Impact of Chorioamnionitis on Exhaled Nitric Oxide and Endotracheal Aspirate Levels of Nitrites–Nitrates and Interleukin-8 in Mechanically Ventilated Preterm Neonates

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Summary. Objectives: To assess the influence of maternal chorioamnionitis on early exhaled nitric oxide (NO) and levels of nitrites–nitrates and interleukin (IL)-8 in endotracheal aspirate fluid in mechanically ventilated preterm neonates. Study Design: Cross-sectional study. Patient–Subject Selection: Between September 2007 and August 2009, 54 mechanically ventilated preterm neonates were included. Patients were divided into two groups according to the presence or absence of maternal chorioamnionitis, and those without chorioamnionitis (controls) were further stratified into two subgroups by birth weight < or ≥2,000 g. Methodology: The ventilator used was a Babylog 8000. The NO level assessed was the plateau value given by the software of the Sievers NOA apparatus. Collection of endotracheal aspirate fluid samples was performed coinciding with routine aspirations and using the dry technique. Results: The two groups of control neonates showed statistically significant differences in exhaled NO expressed as nl/min and normalized exhaled NO expressed as either nl/min or nl/min/kg, so they are not homogeneous and cannot be used in clinical practice. Serum C-reactive protein and endotracheal aspirate levels of nitrites–nitrates were significantly higher in the chorioamnionitis group than in controls (3.6 vs. 1.07 μmol/L; $P = 0.035$). Nitrites–nitrates levels were positively correlated with exhaled NO in ppb ($\rho = 0.367$; $P = 0.006$). Minute exhaled endogenous NO was significantly higher in the chorioamnionitis group (0.48 vs. 0.27 nl/min/kg; $P = 0.021$). Conclusions: In mechanically ventilated preterm infants weighing <2,000 g, maternal chorioamnionitis was associated with an increase of early exhaled NO (nl/min/kg) and serum levels of C-reactive protein and levels of nitrites–nitrates in endotracheal aspirate fluid. **Pediatr Pulmonol. 2011; 46:595–603.** © 2011 Wiley-Liss, Inc.

Key words: chorioamnionitis; exhaled nitric oxide; newborn; preterm; nitrites–nitrates; interleuchin-8.

INTRODUCTION

Maternal chorioamnionitis may cause preterm delivery and be associated with an adverse perinatal outcome, including early-onset sepsis, necrotizing enterocolitis, and lung disease as well as long-term sequelae in the infant.[1–4] Histologic chorioamnionitis is associated with an increased incidence of bronchopulmonary dysplasia (BPD),[5] a chronic lung disease that prolongs hospital stay and causes greater morbidity and mortality of respiratory origin.[6–9]

Chorioamnionitis may injure the fetal lung through the activation of alveolar neutrophils and macrophages. Due to this activation, greater expression of inducible nitric oxide (NO) synthase is to be expected, with elevation of nitrites and nitrates in endotracheal aspirates and of

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minute exhaled NO. Exhaled NO is raised in adults and children with asthma,[10,11] and has also been detected among the gases exhaled in mechanically ventilated neonates.[12–17] Different results of exhaled NO in neonates with BPD have been reported, including lower values in infants breathing spontaneously[18] and higher exhaled NO peak levels in ventilated infants.[19] Differences in postnatal age[16–19] and in the methods for assessing exhaled NO (breathing spontaneously or on assisted ventilation,[6,18] output or minute ventilation obtained using plateau NO values,[18] or peak or maximum values of exhaled NO[19]) may account for the conflicting results. Although recommendations concerning standardized techniques for measurement of exhaled NO have been reported,[20] data for analysis of exhaled NO in mechanically ventilated preterm infants are lacking. Williams et al.[21] studied the influence of ventilatory settings on measurements of exhaled NO and found that increasing peak inflating pressure as well as the inspired oxygen concentration resulted in a decrease in the peak NO levels. The authors emphasized that the conditions of measurement must be standardized in infants receiving respiratory support. In a previous study of our group,[22] the following points were suggested when collecting gas samples from ventilated premature infants to obtain exhaled NO: (1) it would suffice to collect exhaled gas during 1 min, although it is recommended to repeat collection three times;[15] (2) for each sample collection, the parameters to record should be fraction of inspiratory oxygen (FiO$_2$), peak inspiratory pressure (PIP), positive end expiratory pressure (PEEP), respiratory frequency (RF), I/E, volume/minute, and percentage of leakage (the two latter ones, every 15 sec during collection); and (3) the results should be expressed as exhaled NO (ppb plateau value), "minute exhaled NO/kg after correction for leakage" (exhaled NO × volume/min/weight in kg = nl or ppb/min/kg) and "normalized minute exhaled NO/kg after correction for leakage" (after applying the normalization equation).[22]

The cellular composition of tracheal aspirate is the gold standard for defining lung inflammation.[23] Interleukin-8 (IL-8) is produced by alveolar macrophages, fibroblasts, type II pneumocytes, and endothelial cells. Hypoxia, hyperoxia, and endotoxins stimulate the synthesis of IL-8, which is a major neutrophil chemotactic factor in the lung. Histologic chorioamnionitis is associated with an increase of IL-8 in endotracheal aspirate fluid along the first day of life (≥917 pg/ml) and this cutpoint may be used for diagnosis.[24] Preterm infants suffering from BPD can present increased levels of tumor necrosis alpha (TNF-alfa),[25] IL-6,[25] IL-8,[26] and IL-10[26] in endotracheal aspirates.

The present study was designed to assess changes of early exhaled NO and the concentration of nitrites–nitrates and IL-8 in endotracheal aspirate fluid in mechanically ventilated preterm neonates with and without history of maternal chorioamnionitis.

MATERIALS AND METHODS

Design and Patient Population

A cross-sectional study of mechanically ventilated preterm infants was conducted to assess the impact of chorioamnionitis on exhaled NO and the concentration of nitrites–nitrates and IL-8 in endotracheal aspirates. Between September 2007 and August 2009, 54 mechanically ventilated preterm neonates treated in the Neonatology Service of a IIIB level neonatal intensive care unit were eligible. Inclusion criteria were gestational age between 25 weeks 0 days and 36 weeks 6 days, <36 hr of age, presence of mechanical ventilation for the treatment of respiratory distress syndrome (RDS), hemodynamic stability, and written informed consent obtained from the parents or legal guardians. Newborns with pulmonary hypoplasia or other respiratory conditions other than RDS, major congenital malformations, chromosomal abnormalities, sepsis, or treatment with NO donor drugs were excluded. Patients were divided into two groups according to the presence or absence of maternal chorioamnionitis, and those without history of chorioamnionitis (controls) were further stratified into two subgroups according to birth weight <2,000 g or ≥2,000 g. The study protocol was approved by the Ethics Committee of Hospital Clinic of Barcelona, Spain. The parents or legal guardians were informed about the study and provided consent because no blood sampling was needed and procedures performed were not painful and were included in the care of the patients (tracheal aspirates).

Definitions and Procedures

The diagnosis of clinical chorioamnionitis in the pregnant woman who presented with premature labor or premature rupture of the membranes was made in the presence of some combination of uterine pain, fever (≥38°C), leukocytosis (>12,000 cells/mm^3), and increased serum concentrations of C-reactive protein (>30 mg/L) without any clear focus of infection. In these cases, amniotic fluid and placenta were microbiologically cultured, and placenta and umbilical cord histologically analyzed. Chorioamnionitis was diagnosed when clinical data were accompanied by a positive culture from amniotic fluid and placenta (fetal and maternal sites) and histological findings of chorioamnionitis. Polymorphonuclear infiltration in the placenta and umbilical cord was required to establish a diagnosis of histological chorioamnionitis. Mild chorioamnionitis had a few neutrophils (5–10 neutrophils per high-power field) scattered in the subchorionic space and adjacent chorion; moderate chorioamnionitis had many neutrophils (11–30 neutrophils per high-power

field) in the lower half of the chorionic plate and the subchorionic space; and severe chorioamnionitis had dense infiltrates of neutrophils (30 per high-power field) throughout the chorionic plate into the amnion. Mild chorioamnionitis was recorded in seven cases, moderate in three, and severe in five.

RDS was defined in the presence of a typical clinical course and chest radiographic findings, with clinical improvement when exogenous surfactant was administered. RDS was considered non-severe if only one dose of exogenous surfactant was needed and severe if two or more doses of exogenous surfactant or high-frequency oscillatory ventilation were needed as well as in the presence of air leak or if RDS was the cause of death. BPD was defined according to diagnostic criteria of Jobe and Bancalari.[27]

At all times, neonates received appropriate medical care according to his/her clinical condition. Special attention was placed to maintain the inspired gas temperature at $37^{\circ}C$ and maximum humidity of the circuit (100%) using a Fisher–Paykel humidifier. Serial measurements of heart rate, RF, non-invasive blood pressure, and arterial blood gases were performed. Ventilatory parameters included PIP, PEEP, inspiratory time (Ti), effective RF, tidal volume (Vt), minute volume (Vm), and compliance (A). The Clinical Risk Index for Babies (CRIB) score (in infants weighing <1,500 g) and the Score for Neonatal Acute Physiology (SNAP) and SNAP Perinatal Extension (SNAPPE II) score were assessed to check homogeneity of the patients' severity of illness.

All infants were mechanically ventilated using a constant flow, time cycled, pressure-limited device (Babylog 8000 Plus, Dräger Medical AG & Co. KGaA, Lübeck, Germany), which measures all ventilatory parameters displayed in the screen of the device during the expiratory time. Oxygenation was continuously monitored by pulse oximetry to maintain arterial blood oxygen saturation (SaO_2) between 88% and 93% and arterial partial pressure (PaO_2) between 50 and 80 mmHg. For ventilation, we tried to achieve carbon dioxide partial pressure ($PaCO_2$) in the range of 45–55 mmHg and pH >7.20 during the first 96 hr; then, high $PaCO_2$ levels were allowed while maintaining pH >7.20. In order to avoid share stress-induced release of NO from vascular endothelium,[28] gentle ventilation was used.

All newborns were intubated with a double-lumen endotracheal tube (inner diameter 2.7 and 3.2 mm in infants weighing 600–900 g and 1,000–1,500 g, respectively) if a deep resuscitation was needed in the delivery room, or when a FiO_2 >0.30 was required, or in the presence of moderate, severe, or progressive respiratory difficulty in spite of receiving continuous positive airway pressure (CPAP) of 6 cm water. All intubated infants received surfactant (Survanta, Ross Laboratories, Columbus, OH) at 4 ml/kg (100 mg of phospholipids/

kg), which was repeated if needed. Initial ventilatory setting included flow 5–8 L/min, PEEP 5 cm H_2O, RF 60 breaths/min, and FiO_2 to keep SaO_2 in the range of 88–93%. Thereafter, PIP and RF were adjusted to reach the aforementioned pH and $PaCO_2$ levels. Sedation was not routinely used during the study.

NO Sampling

The sample of gas for NO analysis was obtained taking into account that no exogenous surfactant was administered in the previous 6 hr and in the presence of correct humidification of the circuit, correct calibration of the NO apparatus (chemiluminescence technique), and low proportion of NO (<40 ppb) in the compressed wall air. NO sampling was performed as previously described[22] and trying to standardize the ventilation parameters[21] ($FiO_2 = 0.21$, RF = 50 respirations/min, Ti = 0.4 sec, PIP = 20 cm water, PEER = 5 cm water) during the 3 min of NO sampling. This change in the ventilation parameters was well tolerated and in only 12 cases we had to increase FiO_2 to <0.35. The sample of gas in which NO was analyzed was obtained through a ''6 French'' arterial catheter introduced into the endotracheal tube to its tip. This catheter is the narrowest that permits adequate aspiration of the gas sample without generating an alarm in the Sievers NOA apparatus. Furthermore it occupies very little of the endotracheal cross-sectional area, and hence does not affect ventilation of the lung. Three samples of gas during 1 min each were obtained. The mean intra-subject coefficient of variability of exhaled NO levels (in ppb) was calculated from the mean of three 1-min sampling periods in each of the 54 infants. The coefficient of variation was 12.7%.

Influence of Air Contamination

The absence of environmental air contamination in exhaled NO samples was confirmed by the lack of correlation between ambient NO and exhaled NO in ppb (n = 54, $\rho = -0.35$, $P = 0.802$) as well as between inhaled NO and exhaled NO in ppb (n = 54, $\rho = 0.176$, $P = 0.203$). The mean (standard deviation, SD) inspired NO level was 1.27 (0.91) ppb (range 0–4.1).

Calculation of Plateau NO and Minute Exhaled NO

The NO level assessed is not the highest peak, but rather the plateau value given by the software of the Sievers NOA apparatus. This plateau value is the most reliable equivalent of the NO exhaled during expiration (corresponding to the average NO detected between 1/2 and 7/8 of the duration of expiration). Sievers NOA 280i is able to detect from 0.5 ppb to 500 ppm of NO, with a sensitivity of 0.5 ppb, aspirating a gas sample of 10–200 ml/min (100 ml/min in our study) and with a lag time of 1 sec and an electronics response time of 0.067 sec (sampling

rate of 15 Hz) to 90% full scale. This high speed means the apparatus can differentiate between inspired gas (contaminated with compressed air) and expired gas, which adopts the form of a plateau. Minute exhaled NO or minute excretion of NO was calculated using the formula:

$$V'_{NO}(nl/min) = \text{Plateau NO (ppb} = nl/L)$$
$$\times V_E(\text{minute ventilation in L/min})$$

V_E has been previously corrected by the percentage of leakage given by the software of the Babylog 8000 ventilator using the formula:

$$V_E(L/min) = \frac{\text{Volume per minute(L/min)}}{(100-\text{leakage})/100}$$

Finally, minute exhaled NO was divided by the weight in kilograms of the newborn to obtain the minute exhaled NO/kg (nl/min/kg). To convert nl to nmol, it should be remembered that 1 mol = 24.3 L, when environmental temperature is 23°C.

The results can be normalized by applying the formula developed in a previous report[22] as follows: Normalized minute exhaled NO = minute exhaled NO without leakage − minute exhaled NO deviation, being minute exhaled NO deviation = −173.668 × (exhaled NO/100)^3 + 49.513 × (exhaled NO/100) + 12.486 × (VM/100)^3 + 24.075 × (PIP/100) + 12.371 × (RF/100)^3 − 8.126 − average minute exhaled NO.

Collection of Endotracheal Aspirate Fluid Samples

Collection of endotracheal aspirate fluid samples was performed before the administration of exogenous surfactant or at least 6 hr later[29] coinciding with routine aspirations and according to the dry technique, which for research purposes is equally valid than the bronchoalveolar lavage.[30] To obtain the mucus aspirated inside the catheter, the catheter was cleaned with 1 ml of normal saline into a tube and finally purged with air. The entire procedure was repeated to have a 2 ml sample, 1 ml of which was placed in another tube for nitrites–nitrates analysis. Moreover, 1 ml of the same normal saline was placed in another tube as it may be contaminated by different amounts of nitrites–nitrates. Likewise, 0.2 ml for analysis of IL-8 and 0.2 ml for analysis of proteins were also kept. The diluted endotracheal aspirate fluid samples were centrifuged and the supernatant was recovered and frozen at −80°C, as the tube with normal saline only. Samples were delivered to the laboratory on ice.

Measurement of Nitrites–Nitrates, IL-8, and Protein Levels in Endotracheal Aspirates

The concentration of nitrites–nitrates in endotracheal aspirates was measured by a modification of the fluorimetric technique based on the reaction between 2,3-diamino-naphthalene and nitrites in acid medium. Previous filtration in Ultrafee column (UFC4 LGC, Millipore Corp., Bedford, MA) is needed to avoid interferences with serum components. The detection limit of the technique is 0.01–50 μmol/L. Results were expressed in μmol/L and in relation to the concentration of proteins. Levels of IL-8 were measured by a highly sensitive ELISA technique (Diasource Systems) and results were expressed as pg/ml and in relation to the concentration of proteins. Proteins were measured by an automated method to normalize nitrites–nitrates and IL-8 concentrations per gram of proteins in the endotracheal aspirate fluid, so that errors related to differences in the amount of collected samples were avoided.

Variables

In all infants and before the 36 hr of life, the following variables were recorded: gestational age, sex, birth weight, hours of life, history of maternal chorioamnionitis, maternal smoking during pregnancy, cesarean section, severity of illness (CRIB and SNAPPE-II score), concomitant diseases, such as severe RDS, air leak, patent ductus arteriosus, severe periintraventricular hemorrhage (grades 3–4), early sepsis, early exhaled NO (minute exhaled NO/kg [expressed as nl/min/kg], actual and normalized value),[22] and concentrations of nitrites–nitrates (μmol/L and μmol/g proteins) and IL-8 (pg/ml and pg/g proteins) in the endotracheal aspirate fluid.

Statistical Analysis

Continuous variables were presented as mean ± SD if normally distributed and with homogeneous variances or as median and interquartile range (IQR: 25th to 75th percentiles) if not normally distributed or without homogeneous variances. These latter variables were birth weight; C-reactive protein levels; nitrites–nitrates, IL-8, and protein levels in endotracheal aspirates; duration of supplemental oxygen therapy; and all exhaled NO measurements. The influence of environmental NO or NO in compressed air on exhaled NO was analyzed by means of Spearman correlation, as well as other correlations between exhaled NO and other variables, particularly levels of nitrites–nitrates and IL-8 in endotracheal aspirates, and days of supplemental oxygen therapy. The Student's t-test was used for the comparison of parametric variables and the Mann–Whitney U-test for the comparison of non-parametric variables among the different groups according to "maternal chorioamnionitis" (yes/no). Infants with and without history of maternal chorioamnionitis were matched by birth weight. Categorical variables were compared with the chi-square (χ^2) test or the Fisher's exact test. To determine the most useful cut-point of exhaled NO as a marker of history

of chorioamnionitis, the value with the best relation sensitivity–specificity and the highest positive likelihood ratio was chosen. Statistical significance was set at $P < 0.05$.

RESULTS

Control Group

As shown in Table 1, the two groups of neonates without history of maternal chorioamnionitis showed statistically significant differences in the use of antenatal steroids, gestational age, birth weight, and Apgar score at 5 min after birth. In addition (Table 2), there were significant differences in exhaled NO expressed as nl/min and normalized exhaled NO expressed as either nl/min or nl/min/kg. Therefore, the two subgroups were not homogeneous, and the most similar to the study group was the subgroup

with <2,000 g of birth weight. This subgroup will be considered the control group.

Comparison of Infants With and Without History of Maternal Chorioamnionitis

Clinical findings and morbidity according to history of maternal chorioamnionitis are shown in Table 1. Preterm newborns in the chorioamnionitis group compared with controls weighing <2,000 g were less frequently born by cesarean section and mothers were older. Moreover, a significantly higher percentage of infants with history of maternal chorioamnionitis needed resuscitation and endotracheal intubation more frequently and presented with patent ductus arteriosus or severe intraventricular hemorrhage. The only newborn who died from the chorioamnionitis group was a 25 weeks' preterm infant with a

TABLE 1—Characteristics of the Study Groups With and Without Chorioamnionitis

| | Maternal chorioamnionitis | | | | |
| | Absent | | | | |
Variable	≥2,000 g (group 1)	<2,000 g (group 2)	Present (group 3)	P-value (groups 1 vs. 2)	P-value (groups 2 vs. 3)
Total infants	13	26	15		
Mother and labor					
Age, years, mean (SD)	32.8 (3.9)	30.0 (3.9)	33.8 (4.8)	0.185	0.039
Smoking during pregnancy	0	6 (23.0)	0	0.158	0.120
Antibiotic treatment	9 (69.2)	15 (62.5)	13 (92.9)	0.734	0.059
Antenatal steroids	4 (30.8)	22 (84.6)	14 (93.3)	0.003	0.411
Inborn	8 (61.5)	22 (84.6)	15 (100)	0.129	0.278
Cesarean section	8 (61.5)	19 (73.1)	5 (33.3)	0.486	0.012
Multiple gestation	3 (23.1)	11 (42.3)	7 (46.7)	0.409	0.786
Newborns					
Hours of life, mean (SD)	21.8 (8.6)	21.5 (7.3)	17.1 (7.9)	0.997	0.224
Male	10 (76.9)	13 (50)	8 (53.3)	0.112	0.839
Gestational age, weeks, mean (SD)	34.1 (1.6)	29.2 (2.4)	28.1 (2.7)	0.000	0.386
Birth weight, median (IQR)	2,200 (2,160–2,610)	1,020 (970–1,400)	1,100 (850–1,360)	0.000	0.968
Intrauterine growth restriction	0	3 (11.5)	0	0.524	0.457
Resuscitation (endotracheal tube)	0	7 (26.9)	9 (60)	0.105	0.037
Apgar score at 5 min, mean (SD)	9.8 (0.4)	8.0 (1.7)	8.1 (1.5)	0.003	0.959
CRIB score[1], mean (SD)		2.5 (2.3)	2.8 (1.8)		0.746
SNAPPE II score, mean (SD)	11.8 (9.1)	21.5 (13.4)	23.7 (15.9)	0.110	0.885
Morbidity					
Severe respiratory distress syndrome	9 (69.2)	15 (57.7)	11 (73.3)	0.491	0.323
Pneumothorax	0	1 (3.8)	2 (13.3)	0.999	0.543
Patent ductus arteriosus	3 (23.1)	8 (30.8)	10 (66.7)	0.719	0.028
Intraventricular hemorrhage (grades 3–4)	0	0	4 (26.7)		0.013
Early-onset sepsis	0	0	2 (13.3)		0.128
Late-onset sepsis	0	2 (7.7)	3 (20)	0.544	0.336
Necrotizing enterocolitis	0	2 (7.7)	1 (6.7)	0.544	0.999
Supplemental oxygen, days, median (IQR)	2 (1–4)	3 (1–15)	3 (1.5–8.5)	0.208	0.883
Bronchopulmonary dysplasia	0	6 (23.1)	3 (20)	0.081	0.999
Retinopathy of prematurity	0	4 (15.4)	4 (26.7)	0.281	0.434
Deaths	0	1 (3.8)	1 (6.7)	0.999	0.999

IQR, interquartile range (25th to 75th percentile).
Data as absolute numbers and percentages in parenthesis unless otherwise stated.
[1]In infants weighing <1,500 g.

TABLE 2— Inflammatory Markers in Endotracheal Aspirate Fluid and Exhaled NO in the Study Groups With and Without Chorioamnionitis

	Maternal chorioamnionitis				
	Absent		Present (group 3)	P-value (groups 1 vs. 2)	P-value (groups 2 vs. 3)
Variable	≥2,000 g (group 1)	<2,000 g (group 2)			
Total infants	13	26	15		
Inflammatory markers					
C-reactive protein <24 hr life, mg/L	0 (0–0)	0 (0–1)	1 (0–10.5)	0.508	0.035
C-reactive protein <48 hr life, mg/L	3 (1–13)	2 (1–3)	9 (1–25.5)	0.738	0.140
Nitrites–nitrates, μmol/L	2.4 (0.75–4.0)	1.1 (0.6–2.2)	3.6 (1.1–5.1)	0.178	0.035
IL-8, pg/ml	477 (135–5,660)	497 (191–1,181)	1,161 (241–2,485)	0.627	0.277
Proteins, mg/dl	0.25 (0.08–0.46)	0.21 (0.11–0.39)	0.29 (0.19–044)	0.879	0.192
Nitrites–nitrates, μmol/L/g proteins	17.9 (4.6–31.5)	6.1 (1.6–15.4)	11.8 (8.0–17.5)	0.178	0.222
IL-8, pg/ml/g protein	2,769 (1,871–25,381)	3,032 (351–10,964)	5,918 (1,116–12,148)	0.259	0.619
Exhaled NO, expressed as					
ppb	1.48 (0.90–1.96)	1.03 (0.56–1.78)	1.33 (1.01–2.89)	0.308	0.056
nl/min	0.71 (0.38–0.91)	0.32 (0.18–0.47)	0.46 (0.28–0.92)	0.007	Not calculated[1]
nl/min/kg	0.30 (0.17–0.41)	0.27 (0.16–0.48)	0.48 (0.27–0.77)	0.670	0.021
Normalized exhaled NO, expressed as					
nl/min	1.23 (0.71–1.41)	1.48 (1.26–1.55)	1.55 (1.52–1.62)	0.038	Not calculated[1]
nl/min/kg	0.45 (0.33–0.57)	1.22 (0.92–1.54)	1.54 (1.13–1.79)	0.000	Not calculated[1]
Peak of exhaled NO, ppb	2.24 (1.69–2.50)	1.72 (1.07–2.41)	2.14 (1.62–4.29)	0.159	0.032

Data as median an interquartile range (IQR, 25th to 75th percentile).
[1]Because of lack of homogeneity of the control group (i.e., statistically significant differences between groups 1 and 2).

birth weight of 810 g and a CRIB score of 4, who was intubated only during the first day of life after receiving two doses of surfactant. Seven days after birth he suffered from necrotizing enterocolitis with intestinal perforation and died. The only newborn who died from the control group was a 25 weeks' preterm infant with a birth weight of 740 g and a CRIB score of 4, who developed a very severe BPD and died 49 days after birth.

The values of inflammatory markers and exhaled NO according to history of maternal chorioamnionitis are shown in Table 2. C-reactive protein was higher in the chorioamnionitis group. IL-8 levels, either in absolute values or divided by the protein content of the endotracheal aspirate were also higher in this group, although statistically significant differences were not reached. However, the levels of nitrites–nitrates were significantly higher than in controls ($P = 0.035$) and were positively associated with exhaled NO in ppb (n = 54, $\rho = 0.367$, $P = 0.006$).

Minute exhaled endogenous NO, expressed in nl/min/kg, was significantly higher in the chorioamnionitis group. The most useful cutoff for exhaled NO as a marker of history of maternal chorioamnionitis was 0.285 nl/min/kg, with a sensitivity of 73.3%, specificity of 57.7%, positive predictive value of 50.0%, negative predictive value of 78.9%, accuracy of 63.4%, positive likelihood ratio of 1.733, negative likelihood ratio of 0.462, and area under the curve of 0.718 ($P = 0.021$).

Peak values of exhaled NO were significantly higher in the chorioamnionitis group than in controls ($P = 0.032$) and also showed a significant correlation with nitrites–nitrates levels in the endotracheal aspirate fluid (n = 54, $\rho = 0.322$, $P = 0.018$).

DISCUSSION

In the healthy newborn, exhaled NO is not affected by the gestational age,[13,16,29,31,32] birth weight,[33] or mode of delivery,[29] although there is an increase in exhaled NO during the first week of life.[16,29] Exhaled NO is not related either with serum levels of nitrites and nitrates,[13,24] but there may be a relationship with nitrites and nitrates that are present in the low respiratory tract.[33] In addition, there are two trends in the publications of research groups working on expired or exhaled NO in newborns: those authors who work only with concentrations (ppb)[6,12–14,16,17,34] and those as our group who prefer to adapt the methodologies to present the results as "total excretion of exhaled NO" (nl/min/kg).[17,29,31,32,35,36] Olsen et al.[15] in 2003, measured exhaled NO in eight preterm infants with RDS and showed a decline of median minute excretion of NO by 48–72 hr. Williams et al.[16] measured nasal and lower airway NO level in very immature infants (median gestational age 27 weeks) and found that nasal and lower airway NO levels did not correlate significantly with gestational age, but lower airway NO levels correlated with postnatal age.

As far as we are aware, the relationship between maternal chorioamnionitis and possible increases in nitrites–nitrates levels in endotracheal aspirates and

exhaled NO during the first days of life have not been previously investigated. In the present study, these parameters were examined exclusively in mechanically ventilated preterm infants with RDS. The increase of plateau and peak exhaled NO and their positive correlation with endotracheal nitrites–nitrates suggest that both factors are related and due to the same inflammatory process. It has been shown that nitrate concentration in bronchoalveolar lavage fluid is elevated in infants who developed chronic lung disease of prematurity.[33] In the present study, mean IL-8 values were higher in the chorioamnionitis group than in the control group (1,161 vs. 497 pg/ml), although differences were not statistically significant ($P = 0.277$), probably because of the high dispersion of values. It is likely that changes in IL-8 would have been more apparent with a larger sample size.

The birth weight cutoff point of 2,000 g was empirically chosen in an attempt to homogenize the sample in order to analyze the results of exhaled NO in different forms (including ppb, nl/min, nl/min/kg, and normalized values). It was observed that plateau exhaled NO expressed in nl/min was significantly different between the two control subgroups; therefore, this measurement is not useful in clinical practice. The same findings were observed for normalized plateau exhaled NO expressed as either nl/min or nl/min/kg. A comparison of the chorioamnionitis group with the control subgroup of <2,000 g seems reasonable as both groups were homogeneous regarding gestational age and birth weight. However, if comparisons would have been performed with the 15 infants in the chorioamnionitis group and all 39 controls, heterogeneity of controls would probably account for the disappearance of statistically significant differences for nitrites–nitrates (μmol/L) and peak of exhaled NO (ppb) between infants in the chorioamnionitis and controls of <2,000 g. In fact, a positive aspect of the present study was the stratification of infants in the control group according to birth weight, which allowed comparing infants in the chorioamnionitis group with only those preterm babies without history of chorioamnionitis and similar features of immaturity and birth weight. This stratification also revealed that in controls, the expression of exhaled NO in nl/min, both in actual and normalized values was not homogeneous. When exhaled NO was expressed according to the birth weight, actual values of exhaled NO appeared homogeneous, in contrast with normalized values which are even spread out more. Therefore, the attempt to normalize exhaled NO reported in a previous study,[22] unfortunately is not useful in clinical practice. In this respect, plateau of exhaled NO in ppb and nl/min/kg and peak of exhaled NO in ppb seem to be the only useful parameters in clinical practice. Another positive aspect was the absence of subclinical chorioamnionitis in the control group, because in that group the histology of the placenta had to be normal.

The present findings should be interpreted taking into account some limitations of the study, particularly the exclusion of perinatal sepsis; in this case, marked increases in endogenous NO with secondary increase of exhaled NO have been reported.[37] In addition, the study was restricted to the first 36 hr of life, and may be thereafter other results would be obtained.

One difficulty when attempting to analyze exhaled NO of bronchoalveolar origin is the risk that the determination could be invalidated due to NO contained in the gas inhaled. This is not the case when using the "breath program" software which accompanies the Sievers Nitric Oxide Analyzer (NOA 280i) in the mode designated "use pressure for breath detection mode," and with "on-line sampling." It is considered that the gas in the region between 1/2 and 7/8 of the duration of expiration is equivalent to the third and fourth quartiles of the expiration time, when the gas obtained is exclusively bronchoalveolar,[38] with no possibility of external contamination. The manufacturers of the NOA 280i only recommend the use of medicinal air (NO-free) when environmental NO levels exceed 40–50 ppb. Our compressed air NO levels never reached these values (range 0–4.1 ppb). For repeated measurements of exhaled NO in each expiration, it should be available a chemiluminescence analyzer of rapid response (≤0.2 sec) and at a flow rate of >1.7 ml/sec (Sievers NO analyzer [NOA 280] with a response time of 0.2 sec and a minimum sample of 100–300 ml/min).

In our previous study,[22] it was shown that the variable volume per minute was highly influenced by the percentage of leakage given by the ventilator. Although the leakage of gas around the endotracheal tube is low, the sample of gas aspirated by the analyzer is considered as a leak by the ventilator. This leakage can be corrected mathematically, and indeed this should always be done. Therefore, in the present study minute exhaled NO was obtained multiplying the plateau NO value (plateau between 1/2 and 7/8 of expiration) by the volume per minute, as Olsen et al.[15] have previously reported, but volume per minute was previously corrected by the percentage of leakage.

In the present study, exhaled NO obtained at the tracheobronchial level and during the expiratory phase was expressed as total exhaled NO in nl/min/kg, and this value was also normalized according to setting of ventilation parameters.[22] Median values of exhaled NO were 0.48 and 0.27 nl/min/kg in infants with and without history of maternal chorioamnionitis, respectively. These figures are similar to those reported by Olsen et al.[15] who showed that minute excretion of NO (divided by the weight) in premature infants with RDS and mechanically ventilated at 24 hr of life was 0.405 nl/min/kg. In our group of chorioamnionitis, higher values of exhaled NO in nl/min/kg were found. A value of exhaled NO >0.285 nl/min/kg would be suggestive of history of maternal

chorioamnionitis with a 50% positive predictive value. This low figure and the fact that exhaled NO should be measured in the postnatal period preclude the clinical use of this parameter as a diagnostic criterion of chorioamnionitis. The increase in exhaled NO in nl/min/kg and in nitrites–nitrates levels in the endotracheal aspirate fluid, and the positive correlation between both parameters, would support the common origin of NO in the lower respiratory airway as a result of the inflammatory process.

On the other hand, a significant higher incidence of BPD in the chorioamnionitis groups was not observed, which may be explained by the use of antibiotic treatment and antenatal corticoids for fetal maturation, all of which attenuates the inflammatory process.[39] Lahra et al.[40] even suggested that fetal inflammatory response during chorioamnionitis is a protective factor for chronic lung disease. May et al.[41] reported the peak exhaled NO values obtained on days 3, 5, 7, 14, 21, and 28 after birth in 80 preterm infants of <32 weeks of gestation, depending on the appearance of BPD. In the 20 preterm infants who did not develop BPD, mean (SD) peak exhaled NO level at 3 days was 4.8 (2.5) ppb. In our study, mean peak exhaled NO level in the chorioamnionitis group was 2.14 ppb (range 1.62–4.29), which is somewhat lower. This may be due to age differences at the time of sampling (mean 17.1 hr in our study and 3 days in the study of May et al.[41]).

We conclude that maternal chorioamnionitis is associated with an increase of early exhaled NO (nl/min/kg) in mechanically ventilated preterm infants weighing <2,000 g. Chorioamnionitis is also associated in the newborn with an increase of C-reactive protein and of endotracheal nitrites–nitrates levels but not of early IL-8. Although an early single measurement of exhaled NO has little clinical applicability as a marker of lung inflammation, repeated measurements during the first days or weeks of life when the infant is intubated and mechanically ventilated showing increasing values may be useful to suspect BPD. May et al.[41] have shown that an increase exhaled NO along the first 28 days of life was related to BPD, and that there was a linear trend in exhaled NO results as severity of BPD increased. In these circumstances, treatment could be prescribed to improve the prognosis of this chronic lung disease.

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