Transient Defect in Nitric Oxide Generation after Rupture of Fetal Membranes and Responsiveness to Inhaled Nitric Oxide in Very Preterm Infants with Hypoxic Respiratory Failure

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Objective To study antenatal risk factors and inflammatory responses during hypoxic respiratory failure (HRF) in infants of very low gestational age (VLGA, ≤32.0 weeks).

Study design Of a cohort of 765 VLGA infants, 144 required mechanical ventilation. Airway specimens from these patients were prospectively studied. Infants who developed HRF (oxygenation index >25) with echocardiographic diagnosis of pulmonary hypertension were treated with inhaled nitric oxide (iNO). Three gestation comparison groups were formed on the basis of specific antenatal complications: prolonged preterm rupture of membranes (PPROM), spontaneous preterm birth, and preeclampsia. Chest radiographs were studied and airway specimens were analyzed for concentrations of tumor necrosis factor-α, interleukin (IL)-6, IL-8, IL-10, IL-12p70, IL-1β, and nitrite + nitrate over 4 days.

Results Seventeen (2.2% of all VLGA infants) developed HRF. In all 17 cases, PPROM complicated the antenatal course; these infants responded to iNO, regardless of infection or PPROM. The chest radiographs of HRF and non-HRF PPROM infants were similar. Airway proinflammatory cytokines and nitrite + nitrate levels were low in infants with HRF, but they increased during iNO treatment and remained elevated after discontinuation of iNO. Each of the 3 comparison groups had different and characteristic patterns of airway cytokines and nitrite + nitrate levels.

Conclusions Seven percent of VLGA infants with preterm rupture of membranes and 15% of those with PPROM developed HRF, characterized by pulmonary hypertension that acutely responds to iNO. These infants may have a transient deficiency in the inflammatory response, including a defect in nitric oxide generation in airspaces. *(J Pediatr 2012;161:397-403)*.

See editorial, p 379

Prolonged preterm rupture of the fetal membranes (PPROM) decreases the risk of respiratory distress syndrome (RDS).[1] However, some infants with early PPROM develop hypoxic respiratory failure (HRF) that is refractory to exogenous surfactant. According to preliminary reports, some preterm infants with severe respiratory failure respond favorably to inhaled nitric oxide (iNO).[2-6]

Nitric oxide (NO) is a ubiquitous signaling molecule with multiple functions. An increase in NO production contributes to increased pulmonary blood flow at birth, allowing effective gas exchange.[7] NO, as a free radical, and its oxidation products, including nitrite and nitrate, participate in multiple interactions that have physiological or pathological consequences. According to randomized trials involving small preterm infants, iNO had a variable influence on the severity of respiratory distress.[8,9] In these trials, there were marked differences in the degree of prematurity (from <29 up to 34 gestation weeks), antenatal risk factors, race, and severity of respiratory disease, which ranged from mild respiratory distress to HRF.[10] The starting dosage (from 5 parts per million [ppm] up to 40 ppm) and duration of treatment (from 2 days to 3 weeks) with iNO and the concurrent administration of other treatment strategies also varied. Furthermore, the age of the infant at the onset of iNO treatment ranged from 12 hours to 21 days.[11] According to a meta-analysis, iNO therapy was not proved to be of benefit.[10]

Little information is available about early pulmonary cytokine homeostasis[12-15] or NO metabolism in preterm infants.[16] In the present prospective study involving the airway inflammatory responses of infants born very preterm, our aim was to

BPD	Bronchopulmonary dysplasia	PPHN	Persistent pulmonary hypertension
HRF	Hypoxic respiratory failure	ppm	Parts per million
IL	Interleukin	PPROM	Prolonged preterm rupture of fetal membranes
iNO	Inhaled nitric oxide		
iNOS	Inducible nitric oxide synthase	RDS	Respiratory distress syndrome
NO	Nitric oxide	VLGA	Very low gestational age
OI	Oxygenation index		

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Table I. Characteristics of the study patients (n = 17/group)

	HRF-iNO (group A)	PPROM (group B)	Spontaneous birth (group C)	Preeclampsia (group D)
Birth weight, mean (SD), g	1131 (647)	1134 (704)	1170 (339)	979 (431)
Gestation, mean (SD), wk	27.8 (2.3)	27.8 (2.1)	27.7 (2.1)	28.2 (2.2)
Boys/girls, n	9/8	9/8	12/5	14/3
PPROM, n	17	17	0	0
Duration, median (range), d	35 (2-77)	11 (1-63)	-	-
Onset <24 wk, n (%)	11 (65 %)	2 (12 %)	-	-
Antenatal steroid doses, 0/1/2/3*	1/2/13/1	2/3/11/1	1/8/8/0	1/0/16/0
Apgar 1/5 min, median (range)	5 (1-8)/5 (1-8)	5 (2-8)/7 (4-9)	6 (2-8)/6 (2-9)	5 (1-9)/6 (1-9)
Inotropes ≤24 h, 0/1/2†	1/5/10	7/7/3	3/10/4	9/6/2
RDS, n	17	13	15	16
Chest radiograph scoring, n	15	15		
Baseline, mean (SE)‡	2.67 (0.27)	3.06 (0.25)		
24 h later, mean (SE)§	2.60 (0.19)	2.27 (0.21)		
Surfactant doses, median (range)	2 (1-4)	1 (0-3)	1 (0-3)	2 (0-4)
Positive blood cultures, n	10	6	12	7
Age ≤3 d/>3 d, n	4/6	1/5	1/11	0/7
Ventilation, d, median (range)	4 (1.5-40)	3 (0.3-70)	2 (0.1-15)	7.5 (1-38)
Oxygen, d, median (range)	50 (5-112)	35 (0-150)	38 (1-80)	44 (1-117)
BPD¶ 1/2/3/death, n	1/9/0/2	4/4/2/0	9/2/0/0	4/5/3/0

*Antenatal betamethasone: 0 = no steroid, 1 = 1 dose, 2 = 2 doses, 3 = 2 × 2 doses.
†Inotropes given during the first 24 h: 0 = no inotropes; 1 = dopamine; 2 = dopamine and dobutamine.
‡$P = .29$, HRF vs PPROM, independent samples t test.
§$P = .25$, HRF vs PPROM, independent samples t test.
¶BPD classified according to Jobe & Bancalari[21]: 1 = mild, 2 = moderate, 3 = severe.

describe the risk factors of infants who developed HRF within 12 hours after birth. Our further objective was to define the subgroup of preterm infants who would most likely benefit from early iNO therapy. We hypothesized that a failure of NO synthesis may be of primary importance in the pathogenesis of HRF in preterm infants <32 weeks with PPROM.

Methods

A total of 765 infants born at a very low gestational age (VLGA, ≤32.0 weeks) in Oulu University Hospital (October 1997 to May 2009) and admitted to the neonatal intensive care unit were screened for the present study (Figure 1; available at www.jpeds.com). Airway specimens were recovered from the 144 infants who required mechanical ventilation. Those who developed HRF within 12 hours after birth were evaluated for a trial of iNO. The acute respiratory effects of iNO therapy on 5 infants have been described previously.[3] The 3 comparison groups (PPROM, spontaneous birth without PPROM, and preeclampsia) had similar gestation and received mechanical ventilation and supplemental oxygen for respiratory distress shortly after birth. The ethics committee approved the study, and the parents gave written informed consent.

Definition of Patients and Management

Infants who had HRF (oxygenation index [OI] >25 and arterial-alveolar oxygen pressure ratio <0.10) despite surfactant and effective ventilation underwent an echocardiographic examination. Persistent pulmonary hypertension of the newborn (PPHN) was defined as elevated pulmonary vascular resistance to the point that venous blood was diverted through fetal channels into the systemic circulation, bypassing the lungs and resulting in systemic arterial hypoxemia without any specific changes in the chest radiograph. Echocardiographic criteria of PPHN included elevation of the pulmonary artery pressure estimated from tricuspid valve regurgitation and right-to-left shunt through the fetal channels. Once PPHN was diagnosed using these criteria, 20 ppm iNO was administered via the endotracheal tube while the infant received mechanical ventilation.[17] When the need for supplemental oxygen was <50% of the initial value, iNO weaning was started, and the dosage was decreased stepwise (10 to 5 to 2 to 1 ppm) over 24-75 hours. These infants comprised the HRF-iNO group (group A, Table I). The exclusion criteria were lethal congenital diseases, chromosomal and other severe anomalies, signs of septic shock or very severe intrapartum, and birth asphyxia.

The comparison infants were chosen from the prospective cohort of 127 newborns who required mechanical ventilation and responded to conventional therapy. None of them had evidence of PPHN or received iNO therapy. They were matched pairwise to the HRF-iNO cases on the basis of gestational age (±0.5 week) and birth month/year (mean difference 1.6 years). To study the influence of antenatal factors, 3 comparison groups were defined on the basis of the following antenatal complications: (1) PPROM (group B); (2) spontaneous preterm birth without premature rupture of membranes (group C); and (3) maternal preeclampsia (group D) (Table I). The criteria for preeclampsia included maternal hypertension with increasing proteinuria, edema, and imminent compromise of fetoplacental circulation. When >1 patient fulfilled the above selection criteria, the infant with the least difference in the length of gestation was chosen. The comparisons were selected before the airway samples were analyzed.

Infants who required ventilation shortly after birth and >35% oxygen were given an initial dose of surfactant

Table II. Influence of sepsis and the onset of PPROM on the response to inhaled NO therapy showing mean (SD) OI* before and after the onset of iNO

Time, before and after iNO	−2 h	−1 h	+1 h	+3 h	+6 h	+18 h
Blood culture positive, n = 4	24.5 (10.3)	52.5 (20.4)	9.7 (5.4)	7.8 (1.3)	4.1 (1.5)	4.1 (2.2)
Blood culture negative, n = 13	25.0 (15.2)	38.0 (14.5)	8.0 (7.4)	9.1 (11.4)	6.9 (4.6)	6.2 (5.8)
Onset of PPROM <24 wk, n = 11	30.2 (11.4)	48.9 (16.0)	8.5 (7.9)	10.5 (12.2)	6.9 (5.1)	6.5 (6.1)
Onset of PPROM ≥24 wk, n = 6	20.6 (8.9)	31.2 (12.8)	8.3 (5.0)	6.1 (2.0)	4.9 (1.3)	4.3 (2.7)

*OI = mean airway pressure × percent of inspired oxygen/arterial oxygen tension.

(Curosurf, 100 mg/kg; Chiesi Pharmaceuticals, Parma, Italy). Some infants born before 28 weeks of gestation received surfactant in the delivery room. If an infant being treated for RDS required an increase in supplemental oxygen to 30%-35%, despite hemodynamic stabilization, a second dose was given. In accordance with the protocol, the ventilator settings, supplemental oxygen, blood gases, blood pressures, requirements for inotropes, and results of blood cultures were prospectively recorded. A pediatric radiologist unaware of group assignment reviewed the baseline and 24-hour chest radiographs of the HRF-iNO and PPROM groups and graded the findings on a scale of 1-5, as previously described.[18]

Analysis of Cytokines, Nitrite, and Nitrate

Airway specimens were obtained from all study infants within the first 4 days after birth: first shortly after intubation, and thereafter when deemed to be necessary.[19] Normal saline (0.5 mL/kg) was instilled into the airways through a suction catheter distal to the tip of the endotracheal tube. Thereafter, the catheter was connected to a suction trap. The procedure was repeated as needed. Concentrations of interleukin (IL)-12p70, tumor necrosis factor-α, IL-6, IL-8, IL-10, and IL-1β were analyzed with the BD Biosciences (San Diego, California) Cytometric Bead Array kit. Bead populations with distinct fluorescence intensities for specific soluble proteins were measured by flow cytometry. Nitrite + nitrate was quantified with the Nitrate/Nitrite Fluorometric Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan).[20]

Statistical Analysis

Comparisons of cytokine and nitrite and nitrate concentrations between the 4 groups were performed with the Kruskall-Wallis test. Differences in the cytokine levels were further evaluated by comparing the results of the HRF-iNO group with each of the 3 comparison groups using the Mann-Whitney *U* test or *t* test with Bonferroni correction. Statistical analysis was performed with PASW Statistics 18 for Windows (SPSS Inc, Chicago, Illinois). Significance was set at $P < .05$.

Results

Of the very premature infants screened for the study, 17 infants, representing 2.2% of the population, fulfilled the prospectively established criteria of HRF. The antenatal period of all 17 infants was complicated by PPROM and oligohydramnios. Altogether, 16 infants received antenatal steroid. Eight were born vaginally and 9 were born by cesarean section during spontaneous labor. Surfactant therapy (1-4 doses, mean age for the first dose 1.4 hours) had either no effect or a transient effect on the severity of respiratory failure. After the diagnosis of PPHN was made, iNO therapy was started at a median age of 4.0 hours (range 1.5-16.5 hours).

Shortly after the onset of iNO therapy, the OI decreased and the arterial-alveolar oxygen pressure ratio increased in each case, allowing for decreases in oxygen and airway pressure requirements (**Figure 2**; available at www.jpeds.com). The decreases were similar in infants with negative blood cultures and those with positive blood cultures. In addition, the OI of infants resulting from pregnancies complicated by PPROM at or before 24 weeks of pregnancy and those with PPROM after 24 weeks responded favorably to iNO treatment (**Table II**). The median duration of iNO therapy was 35.0 hours (lower quartile 27.5 hours, upper quartile 49.5 hours). Two of the infants died after successful early iNO therapy and stabilization. One developed overwhelming septicemia (*Enterobacter cloacae*) at the age of 9 days. The other infant died at the age of 2 days with a large intracerebral hemorrhage after drainage of a tension pneumothorax.

Antenatal glucocorticoid treatment was given to 47 (92%) of comparison mothers (**Table I**). In the PPROM group, 1 infant was delivered prior to the onset of labor due to imminent intrauterine death. Of the PPROM cases, 11 were delivered by cesarean delivery during spontaneous labor and 5 were delivered vaginally. The majority of the comparison infants (n = 44; 86%) had RDS, and some were classified as having transient respiratory distress (n = 7; 14%). Surfactant was given to 46 (90%) comparison infants at a mean age of 2.1 hours. Mean early OI in the 3 comparison groups at ages comparable to the HRF group just prior to starting iNO revealed no differences: 6.8 (range 3.6-20.9) in group B, 6.6 (range 3.9-13.6) in group C, and 8.1 (range 2.5-21.5) in group D. The primary ventilatory mode was synchronized intermittent mandatory ventilation with patient-conducted flow trigger. Seven infants in the HRF-iNO group were treated with high-frequency oscillatory ventilation.

Altogether, 33 (65%) comparison infants developed mild (n = 17), moderate (n = 11), or severe (n = 5) bronchopulmonary dysplasia (BPD) as defined by Jobe and Bancalari.[21] One infant with severe BPD in the preeclampsia group was discharged home with supplemental oxygen. Of the infants

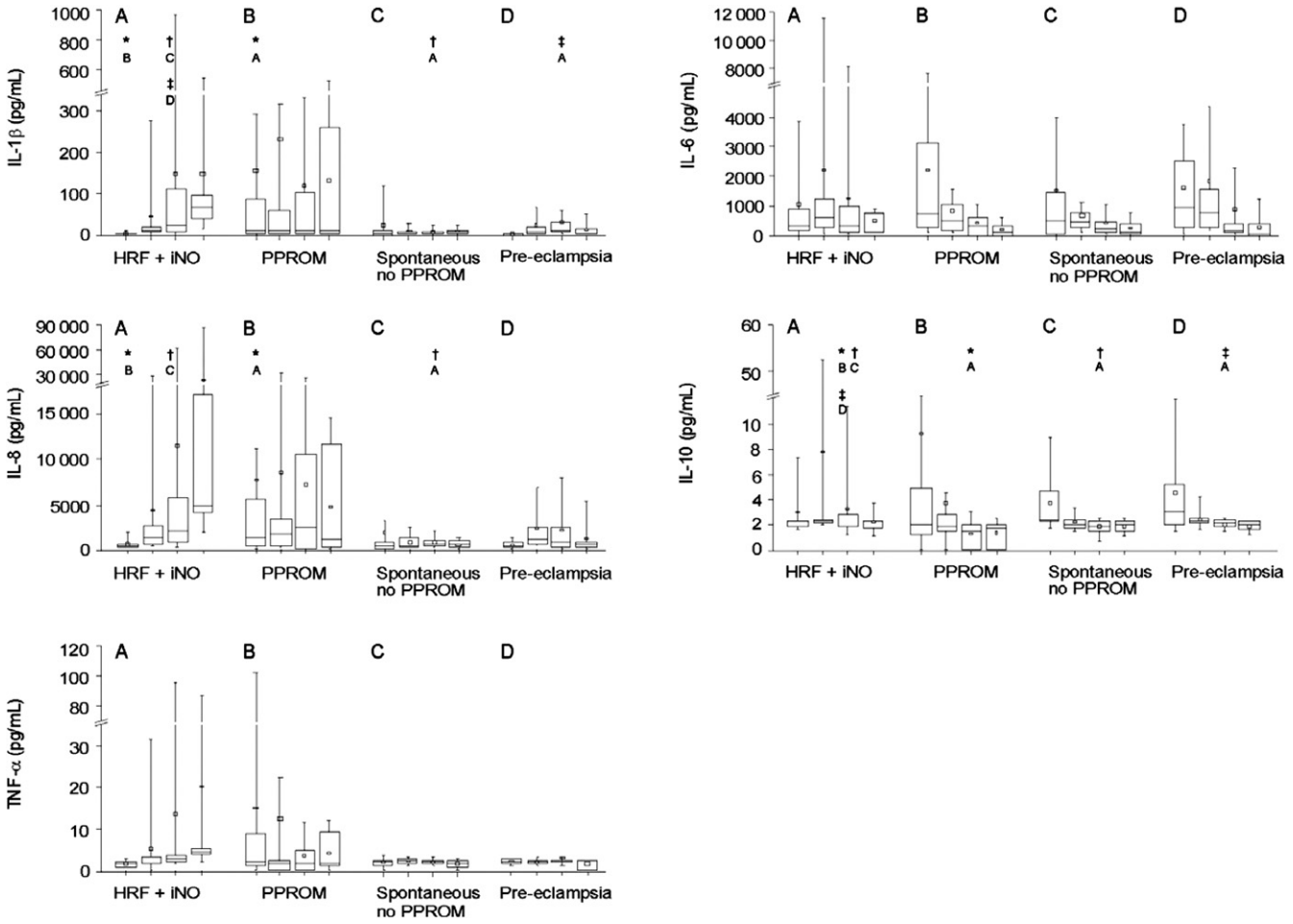

Figure 3. Concentrations of cytokines in airway specimens from the 4 groups of infants (n = 17 per group). Cytokine concentrations are shown as *box plots*: medians, IQRs, 10th and 90th percentiles, and means. The 4 categories of the airway specimens in groups A to D have been explained in detail in the Results. The cytokine concentrations of the HRF + iNO group and comparison groups B, C, and D were compared separately as described in the Methods. Significant differences ($P < .05$) are shown by both a symbol (*, †, or ‡) and the letter of the comparison group.

in the HRF-iNO group, 2 died, 10 (67% of survivors) developed mild-to-moderate BPD, and none had severe BPD. Sepsis during the first hospitalization was diagnosed in 25 (49%) comparison infants, and early sepsis was diagnosed in 2 (4%) comparison infants; the corresponding figures among infants with HRF were 10 (59%) and 4 (24%), respectively.

Fetal chest compression due to early oligohydramnios may cause pulmonary hypoplasia and HRF. All of the infants with HRF also had PPROM. The HRF-iNO group was therefore primarily compared with comparison group B (PPROM). Although the duration of PPROM tended to be longer in the HRF-iNO group compared to group B, the difference was not significant. The 2 groups of infants born from pregnancies complicated by PPROM (HRF-iNO and PPROM) were further compared. Analysis of the chest radiographs revealed no detectable differences between the 2 PPROM groups (**Table I**).

The airway specimens were divided into the following 4 categories on the basis of iNO therapy: (1) before starting iNO therapy; (2) during the first half of the iNO therapy; (3) during the second half of the iNO therapy; and (4) after stopping iNO therapy, up until 4 days of age. The airway specimens from the comparison groups were divided into 4 categories based on the following postnatal ages: (1) 0-8 hours; (2) 8.1-16 hours; (3) 16.1-24 hours; and (4) 24.1-96 hours (**Figure 3**). Comparisons between the HRF-iNO and comparison groups revealed no significant differences in the postnatal ages of these 4 categories.

During iNO treatment, the severity of respiratory distress decreased acutely and the concentrations of IL-1β and IL-8 increased. After discontinuation of iNO, there was no relapse of respiratory failure, and cytokine/chemokine concentrations were maintained at high levels. There were significant differences in the levels of the individual cytokines and the chemokine IL-8 among the 4 groups of infants (**Figure 3**).

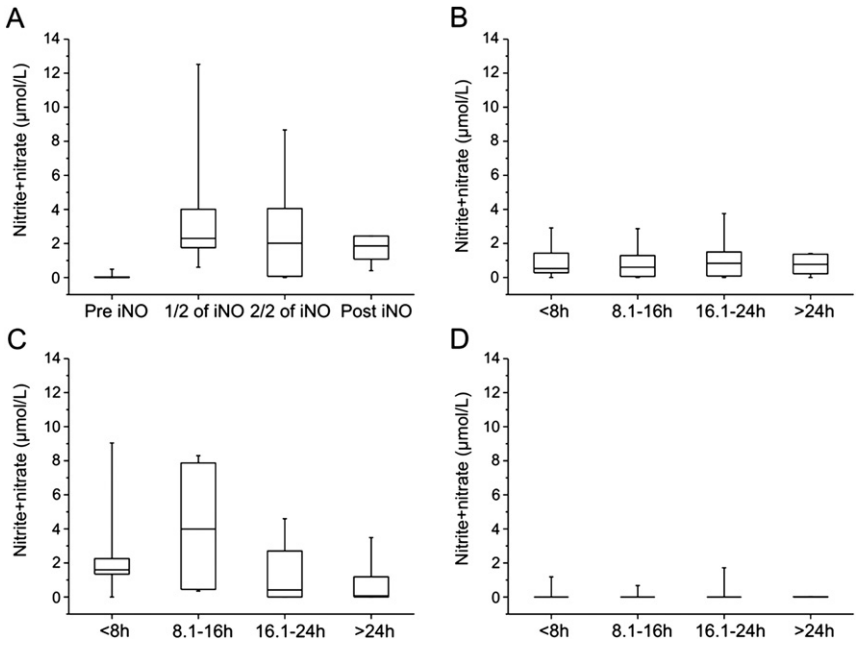

Figure 4. *Box plots* showing concentrations of nitrite + nitrate in airway specimens shortly after birth. **A,** HRF + iNO; **B,** PPROM; **C,** spontaneous birth without PPROM; and **D,** preeclampsia. In group A, shortly before iNO therapy, the concentration of nitrite + nitrate was lower than in group B or C. During early iNO therapy (1/2 iNO), the concentration in group A was higher than in group B. After the onset of iNO treatment, nitrite + nitrate concentrations were higher in group A than in group D ($P < .05$).

IL-12p70 levels were low in all groups, and no differences in IL-12p70 levels were detected among the 4 groups (data not shown). After the difference in levels of a specific cytokine among the 4 groups was established (Kruskal-Wallis test), we compared the levels of each cytokine separately between the HRF-iNO group and each of the 3 comparison groups. Before iNO therapy, the mean levels of IL-1β and IL-8 in airway specimens were lower in the HRF-iNO group than in group B, whereas during the late iNO treatment period, IL-10 levels were higher in the HRF-iNO group than in group B. During the late iNO treatment period, the levels of IL-1β and IL-10 were higher in the HRF-iNO group than in groups C and D; in addition, chemokine IL-8 was higher in the HRF-iNO group than in group C infants born after spontaneous onset of labor (Figure 3).

Because the requirement for iNO was the key distinguishing feature between the 2 groups exposed to PPROM, we compared the concentrations of nitrite + nitrate in the airway specimens. As shown in Figure 4, in the HRF-iNO group, shortly before iNO therapy began, the concentration of nitrite + nitrate was below the detection level (<0.3 μmol/L; ie, lower than in group B). During early iNO therapy (iNO at 20 or 10 ppm), the concentrations in airway specimens increased to a median of 2.3 μmol/L (IQR 1.8-4.0). After discontinuation of iNO, the median level of nitrite + nitrate was maintained at 1.8 μmol/L (IQR 1.1-2.4). In the PPROM group, nitrite + nitrate levels were continuously detectable and there were no trends. To investigate whether a low nitrite + nitrate concentration is a specific biomarker that identifies infants with HRF, the levels were recorded in comparison groups C and D. In infants born after pregnancies complicated by severe preeclampsia (group D), the levels of nitrite + nitrate were continuously low or below the detection level. In contrast, in infants born after spontaneous onset of preterm labor (group C), the concentrations of nitrite + nitrate were higher. Figure 4 shows comparisons between the HRF-iNO group and each of the 3 comparison groups.

Discussion

We show that 2.2% of infants born very preterm developed HRF shortly after birth, excluding cases of lethal malformation and severe asphyxia. All cases of HRF differed from the gestational age-matched comparisons with respiratory distress, as they were exposed to PPROM and had a characteristic development of inflammatory markers in the airways after birth. Infants with early HRF demonstrated a transient delay in airway inflammation, including low concentrations of NO metabolites. iNO acutely increased gas exchange, allowing for a decrease in both oxygen and ventilation requirements without evidence of relapse.

Severe lung hypoplasia (eg, a severe form of oligohydramnios or lethal chondrodysplasia) can present as pulmonary hypertension and HRF that is resistant to current therapies. In the present study, chronic oligohydramnios may have interfered with fetal lung growth in infants with HRF. Infection

is another potential cause of severe respiratory failure. To evaluate the role of infection and the effect of oligohydramios, we studied the acute response to iNO therapy in infants with established sepsis (n = 4) and in those exposed to PPROM starting earlier than 24.0 weeks of gestation (n = 11). A favorable effect of iNO was evident regardless of major risk factors, such as early sepsis or prolonged PPROM starting before 24.0 weeks (**Table II**). There were no detectable differences in chest radiographs between the cases with HRF and the gestational age-matched comparisons with similar exposure to PPROM. Although PPROM was a sensitive biomarker for HRF in the present cohort, it was not specific.

The beneficial influence of iNO was likely a result of pulmonary smooth muscle relaxation, which permitted critical alveolar perfusion, gas exchange, and improved ventilation. Acute dilatation of pulmonary resistance vessels in normal neonatal adaptation is managed by several physiological stimuli and specific mediators.[22] Here, we found that in pregnancies complicated by PPROM, undetectable nitrite + nitrate levels were associated with HRF. This supports the hypothesis that NO deficiency has a role in pathogenesis. The possibility that low nitrite + nitrate levels were due to excessive formation of peroxynitrite, the detrimental peroxidation of NO, is unlikely because we failed to detect significant quantities of the biomarker of peroxynitrate (nitrotyrosine) in the alveoli of infants who had died of early HRF.[19] It is also possible that NO was not available to compensate for either deficient complementary vasodilators or an inappropriate increase in vasoconstrictors, both of which may cause PPHN.[23] The higher levels of airway nitrite + nitrate observed in infants without HRF despite exposure to PPROM are consistent with this possibility.

All 3 isoforms of NO synthase are present in the preterm lung.[24] Deficiencies in endothelial NO synthase have roles in both vascular remodeling and pulmonary adaptation.[25] The activation of endothelial NO synthase at birth is likely an important factor in pulmonary vascular adaptation. The expression of inducible nitric oxide synthase (iNOS) is constitutively low, but this enzyme is induced in monocytes/macrophages as a result of inflammatory stimuli.[26] Cells from airway specimens and alveolar macrophages sampled after the early death of preterm infants with HRF revealed no immunoreactive iNOS, whereas in infants who died of late sepsis, alveolar macrophages had prominent iNOS staining.[19] The functional significance of these observations is unknown. On the basis of the present cohort, 7% of VLGA infants with prelabor rupture of fetal membranes and 15% of those with PPROM (≥7 days) developed HRF. The possibility that an early delay in NO generation is a potential genetic trait that influences the risk of HRF remains to be studied.

We did not expect to find mostly undetectable levels of nitrite + nitrate in airway samples from infants born after severe preeclampsia. However, this finding is consistent with evidence showing defective synthesis of NO in severe preeclampsia.[27] The low levels of nitrate + nitrite in fetal airways may reflect low iNOS protein levels or low NO precursor uptake into monocytes that were isolated from infants born from preeclamptic pregnancies compared to those from normal pregnancies.[28] Despite low nitrite + nitrate levels in airway specimens, infants born after severe preeclampsia did not develop HRF. Neonatal reversal of severe intrauterine hypoxemia in preeclampsia may contribute to the network of smooth muscle constrictors, dilators, growth factors, and cytokines that regulate alveolar perfusion and gas exchange during the perinatal transition.[22]

Our analysis of the early acute responses of inflammatory cytokines in ventilated infants revealed that the gestational age-matched subjects had individual patterns that may depend on the antenatal complications that caused very preterm birth (**Figure 3**). The 2 groups with PPROM showed robust inflammatory cytokine responses, with the exception of an early delay in the infants who developed HRF. Acute inflammation is associated with severe injury,[29] deterioration of lung function,[30] and an increase in pulmonary vascular resistance.[31] Indeed, an excess of cytokines in the postnatal period is associated with an increased risk of BPD.[12,14] On the other hand, inappropriately low cytokine responses during oxidant stress, trauma, or infection may delay the induction of protective mechanisms.[19,32]

There are some limitations to the present study. The number of affected infants was small despite recruitment of 765 VLGA infants. Randomized studies would confirm the efficacy and safety of iNO to treat HRF. Trials studying either the dose response of iNO or comparing different vasodilators would provide important information.

In conclusion, very preterm infants who developed HRF within a few hours of birth were all exposed to PPROM. During HRF, a transient suppression in nitrite + nitrate and inflammatory cytokine levels was evident. During iNO-induced remission, nitrite + nitrate concentrations increased as expected. The elevated nitrite + nitrate and inflammatory cytokine levels were maintained during recovery after discontinuation of iNO. Defining the cause and prevention of deficient NO production and limiting the excessive inflammatory response during the recovery period may help to prevent HRF and chronic lung disease in very preterm infants. ■

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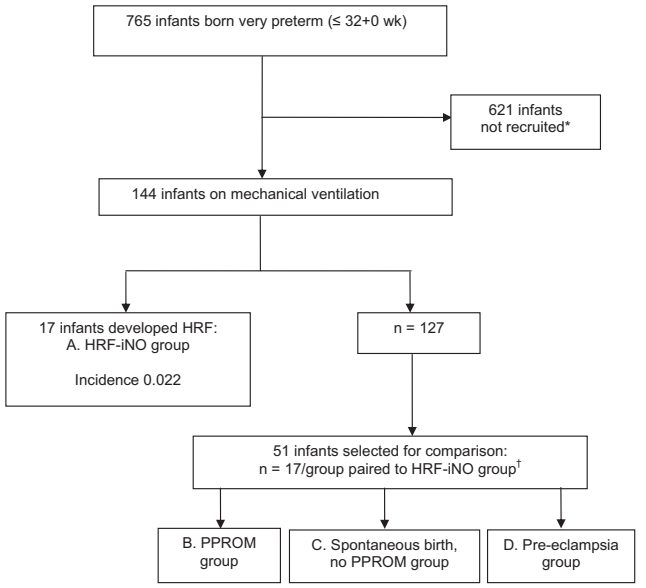

Figure 1. Enrollment of the study population. *Reasons for not being recruited: not intubated, recruited for another study, no consent, lethal malformation, and severe birth asphyxia. †Selected on the basis of similar length of gestation (±0.5 week) and month/year of birth (mean difference 1.67 years).

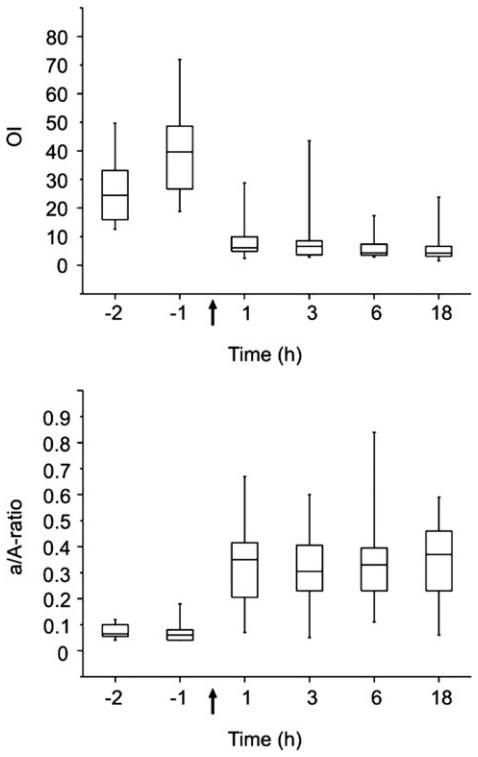

Figure 2. *Box plots* showing medians, IQR, and ranges of OI and arterial-alveolar (a/A) oxygen pressure ratios for HRF + iNO infants before and after the onset of iNO therapy (*arrow*).