

Low Exhaled Nitric Oxide in School-Age Children with Bronchopulmonary Dysplasia and Airflow Limitation

Eugenio Baraldi, Gea Bonetto, Franco Zacchello, and Marco Filippone

Department of Pediatrics, School of Medicine, University of Padova, Padova, Italy

Bronchopulmonary dysplasia (BPD), the chronic lung disease of prematurity, may be associated with long-term airflow limitation. Survivors of BPD may develop asthma-like symptoms in childhood, with a variable response to β_2 -agonists. However, the pathologic pathways underlying these respiratory manifestations are still unknown. The aim of this study was to measure exhaled nitric oxide (F_{ENO}) and lung function in a group of 31 school-age survivors of BPD. They showed variable degrees of airflow obstruction (mean FEV_1 $77.8 \pm 2.3\%$ predicted) unresponsive to β_2 -agonists in 72% of the subjects. Their F_{ENO} values (geometric mean [95% confidence interval]: $7.7 [\pm 1.1]$ ppb) were significantly lower than in a group of healthy matched control subjects born at term ($10.7 [\pm 1.1]$ ppb, $p < 0.05$) and a group of preterm children without BPD ($9.9 [\pm 1.1]$ ppb, $p < 0.05$). The children with BPD were also compared with a group of 31 patients with asthma with a comparable airflow limitation (FEV_1 $80.2 \pm 2.1\%$ predicted) and showed F_{ENO} values four times lower than in those with asthma ($24.9 [\pm 1.2]$ ppb, $p < 0.001$). In conclusion, unlike children with asthma, school-age survivors of BPD have airflow limitation associated with low F_{ENO} values and lack of reversibility to β_2 -agonists, probably as a result of mechanisms related to early life structural changes in the airways.

Keywords: airway remodeling; asthma; bronchopulmonary dysplasia; exhaled nitric oxide; flow limitation

Bronchopulmonary dysplasia (BPD) was first described in premature neonates surviving respiratory distress syndrome, as a consequence of chronic lung injury induced by mechanical ventilation and exposure to high oxygen concentrations (1). Several decades later, BPD still represents a major problem in neonatal medicine despite remarkable advances in the preventive care, treatment, and monitoring of premature babies. Different stimuli acting at different postmenstrual ages may give rise to different forms of BPD, pathologically ranging from the classic pattern of pulmonary fibrosis and extensive airway remodeling to the alveolar simplification typical of "new BPD" (2–5). In most cases, however, regardless of the etiologic pathway, there is an early intense inflammatory response that persists over the first weeks (2, 3). This early event may have far-reaching consequences, extending into childhood and beyond (6). Several studies have reported significant bronchial obstruction and airway hyperresponsiveness in subjects with BPD during their childhood (6–11) and early adulthood (12). Unfortunately, the mechanisms behind these abnormalities are still not entirely understood, as the airway pathology of BPD beyond infancy has not been investigated, and no studies exist on bronchial biopsies in children with BPD. Survivors of BPD often behave like children with asthma, with recurrent wheezing, shortness of breath, and airflow limitation, and they are treated with β_2 -agonists and glucocorticoids (3, 13).

However, although eosinophil-mediated epithelial damage is a major mechanism of respiratory impairment in asthma (14), only scanty information is available on the nature and pathogenetic relevance of airway inflammation in survivors of BPD beyond infancy.

The recent development of noninvasive methods for assessing airway inflammation, for example, exhaled nitric oxide (F_{ENO}) measurement and exhaled breath condensate analysis, has greatly increased our understanding of the pathogenesis of several lung diseases (15). F_{ENO} is the most extensively studied marker. It is thought to reflect airway inflammation in asthma (15–18) and has recently been extended to the noninvasive assessment of disease activity in several other lung diseases, such as cystic fibrosis and chronic obstructive pulmonary disease (15). The aim of this study was to evaluate F_{ENO} and lung function indices at school age in a cohort of survivors of BPD, comparing them with a group of matched healthy children and a group of preterm children without BPD. As control subjects with a similar degree of airflow limitation, we also included a group of children with asthma matched for airway function.

METHODS

Subjects

Children with BPD. Thirty-one school-age children with BPD were recruited to take part in the study protocol from a population of subjects born at gestational ages under 31 weeks and with birth weights less than 2,000 g, admitted to the Neonatal Intensive Care Unit at the Department of Pediatrics in Padova between January 1990 and December 1994. To select a homogeneous group of children with BPD, we considered only patients still needing mechanical ventilation 7 days after birth because of severe neonatal respiratory failure. Patients with major congenital anomalies or suspected lung hypoplasia were excluded. BPD was defined as clinical signs of respiratory distress, chest radiograph abnormalities, and oxygen dependence at 28 days of life (1). Pertinent neonatal data and the characteristics of the subjects at the time of the school-age assessment are shown in Tables 1 and 2, respectively. At the time of inclusion in the study, all of the patients were in stable conditions and had not used steroids for at least 1 month. None of the patients was being treated with leukotriene receptor antagonists.

Preterm children without BPD. Thirty-one children born prematurely who did not develop BPD were recruited as control subjects for the BPD group (Table 2). All the children were admitted to the Neonatal Intensive Care Unit of our Department at birth because of prematurity. They were matched with children with BPD for birth weight and gestational age. They were with mild or without respiratory distress syndrome at birth, requiring no mechanical ventilation beyond the seventh day of life and oxygen supplementation beyond 2 weeks of postnatal age. Thus, none was diagnosed as having BPD according to the previously mentioned criteria, nor were they given postnatal steroids. Their neonatal data are shown in Table 1.

Healthy control children. Thirty-one healthy children born at term with no history of asthma or atopy and no respiratory symptoms in the previous 4 weeks were recruited as control subjects. They were matched for sex and age with the children with BPD (Table 2).

Children with asthma. We enrolled 31 children with asthma with anthropometric and spirometric features comparable with those of the subjects with BPD (Table 2). They were recruited among patients attending the pulmonology/allergy outpatient clinic. The diagnosis of asthma was based on clinical history, symptom frequency, physical

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Correspondence and requests for reprints should be addressed to Eugenio Baraldi, Department of Pediatrics, Via Giustiniani 3, 35128 Padova, Italy. E-mail: baraldi@pediatria.unipd.it

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TABLE 1. NEONATAL DATA OF BRONCHOPULMONARY DYSPLASIA AND PRETERM CHILDREN WITHOUT BRONCHOPULMONARY DYSPLASIA

	BPD	Preterm Non-BPD
Gestational age at birth, wk	28.6 ± 0.3	28.9 ± 0.4
Birth weight, g	1081 ± 57	965 ± 40
Ventilator treatment, d	26 ± 3.4	2.6 ± 0.7
Supplemental oxygen, d	115 ± 22	4.4 ± 1.1
Surfactant-treated newborns	24/31	7/31
Postnatal steroids	20/31	0/31

Definition of abbreviation: BPD = bronchopulmonary dysplasia.
Values are expressed as mean (SEM).

examination, and pulmonary function parameters, according to international guidelines (19). Nineteen children had intermittent asthma, and 12 had moderate persistent asthma. None of the children had used inhaled or oral glucocorticosteroids for at least 1 month. Children with persistent asthma were not under treatment either because they spontaneously discontinued long-term controller medication before the planned visit or because they were referred for the first time to our clinic without treatment. None of the patients was being treated with leukotriene receptor antagonists. Patients were excluded from the study if they had developed a respiratory infection or had an episode of asthma exacerbation in the previous 4 weeks.

The study was approved by the local ethics committee, and all parents gave their verbal informed consent.

Study Design

A detailed clinical history was obtained, and a complete physical examination was performed before FE_{NO} measurement and spirometry. At least one parent was asked to attend the pulmonary function tests and allergometric studies. Spirometry was always performed after FE_{NO} measurement. Then the skin prick test was done. Each child was adequately instructed and trained before taking FE_{NO} measurements and testing pulmonary function.

Fractional Exhaled NO Measurement

FE_{NO} was measured with an online method using a computerized system (EBA Aerocrine, Stockholm, Sweden) following the American Thoracic Society's recommendations (18). Subjects inhaled NO-free air through the mouth to total lung capacity and exhaled through a dynamic flow restrictor with a target flow of 50 ml/second for at least 6–7 seconds (20). No nose clip was used. FE_{NO}, expressed as ppb, was calculated as the mean of three measurements that agreed to within 10% of the mean value.

Spirometry and Reversibility to β_2 -Agonists

Lung function was analyzed by flow-volume spirometry (Biomedin, Padova, Italy). FVC, FEV₁, and forced expiratory flow rate between 25% and 75% (FEF_{25–75%}) of FVC were measured and expressed as percentages of the standardized values predicted for normal children according to sex and height (21). The best of three maneuvers was recorded. Spirometry was also performed after administering 300 μ g of inhaled salbutamol by metered dose inhaler with a spacer device

(Aerochamber, Trudell, Canada). Reversibility to β_2 -agonists was defined as a more than 12% increase in FEV₁ after salbutamol inhalation.

Skin Prick Tests

All subjects underwent skin prick testing with a panel of common inhalant allergens: mixed grass pollen, *Parietaria*, *Artemisia vulgaris*, *Dermatophagoides pteronissynus* and *Dermatophagoides farinae*, *Alternaria*, dog and cat (Lofarma, Milano, Italy). Glycerine with histamine 1:1,000 was used as a positive control and glycerine alone as a negative control. The skin tests were done on the volar side of the forearms and were considered positive if they resulted in a wheal reaction greater than 3 mm.

Statistical Analysis

Results are expressed as mean ± SEM for normally distributed data. FE_{NO} values were logarithmically transformed to normalize the distribution of the data and were expressed as a geometric mean with their 95% confidence intervals. One-way analysis of variance was then used to detect the overall difference between groups; the comparison between groups with a similar variance was performed using the Student-Newman-Keuls test. Correlations were evaluated by Pearson's test. Statistical significance was assumed for p values of less than 0.05. Statistical analysis was performed using SigmaStat version 3.0.

RESULTS

All survivors of BPD were intubated at birth and mechanically ventilated for respiratory distress syndrome. The mean (SEM) mechanical ventilation and oxygen dependency duration were, respectively, 26 ± 3.4 days and 115 ± 22 days (Table 1). Surfactant was routinely introduced in our unit from 1991 onward, and thus, 24 neonates received surfactant on a rescue basis, and 7 did not. Twenty of the 31 children in the study received postnatal glucocorticoid therapy. Twenty-three children had no neurologic sequelae, and eight had mild to moderate degrees of neurodevelopmental delay.

The preterm children without BPD had the same gestational age and birth weight as the children in the group with BPD (Table 1) but a markedly shorter time on mechanical ventilation and oxygen dependence ($p < 0.001$). Eighteen of them had never started mechanical ventilation or had received ventilatory support for less than 24 hours. Surfactant was used in seven cases.

Survivors of BPD, preterm children without BPD, those with asthma, and healthy control subjects did not differ in age, sex, and height (Table 2), whereas weight was significantly lower in patients with BPD than in healthy control subjects or those with asthma ($p = 0.004$ and 0.007 , respectively).

The skin prick test was positive in four children in the group with BPD, in 5 preterm children without BPD, and in 25 children with asthma.

FE_{NO}

All 124 children enrolled in the study were able to perform FE_{NO} measurement. Children with BPD had significantly lower FE_{NO}

TABLE 2. STUDY POPULATION

	BPD (n = 31)	Asthma (n = 31)	Preterm Non-BPD (n = 31)	Healthy (n = 31)
Age, yr	8.6 ± 0.3	8.6 ± 0.5	8.7 ± 0.3	8.4 ± 0.4
Males/females	14/17	14/17	14/17	14/17
Weight, kg	26.4 ± 1.5	32.7 ± 1.7	26.5 ± 1.1	31.4 ± 1.5
Height, cm	128.5 ± 2.1	134.8 ± 2.9	127.4 ± 2	131.5 ± 2

For definition of abbreviations see Table 1.
Values are expressed as mean (SEM).

levels than healthy control subjects or preterm children without BPD ($p < 0.05$). Geometric mean values were 7.7 ppb (95% confidence interval, 6.6–8.8), 10.7 ppb (95% confidence interval, 9.6–11.8), and 9.9 ppb (95% confidence interval, 8.8–11), respectively. $F_{E_{NO}}$ was significantly higher in children with asthma (24.9 [23.7–26.1] ppb) than in any of the other groups ($p < 0.001$) (Table 3 and Figure 1). No significant correlation was found between $F_{E_{NO}}$ and FEV_1 , FVC, and FEF_{25-75} in children with BPD ($p = 0.9, 0.9, 0.4$, respectively), preterm children (0.3, 0.6, and 0.9), and the groups as a whole ($p = 0.7, 0.3$, and 0.1). $F_{E_{NO}}$ values were unrelated to the time of oxygen dependence or mechanical ventilation ($p = 0.09$ and 0.1, respectively).

There was no difference in $F_{E_{NO}}$ between children who did or did not receive surfactant (7.7 ± 1.1 and 7.6 ± 1.1 ppb, respectively; $p = 0.9$) or between children who were or were not given postnatal glucocorticoid therapy (7.5 ± 1.1 and 8.0 ± 1.2 ppb, respectively; $p = 0.7$).

Spirometry

Spirometry test results showed evidence of airflow limitation in the BPD group by comparison with the control group (Table 3), as demonstrated by significantly lower values of FEV_1 , FEF_{25-75} , and FEV_1/FVC ratio ($p < 0.001$). Spirometric values in the BPD group varied considerably, ranging from normal to markedly reduced. Nine of the subjects with BPD had FEV_1 values of 70% or less of predicted. Children with BPD and children with asthma had a similar degree of airflow limitation (FEV_1 77.8 vs. 80.2%, $p = 0.4$), as shown in Table 3.

FEV_1 and FEF_{25-75} were significantly higher in the preterm children without BPD than in the children with BPD and were significantly lower than in the group of healthy control subjects (Table 3).

Reversibility to salbutamol was tested in all but two patients with BPD: airflow limitation was not reversible ($\leq 12\%$) in 21 patients (72%) (mean increase in FEV_1 , $5.8 \pm 0.9\%$). The other eight children showed a mean increase in FEV_1 of $18.8 \pm 2.7\%$. In the group of children with BPD as a whole, the mean increase in FEV_1 after salbutamol was $9.4 \pm 1.5\%$. No difference was found in $F_{E_{NO}}$ values between children with BPD whose airflow limitation did and did not respond to salbutamol inhalation ($6.6 [\pm 1.1]$ ppb vs. $8.1 [\pm 1.1]$ ppb respectively, $p = 0.3$). No correlation was found between ΔFEV_1 and $F_{E_{NO}}$ levels ($p = 0.7$) or between FEV_1 at the baseline and after salbutamol ($p = 0.1$). In the preterm group without BPD, FEV_1 increased by 12% or more in 5 of the 30 cases (17%) tested.

DISCUSSION

This is the first study reporting low $F_{E_{NO}}$ levels in a group of school-age children with BPD with varying degrees of airflow obstruction by comparison with healthy control subjects, preterm subjects without BPD, and FEV_1 -matched children with asthma. Although BPD and asthma share some clinical and functional features, the remarkable difference in $F_{E_{NO}}$ values suggests that the airflow limitation in these two obstructive lung diseases of childhood is related to distinct pathophysiologic pathways that ought to be properly identified.

The early stages of BPD are almost invariably characterized by an intense inflammatory response (3), followed by chronic inflammation and airway remodeling (3, 22, 23). Significant early airway changes often have far-reaching consequences: as in the previous literature (6–11), most of the children we studied at school age had some degree of airflow limitation (mean FEV_1 , 77%), which failed to improve after salbutamol inhalation in 72% of cases. Only sparse information is available on the mechanisms underlying the long-term clinical and functional manifestations of BPD. Some of the clinical features and the abnormal airway physiology (airflow limitation and airway hyperresponsiveness) that BPD shares with bronchial asthma might suggest a common mechanism responsible for lung function impairment (3, 13). Unfortunately, studies on airway pathology in BPD beyond infancy are currently lacking, although clarifying this issue would have important prognostic and therapeutic implications. In fact, children with BPD are frequently treated empirically with asthma medication, although there is no evidence to support this common practice. For these reasons, we aimed to noninvasively assess the presence of airway inflammation in children with BPD by measuring their $F_{E_{NO}}$. In the lungs, NO plays a key role in the physiologic regulation of vessels and airway tone, and it can be altered in several heart–lung diseases (15). In patients with asthma, $F_{E_{NO}}$ is considered an indirect marker of eosinophilic airway inflammation (16, 17, 24, 25). Part of the NO measured in exhaled breath may be produced in the vascular endothelium, however, and $F_{E_{NO}}$ measurement has recently also been proposed as a marker of pulmonary endothelial dysfunction (26).

Our data show that flow limitation at school age in survivors of BPD was not associated with an increased NO production. This is consistent with the $F_{E_{NO}}$ results that we had previously obtained in a smaller group of children with BPD enrolled in a longitudinal pulmonary function study (11).

The comparison with children with asthma with a similar airflow limitation indicates a clear difference in the pathophysiology of flow limitation between these two obstructive lung diseases.

TABLE 3. SPIROMETRIC VALUES AND EXHALED NITRIC OXIDE

	BPD (n = 31)	Asthma (n = 31)	Preterm Non-BPD (n = 31)	Healthy (n = 31)
FVC % predicted	85.9 $\pm$ 2.5*	88.4 $\pm$ 2.4*	96.2 $\pm$ 2.2 [§]	101.7 $\pm$ 2.5
FEV_1 % predicted	77.8 $\pm$ 2.3*	80.2 $\pm$ 2.1*	90.3 $\pm$ 2.8 [§]	100.1 $\pm$ 2.3
FEV_1/FVC , %	81.8 $\pm$ 2*	83 $\pm$ 1.7 [§]	84.3 $\pm$ 5.6 [†]	89.4 $\pm$ 1
FEF_{25-75} % predicted	63.9 $\pm$ 4*	72.3 $\pm$ 4.2*	83 $\pm$ 5.6*	110.9 $\pm$ 5.1
$F_{E_{NO}}$, ppb	7.7 ^{††} (6.6–8.8)	24.9* (23.7–26.1)	9.9 (8.8–11)	10.7 (9.6–11.8)

Definition of abbreviations: BPD = bronchopulmonary dysplasia; $FEF_{25-75\%}$ = forced expiratory flow rate between 25% and 75%. Values are expressed as mean (SEM) for normally distributed data and as geometric mean (95% confidence intervals) for not normally distributed data.

* $p < 0.001$ compared with healthy children.

[†] $p < 0.05$ compared with healthy children.

^{††} $p < 0.05$ compared with preterm children without BPD.

[§] $p < 0.01$ compared with healthy children.

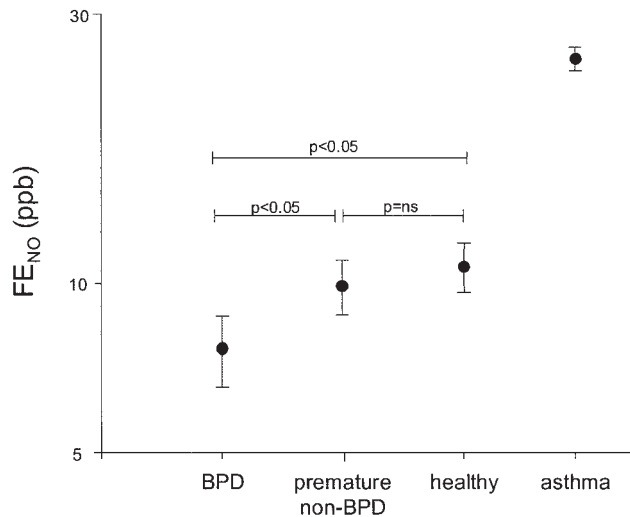


Figure 1. Geometric mean and 95% confidence intervals of exhaled nitric oxide (FE_{NO}) levels in children with bronchopulmonary dysplasia (BPD), preterm children without BPD, healthy control subjects, and those with asthma on a log scale.

We chose to compare children with BPD and children with asthma with similar airflow limitations to prevent any bias in FE_{NO} measurement related to possible differences in airway caliber. In addition, we compared children with BPD with a group of preterm children without BPD to exclude any influence of prematurity per se on FE_{NO} . Preterm children without BPD also had a significantly worse lung function than healthy control subjects but normal FE_{NO} values (Table 3).

Mieskonen and colleagues recently showed significant bronchial obstruction but normal FE_{NO} levels in a group of school-age children born prematurely compared with healthy control subjects. Similar results were obtained by separately analyzing a subgroup of nine children with BPD (27). In our study, FE_{NO} values in children with BPD were slightly but significantly lower than in healthy controls, a result that may have been obscured in the Mieskonen study by the small number of children and the high expiratory flow (100–200 ml/second) used in their FE_{NO} measurement. As recommended by international guidelines (18), we used an expiratory flow rate of 50 ml/second, which is highly reproducible and considered the most sensitive flow for discriminating among subjects (28).

A defective NO synthesis or release in children with BPD could be related to different mechanisms. The more obvious explanation, a reduction in FE_{NO} related to a smaller lung size of children with BPD, is unlikely for two reasons: children with BPD have a FE_{NO} that is also lower than in equally premature children without BPD, and an insignificant relationship was found between vital capacity and FE_{NO} values. A defective NO synthesis and/or diffusion in the airway lumen could be a sequela of the epithelial damage occurring in the early phases of BPD. A complementary explanation for the lower FE_{NO} in patients with BPD may lie in the “vascular hypothesis” recently proposed to explain some features of BPD (29, 30), according to which the early lung injury in infants with BPD results in a dysmorphic vascular growth, with a reduction in the pulmonary vascular bed. As a result, an impaired endothelial release or reduced diffusion of NO from the endothelium into the airways would account for the lower levels of FE_{NO} . However, at this regard, a potential limit of our study is that we did not use multiple flows analysis of FE_{NO} to separate conducting-airway NO output from alveolar

NO production. A similar phenomenon, with reduced FE_{NO} values, has been described in patients with chronic obstructive pulmonary disease with cor pulmonale (31), where there is evidence of NO release being impaired in the pulmonary vasculature (32). In the light of these hypotheses, a reduced FE_{NO} suggests a poor or dysmorphic pulmonary development in survivors of BPD. Airway remodeling is also suggested by the lack of reversibility of airflow limitation in most of our children with BPD (33). This is consistent with the evidence of a considerable airway function tracking we previously found in a group of children with BPD whose degree of spirometric impairment at school age was found closely related to their airflow limitation at 2 years of age (11). This adds concern that moderate to severe BPD may be associated with chronic obstructive lung disease in later life (6), but longer longitudinal studies are needed to establish the clinical and functional relevance of these findings in adult life.

In conclusion, in school-age survivors of BPD, differently from what is observed in children with asthma, airflow limitation is not associated with an increase in FE_{NO} . The low FE_{NO} values we found and the lack of β_2 -agonist reversibility of airflow limitation in most of these subjects suggest that a distinct pathophysiologic mechanism is present in children with BPD. Further studies are necessary to determine the role of NO in the developmental biology of the lung.

Conflict of Interest Statement: E.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; G.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; F.Z. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

- Northway WHJ, Rosan RC, Porter DY. Pulmonary disease following respiratory therapy of hyaline-membrane disease: bronchopulmonary dysplasia. *N Engl J Med* 1967;276:357–368.
- Jobe AH, Bancalari E. NICHD/NHLBI/ORD workshop summary: bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;163:1723–1729.
- Allen J, Zwerdling R, Ehrenkranz R, Gaultier C, Geggel R, Greenough A, Kleinman R, Klijanowicz A, Martinez F, Ozdemir A, *et al.* Statement on the care of the child with chronic lung disease of infancy and childhood. *Am J Respir Crit Care Med* 2003;168:356–396.
- Coalson JJ, Winter VT, Siler-Khodr T, Yoder BA. Neonatal chronic lung disease in extremely immature baboons. *Am J Respir Crit Care Med* 1999;160:1333–1346.
- Clark RH, Gerstmann DR, Jobe AH, Moffitt ST, Slutsky AS, Yoder BA. Lung injury in neonates: causes, strategies for prevention, and long-term consequences. *J Pediatr* 2001;139:478–486.
- Eber E, Zach MS. Long term sequelae of bronchopulmonary dysplasia (chronic lung disease of infancy). *Thorax* 2001;56:317–323.
- Tepper RS, Morgan WJ, Cota K, Taussig LM. Expiratory flow limitation in infants with bronchopulmonary dysplasia. *J Pediatr* 1986;109:1040–1046.
- Bader D, Ramos AD, Lew CD, Platzker ACG, Stabile MW, Keens TG. Childhood sequelae of infant lung disease: exercise and pulmonary function abnormalities after bronchopulmonary dysplasia. *J Pediatr* 1987;110:693–699.
- Pelkonen AS, Hakulinen AL, Turpeinen M. Bronchial lability and responsiveness in school-children born very preterm. *Am J Respir Crit Care Med* 1997;156:1178–1184.
- Jacob SV, Coates AL, Lands LC, Mac Neish CF, Riley SP, Hornby L, Outerbridge EW, Davis GM, Williams RL. Long-term pulmonary sequelae of severe bronchopulmonary dysplasia. *J Pediatr* 1998;133:193–200.
- Filippone M, Sartor M, Zacchello F, Baraldi E. Flow limitation in infants with bronchopulmonary dysplasia and respiratory function at school age. *Lancet* 2003;361:753–754.
- Northway WHJ, Moss RB, Carlisle KB, Parker BR, Popp RL, Pitlick PT, Eichler I, Lamm RB, Brown BW. Late pulmonary sequelae of bronchopulmonary dysplasia. *N Engl J Med* 1990;323:1793–1799.

13. Mai XM, Gaddlin PO, Nilsson L, Finnstrom O, Bjorksten B, Jenmalm MC, Leijon I. Asthma, lung function and allergy in 12-year-old children with very low birth weight: a prospective study. *Pediatr Allergy Immunol* 2003;14:184–192.
14. Busse WW, Lemanske RF. Asthma. *N Engl J Med* 2001;344:350–362.
15. Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. *Am J Respir Crit Care Med* 2001;163:1693–1722.
16. Payne DN, 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;164:1376–1381.
17. Van den Toorn LM, Overbeek SE, de Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. *Am J Respir Crit Care Med* 2001;164:2107–2113.
18. American Thoracic Society. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children: 1999. *Am J Respir Crit Care Med* 1999;160:2104–2117.
19. National Institutes of Health and National Heart, Lung, and Blood Institute. Guidelines for the diagnosis and management of asthma. Washington, DC: National Institutes of Health; 1997. Publication No. 97–4051.
20. Baraldi E, Scollo M, Zaramella C, Zanconato S, Zacchello F. A simple flow-driven method for on-line measurement of exhaled NO starting at the age of 4 to 5 years. *Am J Respir Crit Care Med* 2000;162:1828–1832.
21. Polgar G, Promadhat V. Pulmonary function testing in children: techniques and standards. Philadelphia: W. B. Saunders; 1974.
22. Stocker JT. Pathologic features of long-standing “healed” bronchopulmonary dysplasia: a study of 28 3- to 40-month-old infants. *Hum Pathol* 1986;17:943–961.
23. Odzemir A, Brown MA, Morgan WJ. Markers and mediators of inflammation in neonatal lung disease. *Pediatr Pulmonol* 1997;23:292–306.
24. Baraldi E, Azzolin MN, Zanconato S, Dario C, Zacchello F. Corticosteroids decrease exhaled nitric oxide in children with acute asthma. *J Pediatr* 1997;131:381–385.
25. 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;58:1048–1052.
26. Malmstrom RE, Tornberg DC, Settergren G, Liska J, Angdin M, Lundberg JO, Weitzberg E. Endogenous nitric oxide release by vasoactive drugs monitored in exhaled air. *Am J Respir Crit Care Med* 2003;168:114–120.
27. Mieskonen ST, Malmberg LP, Kari MA, Pelkonen AS, Turpeinen MT, Hallmann NMK, Sovijärvi ARA. Exhaled nitric oxide at school age in prematurely born infants with neonatal chronic lung disease. *Pediatr Pulmonol* 2002;33:347–355.
28. Pedroletti C, Zetterquist W, Nordvall L, Alving K. Evaluation of exhaled nitric oxide in schoolchildren at different exhalation flow rates. *Pediatr Res* 2002;52:393–398.
29. Bhatt AJ, Pryhuber GS, Huyck H, Watkins RH, Metlay LA, Maniscalco WM. Disrupted pulmonary vasculature and decreased vascular endothelial growth factor, Flt-1 and TIE-2 in human infants dying with bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;164:1755–1756.
30. Abman SH. Bronchopulmonary dysplasia: “a vascular hypothesis.” *Am J Respir Crit Care Med* 2001;164:1971–1980.
31. Clini E, Cremona G, Campana M, Scotti C, Pagani M, Bianchi L, Giordano A, Ambrosino N. Production of endogenous nitric oxide in chronic obstructive pulmonary disease and patients with cor pulmonale. *Am J Respir Crit Care Med* 2000;162:446–450.
32. Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1995;333:214–221.
33. Rasmussen F, Taylor DR, Flannery EM, Cowan JO, Greene JM, Herbison GP, Sears MR. Risk factors for airway remodeling in asthma manifested by a low postbronchodilator FEV₁/vital capacity ratio: a longitudinal population study from childhood to adulthood. *Am J Respir Crit Care Med* 2002;165:1480–1488.