorf, Switzerland), and the child was considered allergic by a RAST class ≥ 2 (≥ 0.7 kU/L) for at least 1 of the tested inhaled or food allergen. Peripheral blood eosinophils were determined in a venous blood sample by a fully automated blood cell counter (SE 9000; Sysmex, Digitana, Horgen, Switzerland) and expressed as the percentage of total leukocyte counts.

Statistical analysis

Results were expressed as mean (SD) or, if not normally distributed or in the case of categorical variables, as median (interquartile range). Levels of FeNO were logarithmically (base₁₀) transformed because of skewed distribution.

Differences in FeNO and clinical and anthropometric data between groups were assessed with 1-way ANOVA for normally distributed data or Kruskal-Wallis 1-way ANOVA on ranks for nonnormally distributed data. *Post hoc* comparisons were tested with the Tukey test. Multiple linear regression and backward stepwise regression were used to test for significant associations between FeNO in groups 1, 2, and 3 and factors known to influence FeNO levels

TABLE I. Definition of diagnostic groups: Clinical index to define the risk of asthma in young children¹⁰

Major criteria	Minor criteria
1. Parental physician-diagnosed asthma	1. Physician diagnosis of allergic rhinitis
2. Physician diagnosis of atopic dermatitis	2. Wheezing apart from colds
	3. Eosinophilia ($\geq 4\%$)
Group 1	
Children with chronic persistent or recurrent cough but no history of wheeze	
Group 2	
"Loose index for the prediction of asthma at school age" ¹⁰ : children with nonfrequent recurrent wheeze, <3 episodes/y, and 1 of the major criteria or 2 of the 3 minor criteria	
Group 3	
"Stringent index for the prediction of asthma at school age" ¹⁰ : children with frequent recurrent wheeze, ≥ 3 episodes/y, and at least 1 major or 2 minor criteria	

(age, sex, allergic sensitization, parental smoking, and the use of inhaled corticosteroids). A *P* value of $<.05$ was considered significant. All analyses were performed by using SigmaStat for Windows 2.03 (SPSS Inc, Chicago, Ill).

RESULTS

Group 1 (children with cough) consisted of more girls ($P = .023$) and older children ($P < .026$) compared with groups 2 and 3. Allergic sensitization as assessed by RAST was more frequent in group 3 compared with groups 1 and 2. No differences between groups were noted for percentage of blood eosinophils or prenatal or postnatal passive smoke exposure. Less than 1/3 of the children were on current inhaled corticosteroid (ICS) treatment at the time of FeNO measurement, significantly more in groups 2 and 3 than in group 1 (Table II).

The overall median FeNO value (interquartile range) was 7.5 ppb (8.7). Children from group 3 had significantly higher FeNO levels compared with group 1 ($P = .001$) and group 2 ($P < .001$), whereas children from group 1 and group 2 showed similar FeNO levels ($P = .49$; Table II; Fig 1). In neither group 2 nor group 3 was there a significant correlation between FeNO and number of wheezing episodes ($r = 0.16$, $P = .073$ for group 2 and $r = -0.11$, $P = .32$ for group 3, respectively). When FeNO and the number of wheezy episodes were analyzed for both groups together (group 2 and 3 merged), there was a significant but weak correlation ($r = 0.2$; $P = .01$) between the number of wheezy episodes and FeNO. Regression analysis revealed that phenotype as defined by the disease groups 1 to 3 was the only significant factor to influence FeNO levels, with none of the investigated demographic or clinical variables (age, sex, number of wheeze episodes, passive smoke exposure, allergic sensitization, blood eosinophil count, and treatment (ICS and or leukotriene receptor antagonists) influencing FeNO. However, when the different subgroups were analyzed separately according to their treatment with or without ICS, we found higher FeNO levels in ICS-naïve and ICS-treated children from group 3 with allergic sensitization as defined by a positive RAST test (14.2 [16.95] ppb and 7.3 [8.45] ppb, respectively; $P = .017$). In all other children (groups 1 and 2 and children from group 3 without allergic sensitization), FeNO levels were similar in both children receiving ICS treatment and steroid-naïve children ($P = .385$, $P = .584$, and $P = .27$, respectively). Further, when the different subgroups were

TABLE II. Patient characteristics for the subgroups

	Group 1	Group 2	Group 3	<i>P</i> value	Test <i>post hoc P</i> value**
Number	80	190	121		
Sex: female/male	42/38	66/124	46/75	.023†	<i>P</i> _{1,2} = .01 <i>P</i> _{1,3} = .45 <i>P</i> _{2,3} = .071
Age (m)*	30 (25.5)	21 (27)	23 (18.5)	.047	<i>P</i> _{1,2} = .024 <i>P</i> _{1,3} = .026 <i>P</i> _{2,3} = .76
History of hay fever	10%	16.5%	24.8%	.021†	<i>P</i> _{1,2} = .25 <i>P</i> _{1,3} = .015 <i>P</i> _{2,3} = .091
History of food allergy	3.8%	4.2%	14.9%	<.001†	<i>P</i> _{1,2} = .05 <i>P</i> _{1,3} = .022 <i>P</i> _{2,3} = .002
History of atopic eczema	6.3%	9.5%	20.7%	.002†	<i>P</i> _{1,2} = .53 <i>P</i> _{1,3} = .009 <i>P</i> _{2,3} = .009
Allergic sensitization	10%	12.6%	33.4%	<.001†	<i>P</i> _{1,2} = .065 <i>P</i> _{1,3} < .001 <i>P</i> _{2,3} < .001
Percentage of eosinophils‡	2.7 ± 2.08	2.7 ± 1.79	3.82 ± 2.9	.075	
Passive smoke exposure	23.75%	21.05%	25.62%	.63†	
ICS therapy	10%	33.7%	40.5%	<.001†	<i>P</i> _{1,2} < .001 <i>P</i> _{1,3} < .001 <i>P</i> _{2,3} = .18
Leukotriene receptor antagonist therapy	1.25%	4.4%	7.4%	.115†	
β-Agonist use	17.5%	25.3%	29.8%	.38†	
FeNO*	6.5 (5.55)	6.4 (6.5)	11.65 (11.85)	<.001	<i>P</i> _{1,2} = .49 <i>P</i> _{1,3} = .001 <i>P</i> _{2,3} < .001

*Median (interquartile range).

† χ^2 Analysis.

‡Mean ± SD.

analyzed separately, parental-reported passive smoke exposure did not influence the FeNO levels.

DISCUSSION

We have shown that the measurement of exhaled NO differentiates between subgroups of young children less than 4 years of age with wheezy and nonwheezy respiratory symptoms. Children with frequent recurrent wheeze and a stringent asthma predictive index¹⁰ (group 3) showed significantly higher FeNO values than children with nonfrequent recurrent wheeze and a loose asthma predictive index (group 2) or nonwheezy children with chronic or recurrent cough (group 1). Although there was an overlap in the distribution of FeNO values among the 3 groups, our results suggest that wheezy children with a higher risk of persistent asthma at the age of 6 years have higher levels of FeNO, representing airway inflammation, in their first 4 years of life.

Although it is known from previous studies that sex,¹⁹ age,²⁰ and exposure to passive smoke^{21,22} alter FeNO levels in healthy children and children with asthma, the strongest predictor for FeNO levels in our cohort was the phenotype as defined by the 3 disease groups. The lack of the influence of the findings from the allergy tests is most likely to be explained by the fact that the definition of the disease groups used in this study already includes atopy status.¹⁰ The finding is in agreement with results from a recent population-based study in preschool children.²³ When taking into account age, sex, and allergic sensitization,

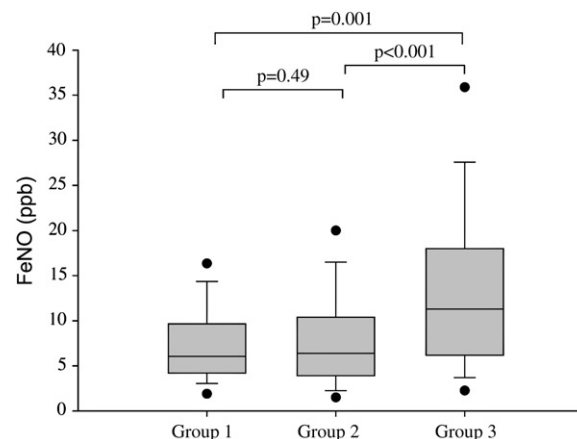


FIG 1. Levels of exhaled NO in infants and children with nonwheezy or wheezy respiratory symptoms. The median is the line bisecting the box, the box limits represent 25th and 75th percentiles, and whiskers extend to the 10th and 90th percentile. The black dots represent outliers.

the use of ICS at the time of FeNO measurement did not influence the results to a significant degree. This finding may be surprising because it is well known that ICS treatment reduces FeNO levels in a dose-dependent manner in patients with asthma.²⁴⁻²⁷ However, this is not the case in all children with asthma.²⁸⁻³⁰ In addition, there is evidence of lack of clinical response to inhaled

steroids in children with transient wheezing.^{3,31} On the other hand, regular ICS treatment reduces wheezing episodes and days on additional asthma medication in atopic and persistent wheezers.³² When children from our study were analyzed for whether they showed allergic sensitization as defined by a positive RAST test, we found significant differences between FeNO levels in children from group 3 with allergic sensitization with or without ICS treatment. This was not the case in the other groups (groups 1 and 2 and children from group 3 without allergic sensitization). This result is in agreement with the findings of Guilbert et al³² and an epidemiologic study in 4-year-old children.²³ FeNO is related to underlying mechanisms of both atopy and airway responsiveness (AR),³³ and it may be hypothesized that AR is the more relevant mechanism in young children.³⁴ FeNO might therefore be less reactive to the ICS doses used in these children.²⁸ In addition, a confounding factor may be the lack of cooperation with inhalation therapy in this age group, which plays a major role in asthma control.³⁵

The task Force of the American Thoracic Society and ERS published updated recommendations on the measurement of exhaled NO in 2005.¹³ Despite the documented usefulness of measuring FeNO in older children,^{14-17,29} surprisingly few studies have been performed in infants and preschool children. Both online and offline measurements of FeNO have been used in this age group without sedation^{19,36,37} and with sedation.³⁸ According to the recommendations, we have measured FeNO with an offline method during quiet, regular breathing in children inhaling NO-free air.¹³ In our study, we included children from 5 to 47 months. Age was not a significant factor influencing FeNO levels in the regression analysis, taking into account all demographic or clinical variables. However, in a separate analysis, we looked at subgroups of children less than 12 months and children from 12 to 47 months. We could show that, whereas in the older children the results were highly statistically significant ($P < .001$), the differences were less powerful in the children younger than 12 months ($P = .016$), and the difference between FeNO levels of children from groups 2 and 3 were not significant ($P = .107$). This finding may be explained by the heterogeneity of wheezing disorders in very young children, the technical limitation of FeNO measurements in this age group, or the evolution of airway inflammation in the first years of life.

The ability of FeNO to differentiate between various groups of young children with respiratory symptoms is in accordance with findings from other studies in preschool children. In 113 children 4 to 14 years of age, FeNO was significantly lower in children without asthma compared with children with asthma. Children with asthma treated with inhaled steroids had lower values than steroid-naïve patients.³⁹ A study in 4-year-olds found higher values of FeNO in children with doctor-diagnosed asthma compared with those without.²³ However, this study could not distinguish between various wheezing phenotypes. In another study, FeNO differentiated steroid-naïve young children with intermittent asthma from healthy children, children without asthma with chronic cough, and children with asthma treated with inhaled steroids.⁴⁰ In infants, it was shown that first-time wheeze is associated with lower FeNO compared with levels in recurrent wheeze.¹⁸ By using a similar technique in sedated children with different airway diseases and nonsedated healthy controls, Gabriele et al⁴¹ showed elevated FeNO levels in atopic wheezing infants age 4.6 to 25.2 months compared with healthy controls and infants with cystic fibrosis or bronchopulmonary dysplasia. Exhaled NO increased during an acute wheezy episode and decreased rapidly

with anti-inflammatory therapy using inhaled steroids. Levels of FeNO in infants are already influenced by intrauterine factors, such as tobacco smoke exposure, which is also associated with an increased risk of wheeze in early childhood, but not with persistent asthma in later childhood.²¹ Exhaled NO has been proven to represent a reliable noninvasive measure for airway inflammation. A close correlation of FeNO and direct and indirect measures of airway inflammation (bronchial biopsies,^{42,43} bronchoalveolar lavages,⁴⁴ and sputum⁴⁵) was found. However, it has also been shown that the relationship between FeNO and asthma may be more complex⁴⁶: FeNO was raised in children with a combination of both atopy and increased AR, independent of symptoms; however, FeNO was not raised in children with only atopy or increased AR. The importance of the measurement of airway inflammation in young children with respiratory symptoms has been proven, because FeNO has been found to be superior to baseline respiratory function and bronchodilator responsiveness in identifying preschool children with probable asthma.³³ According to the asthma predictive index,¹⁰ many of the children from group 3 differed only by the number of wheezy episodes from children of group 2. Therefore, the elevated FeNO levels may also represent greater disease activity and/or severity. With the data we present, we are not able to distinguish between current disease activity, hence actual airway inflammation, and a more general elevated expression of NO synthases within the airway epithelial cells that might be at least hypothetically related to an elevated risk for the persistence of asthma at school age. We found a weak but significant correlation between the number of wheezy episodes and FeNO levels. This may suggest that FeNO reflects disease activity, rather than a general difference between disease groups. When the 2 wheezy groups (groups 2 and 3) were analyzed separately, this correlation disappeared, and the number of wheeze episodes was not relevant in the regression analysis. ICSs are widely used to treat persistent cough and wheeze in young children. The indication for ICS treatment is mostly empirical. The clinical dilemma is to differentiate children with transient nonallergic wheeze from those children who will go on to develop persistent asthma. Treatment with ICS has been shown to fail in the prevention of exacerbations in young children with transient wheeze.^{3,31} On the other hand, regular ICS treatment reduces wheezy episodes and days on additional asthma medication in atopic and persistent wheezers.³² However, ICS treatment does not influence the natural course of disease.³² It is therefore interesting from not only an epidemiologic but also a clinical point of view to be able to distinguish between subgroups of infants and young children with wheezy and nonwheezy respiratory symptoms, and hence to define which child is likely to respond to ICSs.

The results of this large study suggest that the measurement of FeNO serves as an additional parameter for the differentiation between subgroups of children younger than 4 years of age with wheezy and nonwheezy respiratory symptoms. Children with a stringent index for the prediction of asthma at school age showed higher FeNO values than those with a loose index for the prediction of asthma at school age or children with recurrent or chronic cough.

To determine what effect measuring FeNO has on determining subsequent asthma risk in relation to the asthma predictive index and hence to set a cutoff value for FeNO levels and to calculate sensitivity, specificity, and predictive values, the children have to be reassessed at school age. To date, no single parameter in young children with respiratory symptoms has been shown to be predictive for the development of asthma later on. At this stage,

it is likely that an assessment based on clinical history and examination in addition to objective tests such as the measurement of FeNO is needed for a reliable prediction for asthma in later life.

Clinical implications: Our results suggest that the measurement of FeNO in young children serves as an additional parameter for the differentiation between subgroups of preschool children with respiratory symptoms.

REFERENCES

- Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;332:133-8.
- Trindade JC. The importance of diagnosis of allergy in early wheezing. *Pediatr Allergy Immunol* 1998;9:23-9.
- Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson Children's Respiratory Study: 1980 to present. *J Allergy Clin Immunol* 2003;111:661-75.
- Wright AL, Holberg CJ, Martinez FD, Morgan WJ, Taussig LM, Bean J, et al. Breast feeding and lower respiratory tract illness in the first year of life. *Br Med J (Clin Res Ed)* 1989;299:946-9.
- Holberg CJ, Wright AL, Martinez FD, Ray CG, Taussig LM, Lebowitz MD. Risk factors for respiratory syncytial virus-associated lower respiratory illnesses in the first year of life. *Am J Epidemiol* 1991;133:1135-51.
- Martinez FD, Wright AL, Holberg CJ, Morgan WJ, Taussig LM. Maternal age as a risk factor for wheezing lower respiratory illnesses in the first year of life. *Am J Epidemiol* 1992;136:1258-68.
- Aldous MB, Holberg CJ, Wright AL, Martinez FD, Taussig LM, Bean J, et al. Evaporative cooling and other home factors and lower respiratory tract illness during the first year of life. *Am J Epidemiol* 1996;143:423-30.
- Holberg CJ, Wright AL, Martinez FD, Morgan WJ, Taussig LM, Bean J, et al. Child day care, smoking by caregivers, and lower respiratory tract illness in the first 3 years of life. *Pediatrics* 1993;9:885-92.
- Wright AL, Holberg C, Martinez FD, Taussig LM. Relationship of parental smoking to wheezing and nonwheezing lower respiratory tract illnesses in infancy. *J Pediatrics* 1991;118:207-14.
- Castro-Rodriguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med* 2000;162:1403-6.
- Anonymous. Statement of the American Thoracic Society on standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-44.
- Stocks J. Clinical implications of pulmonary function testing in preschool children. *Paediatr Respir Rev* 2006;7(suppl 1):S26-9.
- ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am J Respir Crit Care Med* 2005;171:912-30.
- Griese M, Koch M, Latzin P, Beck J. Asthma severity, recommended changes of inhaled therapy and exhaled nitric oxide in children: a prospective, blinded trial. *Eur J Med Res* 2000;18:334-40.
- Zacharasiewicz A, Wilson N, Lex C, Erin EM, Li AM, Hansel T, et al. Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children. *Am J Respir Crit Care Med* 2005;171:1077-82.
- Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. *Am J Respir Crit Care Med* 2005;172:831-6.
- Rytala P, Metso T, Heikkinen K, Saarelainen P, Helenius I, Haahtela T. Airway inflammation in patients with symptoms suggesting asthma but with normal lung function. *Eur Respir J* 2000;16:824-30.
- Baraldi E, Dario C, Ongaro R, Scollo M, Azzolin NM, Panza N, et al. Exhaled nitric oxide concentrations during treatment of wheezing exacerbation in infants and young children. *Am J Respir Crit Care Med* 1999;159:1284-8.
- Nordvall SL, Janson C, Kalm-Stephens P, Foucard T, Toren K, Alving K. Exhaled nitric oxide in a population-based study of asthma and allergy in schoolchildren. *Allergy* 2005;60:469-75.
- Malmberg LP, Petays T, Haahtela T, Laatikainen T, Jousilahti P, Vartiainen E, et al. Exhaled nitric oxide in healthy nonatopic school-age children: determinants and height-adjusted reference values. *Pediatr Pulmonol* 2006;41:635-42.
- Hall GL, Reinmann B, Wildhaber JH, Frey U. Tidal exhaled nitric oxide in healthy, unsedated newborn infants with prenatal tobacco exposure. *J Appl Physiol* 2002;92:59-66.
- Franklin PJ, Turner S, Mutch R, Stick SM. Parental smoking increases exhaled nitric oxide in young children. *Eur Respir J* 2006;28:730-3.
- Brussee JE, Smit HA, Kerkhof M, Koopman LP, Wijga AH, Postma DS, et al. Exhaled nitric oxide in 4-year-old children: relationship with asthma and atopy. *Eur Respir J* 2005;25:455-61.
- Yates D, Kharitonov S, Robbins R, Thomas P, Barnes P. Effect of a nitric oxide synthase inhibitor and a glucocorticosteroid on exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;152:892-6.
- Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1996;153:454-7.
- Tsai YG, Lee MY, Yang KD, Chu DM, Yuh YS, Hung CH. A single dose of nebulized budesonide decreases exhaled nitric oxide in children with acute asthma. *J Pediatr* 2001;139:433-7.
- Piacentini GL, Bodini A, Costella S, Suzuki Y, Zerman L, Peterson CGB, et al. Exhaled nitric oxide, serum ECP and airway responsiveness in mild asthmatic children. *Eur Respir J* 2000;15:839-43.
- Jatakanon A, Kharitonov S, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. *Thorax* 1999;54:108-14.
- Pijnenburg MWH, Bakker EM, Lever S, Hop WC, De Jongste JC. High fractional concentration of nitric oxide in exhaled air despite steroid treatment in asthmatic children. *Clin Exp Allergy* 2005;35:920-5.
- Buchvald F, Eiberg H, Bisgaard H. Heterogeneity of FeNO response to inhaled steroid in asthmatic children. *Clin Exp Allergy* 2003;33:1735-40.
- Bisgaard H, Hermansen MN, Loland L, Halkjaer LB, Buchvald F. Intermittent inhaled corticosteroids in infants with episodic wheezing. *N Engl J Med* 2006;354:1998-2005.
- Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefer SJ, et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. *N Engl J Med* 2006;354:1985-97.
- Franklin PJ, Turner SW, Le Souef PN, Stick SM. Exhaled nitric oxide and asthma: complex interactions between atopy, airway responsiveness, and symptoms in a community population of children. *Thorax* 2003;58:1048-52.
- Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, Lesouef PN. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. *Am J Respir Crit Care Med* 2001;163:37-42.
- Schuepp KG, Straub D, Moller A, Wildhaber JH. Deposition of aerosols in infants and children. *J Aerosol Med* 2004;17:153-6.
- Artlich A, Jónsson B, Bhiladvala M, Lönnqvist P, Gustafsson L. Single breath analysis of endogenous nitric oxide in the newborn. *Biol Neonate* 2001;79:21-6.
- Ratjen F, Kavuk I, Gartig S, Wiesemann HG, Grasemann H. Airway nitric oxide in infants with acute wheezy bronchitis. *Pediatr Allergy Immunol* 2000;11:230-5.
- Moeller A, Franklin P, Hall GL, Turner S, Straub D, Wildhaber JH, et al. Inhaled fluticasone dipropionate decreases levels of nitric oxide in recurrently wheezy infants. *Pediatr Pulmonol* 2004;38:250-5.
- Visser MJ, de Wit M-CY, van Aalderen WMC, Postma DS, Brand PLP. Exhaled nitric oxide in children measured by tidal breathing method: differences between asthmatics and nonasthmatic controls. *Pediatr Pulmonol* 2000;29:434-7.
- Avital A, Uwyied K, Berkman N, Godfrey S, Bar-Yishay E, Springer C. Exhaled nitric oxide and asthma in young children. *Pediatr Pulmonol* 2001;32:308-13.
- Gabriele C, Nieuwhof EM, Van der Wiel EC, Hofhuis W, Moll HA, Merkus PJFM, et al. Exhaled nitric oxide differentiates airway diseases in the first two years of life. *Pediatr Research* 2006;60:461-5.
- Lim S, Jatakanon A, Meah S, Oates T, Chung KF, Barnes PJ. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in mild to moderately severe asthma. *Thorax* 2000;55:184-8.
- Payne DNR, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001;164:1376-81.
- Warke TJ, Fitch PS, Brown V, Taylor R, Lyons JD, Ennis M, et al. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. *Thorax* 2002;57:383-7.
- Piacentini GL, Bodini A, Costella S, Vicentini L, Mazzi P, Sperandio S, et al. Exhaled nitric oxide and sputum eosinophil markers of inflammation in asthmatic children. *Eur Respir J* 1999;13:1386-90.
- Malmberg LP, Pelkonen AS, Haahtela T, Turpeinen M. Exhaled nitric oxide rather than lung function distinguishes preschool children with probable asthma. *Thorax* 2003;58:494-9.