# UC Davis

## UC Davis Previously Published Works


### Title

Early Use of Inhaled Nitric Oxide in Preterm Infants: Is there a Rationale for Selective Approach?


### Permalink

https://escholarship.org/uc/item/7pn282sh



### Journal

American Journal of Perinatology, 34(05)

### ISSN

0735-1631



### Authors

Chandrasekharan, Praveen
Kozielski, Rafal
Kumar, Vasantha HS
et al.



### Publication Date

2017-04-01

### DOI

10.1055/s-0036-1592346

Peer reviewed



eScholarship.org Powered by the California Digital Library
University of California

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

*Am J Perinatol*. Author manuscript; available in PMC 2017 August 27.

Published in final edited form as:

*Am J Perinatol*. 2017 April ; 34(5): 428–440. doi:10.1055/s-0036-1592346.

# Early Use of Inhaled Nitric Oxide in Preterm Infants: Is there a Rationale for Selective Approach?

**Praveen Chandrasekharan, MD**[1], **Rafal Kozielski, MD**[2], **Vasantha H.S. Kumar, MD**[1], **Munmun Rawat, MD**[1], **Veena Manja, MD**[3,4], **Changxing Ma, PhD**[5], and **Satyan Lakshminrusimha, MD**[1]

[1]Division of Neonatology, Department of Pediatrics, Women and Children's Hospital of Buffalo, Buffalo, New York

[2]Department of Pathology, Women and Children's Hospital of Buffalo, Buffalo, New York

[3]Department of Internal Medicine (Cardiology), University at Buffalo, Buffalo, New York

[4]Department of Epidemiology, McMasters University, Hamilton, Ontario, Canada

[5]Department of Biostatistics, School of Public Health, University at Buffalo, Buffalo, New York

## Abstract

**Background—**Inhaled nitric oxide (iNO) is being increasingly used in preterm infants < 34 weeks with hypoxemic respiratory failure (HRF) and/or pulmonary hypertension (PH).

**Objective—**To evaluate the risk factors, survival characteristics, and lung histopathology in preterm infants with PH/HRF.

**Methods—**Retrospective chart review was conducted to determine characteristics of 93 preterm infants treated with iNO in the first 28 days and compared with 930 matched controls. Factors associated with survival with preterm HRF and smooth muscle actin from nine autopsies were evaluated.

**Results—**Preterm neonates treated with iNO had a higher incidence of preterm prolonged rupture of membrane (pPROM ≥ 18 hours), oligohydramnios and delivered by C-section. In infants treated with iNO, antenatal steroids (odds ratio [OR], 3.7; confidence interval [CI], 1.2–11.3; $p = 0.02$), pPROM (OR, 1.001; CI, 1.0–1.004; $p = 0.3$), and oxygenation response to iNO (OR, 3.7; CI, 1.08–13.1; $p = 0.037$) were associated with survival. Thirteen infants with all three characteristics had 100% (13/13) survival without severe intraventricular hemorrhage (IVH)/periventricular leukomalacia (PVL) compared with 48% survival (12/25, $p = 0.004$) and 16% severe IVH/PVL without any of these factors. Severity of HRF correlated with increased smooth muscle in pulmonary vasculature.

**Conclusion—**Preterm infants with HRF exposed to antenatal steroids and pPROM had improved oxygenation with iNO and survival without severe IVH/PVL. Precisely targeting this subset may be beneficial in future trials of iNO.

Address for correspondence Praveen Chandrasekharan, MD, Division of Neonatology, Department of Pediatrics, Women and Children's Hospital of Buffalo, University at Buffalo, 219 Bryant Street, Buffalo, NY 14222 (pkchandr@buffalo.edu).

**Conflict of Interest**

None.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

**Keywords**

inhaled nitric oxide; hypoxemic respiratory failure; preterm prolonged rupture of membrane; pulmonary hypertension; smooth muscle area ratio

Inhaled nitric oxide (iNO) is a selective pulmonary vasodilator and is effective in term and near-term infants with hypoxemic respiratory failure (HRF) and persistent pulmonary hypertension of the newborn (PPHN).[1] However, iNO does not reduce mortality or bronchopulmonary dysplasia (BPD) in preterm neonates ≤ 34 weeks postmenstrual age (PMA) with HRF.[2] A consensus statement from the National Institutes of Health (NIH) stated that available evidence does not support the use of iNO as an early-rescue regimen in preterm infants < 34 weeks PMA.[3] American Academy of Pediatrics (AAP) strongly recommends against routine or rescue use of iNO in preterm infants.[4] Both these statements cited safety concerns with the use of iNO in extremely low birth weight infants. This cohort of infants had higher mortality and risk of intraventricular hemorrhage (IVH) with iNO therapy for HRF. Despite these recommendations, off-label use of iNO continues to increase in preterm infants in the neonatal intensive care unit (NICU)[5] possibly by instinct or in a desperate attempt when faced with an extremely hypoxemic preterm infant on maximal ventilator support.[6]

Inhaled NO therapy has not been beneficial in preterm infants with HRF in randomized trials.[7–9] A large prospective multicenter randomized controlled trial conducted by Van Meurs et al did not demonstrate any reduction in mortality or BPD with iNO in premature infants with HRF.[10] In fact, the incidence of severe IVH and mortality was higher in the iNO group as compared with placebo in the ≤ 1,000 g birth weight infants. However, a retrospective post hoc analysis of this study suggested that a subset of premature infants with preterm prolonged rupture of membranes (pPROM), oligohydramnios, and pulmonary hypoplasia may have benefitted from iNO therapy.[11] Similar conclusions were derived from other case reports/series linking pPROM to improved survival with iNO in preterm infants[12–14] possibly because of a transient defect in NO generation with pPROM.[15]

The existence and the hemodynamic and histological features of early pulmonary hypertension (PH) and HRF in preterm infants are subjects of controversy.[6,16] Recently, Cheng et al[17] utilized functional echocardiography (fECHO) to assess hemodynamics prior to the use of iNO and demonstrated tricuspid regurgitation and bidirectional/right-to-left shunt at patent ductus arteriosus and patent foramen ovale in preterm infants with PH. Histologically, the intra-acinar arteries in the developing lungs of preterm infants are non-muscular or have a partial muscular layer.[18] Unlike term infants and preterm infants with BPD,[19] where clinical, hemodynamic, and histological features of pulmonary hypertension[20] are well established, there is limited literature in preterm infants with early onset HRF and PH (first 28 days of postnatal age, before the onset of BPD).[18,19] Despite improvements in the use of antenatal steroids, ventilation strategies, and nutrition in preterm infants, the use of iNO in this population has not decreased. We report a single-center retrospective study over a 13-year period looking at clinical and histological characteristics of preterm infants with HRF treated with iNO. We first compared preterm infants with HRF

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

treated with iNO with matched controls to identify risk factors associated with HRF. Next, we evaluated factors that predicted a favorable outcome following iNO therapy in preterm infants. Finally, we correlated severity of HRF to pulmonary vascular remodeling at autopsy.

## Methods

The Children and Youth Institutional Review Board of the State University of New York at Buffalo approved this study. Preterm infants (< 34 weeks PMA at birth) treated with iNO during the first 28 days of postnatal agebetween January 2002 and December 2014 at the Women and Children's Hospital of Buffalo were identified through a neonatal database (Neodata Isoprime Corporation, Lisle, IL). During this period, it was common practice to initiate iNO therapy in preterm infants with persistent hypoxemia (after optimal lung recruitment and surfactant) and/or clinical or echocardiographic evidence of PH. During this 13-year period, the number of deliveries in Erie County, NY has remained constant (range, 11,093–11,387). The number of deliveries at our institution has not changed over this period (2,901–3,175 per year). Admissions to the NICU ranged between 809 to 879 per year during this period.

In this study, we included infants treated with iNO for HRF (defined as need for mechanical ventilation with an oxygenation index [OI] ≥ 10) or PH identified on an echocardiogram. Oxygenation index was calculated as (mean airway pressure [cm $H_2O$] × fraction of inspired oxygen [$FIO_2$] × 100) ÷ partial pressure of oxygen in arterial blood ($PaO_2$) (mmHg). Echocardiographic evidence of PH was based on measurement of the direction of the ductal and foramen ovale shunt, flattening or leftward deviation of the interventricular septum, and tricuspid regurgitation velocity on continuous wave Doppler with simultaneous systemic blood pressure measurement to estimate right-sided pressures.[21] Preterm infants treated prophylactically with iNO to prevent BPD were excluded from the study (this strategy was briefly practiced during 2009–2010 and was discontinued after the release of NIH consensus statement). Infants who had major congenital cardiac and other major anomalies were excluded. Each iNO-treated case was matched with 10 control infants mechanically ventilated for at least 7 days, matched by gestational age (same week PMA), birth weight (within ± 50 g), gender and ± 1 year of birth (if a case was born in 2008, 10 controls of similar PMA and birth weight were chosen with birthdates between January 2007 and December 2009). If 10 preterm infants of the same gender were not available within this time, infants from the opposite gender were chosen.

For the purpose of this study, only mothers who received two doses of betamethasone were considered to have received antenatal steroids. Rupture of membranes ≥ 18 hours prior to delivery was used as the cut-off for definition of pPROM.

The time of initiation of iNO along with indication (PH and/or HRF), blood gas data before and after initiation of iNO, and ventilator settings were collected in the study group. Initiation of iNO within the first 4 weeks of postnatal period was arbitrarily termed as "early" iNO therapy to distinguish patients with BPD and PH. A complete oxygenation response to iNO was defined as an increase in $PaO_2/FIO_2$ by ≥ 20 mm Hg.[21] Major morbidities such as BPD (defined as oxygen dependence at 36 weeks PMA), IVH grade 3

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and 4 and or PVL, necrotizing enterocolitis (NEC)–stage 2 and 3, retinopathy of prematurity (ROP) stage ≥ 3 and/or treatment (laser therapy or anti-vascular endothelial growth factor injection) were collected.

## Lung Histopathology

Sections of the lung tissue obtained at autopsy were evaluated for morphologic changes in arterioles in PH and/or HRF. The histology slides were prepared from formalin-fixed, paraffin-embedded tissue sectioned at 4.0 μm and stained with hematoxylin–eosin. An additional immunohistochemical stain for smooth muscle actin (SMA) was performed. The deparaffinized and rehydrated tissue sections were incubated with SMA primary antibody (Actin, smooth muscle (1A4) prediluted mouse monoclonal antibody, Ventana catalog number: 760–2833, Ventana, Basel, Switzerland) followed by application of the Ventana Roche detection kit according to the automated slide stainer manufacturer's instructions. The slides were counter-stained with hematoxylin. Appropriate negative and positive control tissues were used as an external validation of the immunostaining procedure. Nine preterm infants < 34 weeks PMA who died at < 28 days postnatal age and had an autopsy performed were chosen. Hematoxylin and eosin (H&E)-stained slides were used to assess the patency of the intra-acinar arteries and corresponding SMA-stained sections were used to assess the thickness of the smooth muscle. All slides were scanned at 40× using Aperio scanner (Leica Biosystems, San Diego, CA, USA) and further analysis of scanned whole-slide images was performed using the Aperio ImageScope viewing application. Image analyzer was used to measure the smooth muscle area of the intra-acinar arteriole with diameter < 60 μm. The vessel structure was analyzed at 40×magnification. The two independent analysts (P. K. and M. R.) were blinded to the respiratory course of the patients and measured the area of the smooth muscle. Ten random fields of intra-acinar arteries were chosen to assess the smooth muscle area and the ratio of the area of smooth muscle to the total area of the vessel was calculated.

Arterial blood gases and OI data were not available for some controls and a few cases. A simplified respiratory severity score (RSS = $FIO_2$ ×mean airway pressure [MAP]) was calculated at a specified time (12 noon) when the infant was stable each day. The mean RSS for the entire length of stay of each infant (average of daily RSS values from the date of birth to date of death) was correlated with histological changes.[22,23]

## Statistics

Bivariate analyses were performed to assess the characteristics of infants with PH and/or HRF and their outcomes. Normally distributed continuous variables are expressed as mean and standard deviation (SD) and skewed variables as median and interquartile range (IQR). Chi-square tests were used for categorical variables and unpaired t-test for continuous variables. Cases were further analyzed to study the characteristics of survivors using logistic regression. Multiple logistic regressions were used to calculate odds ratio and adjusted odds ratio (OR) with 95% confidence interval (CI). Variables in regression analysis were selected by forward stepwise regression with statistical significance defined as $p < 0.05$. Patients with missing data points were excluded from the analysis. The ratio of thicknesses of smooth

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

muscle to the whole area of the vessel was correlated to the severity of respiratory illness (RSS) for the entire NICU course as described previously.[24,25]

## Results

### Factors Associated with Pulmonary Hypertension and/or Hypoxemic Respiratory Failure and Treatment with Inhaled Nitric Oxide (Comparison with Controls)

Ninety-three preterm infants with early PH and or HRF were treated with iNO within the first 28 days of postnatal age between 2002 and 2014. They were matched with 930 controls. The characteristics of these infants are shown in Table 1. Infants with PH and/or HRF and treated with iNO had a higher incidence of delivery by cesarean section, pPROM, and oligohydramnios compared with controls. There was no difference in frequency of prenatal corticosteroid use or chorioamnionitis. As expected, infants with PH and/or HRF treated with iNO had longer periods of intubation and ventilation. Preterm infants treated with iNO had a shorter duration of noninvasive ventilation following extubation (Table 2). Infants with PH and/or HRF treated with iNO also had higher rates of BPD, vasopressor use, postnatal steroid use, severe ROP, and mortality compared with controls (Table 2).

### Initiation of Inhaled Nitric Oxide

Fifty-seven percent of the infants treated with iNO were extremely preterm at ≤ 25 weeks PMA at birth. Inhaled NO was initiated at 20 ppm within the first 2 weeks after birth in 84% of patients. The OI prior to initiation of iNO was 32 ± 23.

### Indication for Inhaled Nitric Oxide Therapy

Therapy with iNO was initiated for two indications: preterm neonates with moderate HRF (OI, 25 ± 13) with echocardiographic evidence of PH ($n = 29$) prior to initiation of iNO; and severe HRF (33 ± 15) where iNO was initiated as a desperate measure without a prior echocardiogram ($n = 64$). All infants underwent an echocardiogram within a few hours of iNO initiation and six additional infants in this group also had signs of PH in spite of treatment with iNO. Inhaled NO was initiated at an earlier postnatal age in infants with PH on echocardiogram compared with infants with severe HRF without PH (6 ± 5 vs 9 ± 6 days, $p = 0.02$). Echocardiographic evidence of PH prior to initiation of iNO was not associated with a higher incidence of oxygenation response to iNO (13/29 = 45% vs 27/64 = 42%, $p = 0.8$) or mortality (5/29 = 17% vs 18/64 = 28%, $p = 0.26$). The subset of infants with HRF without prior echocardiogram had a birth weight of 876 ± 490 g, PMA of 26 ± 3 weeks at birth, and OI of 33 ± 26 prior to iNO and were associated with a 28% mortality and 12.5% risk of grade 3 or 4 IVH and PVL. The subset of 29 patients with evidence of PH on echocardiography prior to initiation of iNO were born at 27 ± 3 weeks gestation with a birth weight of 992 ± 564 g and had OI of 24.9 ± 13.3 prior to iNO and were associated with 17% mortality and 10% risk of grade 3 or 4 IVH and PVL.

### Oxygenation Response to Inhaled Nitric Oxide

Forty infants (43%) had a short-term oxygenation response to iNO[3] (20 mm Hg increase in $PaO_2/FIO_2$ ratio) (Table 3). Responders had significantly higher birth weight, gestational

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

age, higher incidence of pPROM, and better survival compared with nonresponders (Table 3).

### Duration of Inhaled Nitric Oxide Therapy

The duration of treatment of iNO was not significantly different between responders and nonresponders (Table 3). Inhaled NO was weaned, but continued at low doses for prolonged period among some survivors. The duration of iNO therapy among preterm infants in this study was 316 ± 255 hours. The median duration of iNO therapy was shorter among responders compared with nonresponders but was not statistically significant (Table 3). Among survivors, the median duration of iNO therapy was 277 hours (IQR, 76–575 hours). In comparison, the mean duration of iNO use among term babies with PPHN (excluding diaphragmatic hernia) at our institution from 2012 to 2014 is 46 ± 45 hours. The current cost of iNO per hour at our institution is $137. The median cost of iNO therapy (using current rates) was $ 37,949 per survivor.

### Factors Associated with Survival

Among preterm infants treated with iNO for PH and/or HRF, the infants who survived had a complete course of prenatal steroids, pPROM, and a short-term response to iNO (Table 4).

Logistic regression analysis after controlling for gestational age and birth weight showed increased odds for survival with complete course of antenatal steroids (OR, 3.7; CI, 1.2–11.3; $p = 0.02$) and short-term response to iNO (OR, 3.7; CI, 1.08–13.1; $p = 0.037$). pPROM was significant by unadjusted OR (1.1 [1.001–1.400, $p = 0.03$]). However, after adjusting for gestational age and birth weight, pPROM (OR, 1.001; CI,1.0–1.004; $p = 0.3$) had an increased tendency toward survival, but was not statistically significant (Fig. 1).

Based on these findings, the mortality and neurologic morbidity along with BPD of individual groups are shown as an algorithmic flow chart in Fig. 2. Preterm infants with PH and or HRF exposed to prenatal steroids and delivered after at least 18 hours of pPROM with a short-term oxygenation response to iNO ($n = 13$) had a 100% survival without severe IVH or PVL. In contrast, preterm infants with PH and/or HRF without prenatal steroids or PROM who did not respond to iNO had a 48% survival with 16% incidence of severe IVH or PVL ($p = 0.004$) (Fig. 2).

### Outcomes Based on Gestational Age

The incidence of BPD was significantly different at different gestational age groups decreasing from 90% in the most immature cases (≤24 weeks PMA) to 53% of$^{3}$28 weeks developing BPD compared with the rest ($p < 0.05$, Table 5). The incidence of NEC, ROP, IVH, and PVL decreased with advancing gestational age but this difference did not reach statistical significance (Table 5).

### Histology

The clinical characteristics of nine infants who died with HRF and underwent autopsy are shown in Table 6. Five of these infants were treated with iNO and four were not (Table 6). The mean RSS for the whole duration of each infant’s hospitalization is shown in this table.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

A representative sample of H&E and the SMA stained lung sections are shown in Fig. 3A,B. An example of a normal intra-acinar artery with partial muscularization from a preterm infant that died due to a nonrespiratory cause is shown in Fig. 3C,D for comparison. The smooth muscle to the total area of the vessel was measured and expressed as smooth muscle area ratio (SMR). The RSS in these nine infants were 9.2 ± 2.6 and the SMR was 0.729 ± 0.048. There was a positive correlation between SMR and RSS (Fig. 4).

## Discussion

The clinical features and pulmonary vascular pathology in delayed PH associated with BPD has been well characterized[19,26] but there is limited literature on early PH and/or HRF, described predominantly in the first few weeks after birth in extremely preterm infants with RDS.[27–30] This single-center case series provides clinical and histological characteristics of preterm infants presenting with HRF with or without PH that were treated with iNO at our institution.

Bedside fECHO findings including determination of pulmonary blood flow and pressure should ideally be performed prior to initiation of iNO to rationalize this expensive therapy. However, similar to many units in the United States, we did not have access this technique. Hence, we report limited information on echocardiography from our cases and attempt to look at the following four clinical factors to determine a precise subgroup of preterm infants with PH and/or HRF with better chance of survival following iNO therapy: 1) presence of documented PH by echocardiogram prior to iNO therapy, 2) prenatal steroid therapy, 3) pPROM, and 4) short-term oxygenation response to iNO (Table 4).

### Echocardiographic Evidence of Pulmonary Hypertension

The existence of PH and response to iNO in preterm infants is a subject of controversy. Evidence of PH may include signs of elevated pulmonary arterial pressure such as tricuspid regurgitation or interventricular septal flattening. In addition, a right-to-left shunt or bidirectional shunt is an important variable relied upon by clinicians to adjudicate PH. Documentation of low pulmonary blood flow (Qp) may also be a sign of increased pulmonary vascular resistance (PVR). Desandes et al[31] reported that presence of low pulmonary blood flow on echocardiogram predicted a short-term (30 minute) positive oxygenation response to iNO in preterm infants.[31] Dani et al could not replicate these findings and showed that only birth weight (and not echocardiographic PH as measured by signs of elevated pressure and not low flow) predicted response to iNO at 6 hours.[32] We could not find an association between echocardiographic evidence of PH prior to initiation of iNO and oxygenation response or mortality. However, we did not have echocardiography in all subjects prior to initiating iNO therapy and may have missed infants with severe HRF with coexisting PH. We also did not measure pulmonary blood flow as done by Desandes et al and may have missed patients with low flow and no evidence of elevated pulmonary arterial pressure. These results may be explained by two speculative mechanisms. Low pulmonary blood flow may be a better predictor of response to iNO than elevated pulmonary arterial pressure.[33] The second possibility is that evidence of PH by echocardiogram only predicts short-term response to iNO (30 minute) but does not predict sustained response at 6

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

hours. For these reasons, we elected to focus on survival without severe IVH and/or PVL in this study. We speculate that clinical improvement seen in infants with HRF without echocardiographic evidence of PH may be due to ventilation–perfusion matching.[34]

## Prenatal Corticosteroids

Preterm infants with PH and/or HRF treated with iNO and exposed to complete course of prenatal corticosteroids had better survival (43/50 = 86% vs 27/43 = 63% without prenatal corticosteroids, Fig. 2) and higher incidence of a positive oxygenation response to iNO (23/50 = 48% vs 16/43 = 37%). The beneficial effects of prenatal glucocorticoids on respiratory outcomes in preterm and term infants (after elective cesarean section)[35] are well known. In preterm lambs, pre-natal betamethasone improves pulmonary blood flow.[36,37] In late preterm lambs with PPHN induced by antenatal ductal ligation, prenatal betamethasone improved oxygenation, reduced pulmonary arterial pressure, and increased systemic blood pressure.[38] Prenatal betamethasone also enhances isoproterenol and prostaglandin E1-mediated relaxation in pulmonary arteries and veins and NO donor-mediated relaxation in pulmonary veins and coronary arteries possibly by increasing soluble guanylate cyclase in vascular smooth muscle.[39–42] We speculate that a similar mechanism may explain its beneficial effects of iNO in preterm infants with PH and/or HRF.

We also observed that the presence of pPROM and oligohydramnios were associated with PH and/or HRF (Table 1). pPROM appears to have contradicting effects on preterm infants. It is possible that pPROM induces a transient defect in NO generation,[15] thereby increasing the risk of PH and/or HRF and overcoming this defect with iNO improves outcome but at the same time affects lung maturity. The recently released Pediatric PH guidelines from the American Heart Association and the American Thoracic Society state: “iNO can be beneficial for preterm infants with severe hypoxemia that is primarily due to PPHN physiology rather than parenchymal lung disease, particularly if associated with prolonged rupture of membranes and oligohydramnios” (Class IIa; Level of Evidence B).[43] Our data agree with this statement and infants treated with iNO for PH and/or HRF, history of pPROM was present in 37% of survivors and only 13% of nonsurvivors (Table 4). In fact, survival was 90% among preterm infants treated with iNO for PH and/or HRF and history of pPROM. Similar to these findings, other investigators have observed the beneficial effect of iNO in preterm infants with HRF and pPROM.[11–14]

## Oxygenation Response to Inhaled Nitric Oxide

The largest randomized trial of iNO in preterm infants with HRF included 210 infants with a birth weight of 840 ± 264 g at 26 ± 2 weeks gestation with a baseline OI of 23 ± 17. Inhaled NO at 5 ppm increased $PaO_2$ by > 20 mm Hg in 57% of subjects (compared with 17% with placebo). The overall mortality rate in the iNO group ($n$ = 210) was 52% with a 39% risk of grade 3 or 4 IVH or PVL.[10] However, this study did not report the mortality or incidence of IVH and/or PVL among preterm infants with short-term oxygenation response. We found significantly higher survival among preterm infants with short-term oxygenation response to iNO (Table 3). The incidence of survival without severe IVH and/or PVL among responders was 2.8% (1 out of 35)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## Cost of Inhaled Nitric Oxide Therapy

The duration of iNO use among preterm infants and associated cost is a source of concern.[44] Our institution has an automated weaning protocol[45] with respiratory therapists weaning iNO if peripheral capillary oxygen saturation[3] is 90% and $FIO_2$ is < 0.6. As mentioned previously, the duration of iNO therapy among preterm infants in this study was prolonged compared with term infants. There was no significant difference in duration of iNO therapy between responders and nonresponders. Treatment with iNO does not restore endothelial nitric oxide synthase (eNOS) function[46,47] and inhibits eNOS activity in term lambs.[48] We speculate that a similar effect in preterm infants may explain the inability to wean them off iNO after initiation.

## Histology

Intra-acinar arteries are usually partially muscularized or not muscularized in term infants.[18,25] Interestingly, preterm infants who died with severe HRF demonstrated completely muscularized intra-acinar arteries. The histological features of these pulmonary arteries was similar to that described in term infants with PPHN[49] and preterm infants with BPD and PROM.[24,25] The smooth muscle thickness did not show a trend with gestational age or postnatal age at the time of death of these infants. We found a positive correlation between the mean RSS for the entire duration of NICU stay to intra-acinar pulmonary arterial smooth muscle thickness and were similar in infants treated with and without iNO (Fig. 4). But these data were based on nine infants and further studies evaluating histological evidence of pulmonary artery remodeling are warranted.

## Limitations

Besides being a single-center retrospective study, we did not have echocardiogram done prior to initiating iNO on all infants with HRF. In addition, we did not have uniform oxygenation index cut-off for initiation of iNO. Over the years, our changes in practice such as earlier extubation, caffeine use, and lesser days of use of invasive mode of ventilation might have affected the overall outcomes. Few infants did not have arterial blood gases and oxygenation indices and we used RSS as an alternative to assesses severity of HRF especially in infants that did not survive and had an autopsy. The cost estimates were based on the current cost of iNO and not at the time of use. The low number of infants with autopsy (9/23 infants) limits the generalizability of these findings. The pulmonary arteries in the autopsy specimen were not perfusion-fixed. Nevertheless, percentage circumference of intra-acinar arteries with SMA is a qualitative assessment of muscularization that is not dependent on the degree of vessel expansion[24] and it positively correlated with the RSS.

We conclude that early HRF in preterm infants is associated with pulmonary vascular remodeling similar to PPHN in term infants. We agree with the recommendation from AAP and consensus panel at NIH that based on currently available evidence from randomized controlled trials there is no indication for rescue therapy with iNO for HRF in preterm infants. However, data from our center, California Perinatal database and Pediatrix database show that nontargeted indiscriminate use of iNO is a common practice. In today's era of precision medicine in conditions such as pulmonary arterial hypertension,[50] it is crucial to identify a specific target preterm population with HRF that can potentially benefit from iNO

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(Fig. 5). Our study suggests that among preterm infants < 34 weeks gestation with HRF, the presence of the following criteria may enhance survival: 1) exposure to a complete course of prenatal steroids, 2) prolonged rupture of membranes, and 3) a positive short-term oxygenation response to a 60-minute trial of iNO. Echocardiographic evidence of increased PVR (low Qp and high pulmonary arterial pressures) was not evaluated in this study but may potentially be an important determinant of survival among preterm infants treated with iNO. A randomized trial to address the benefit of iNO in this subpopulation with echocardiographic evidence of PH is required, although practically difficult due to lack of equipoise among neonatal providers.[51] In addition, effective strategies to wean iNO in infants with HRF, limiting the duration of exposure, and reducing costs require further investigation.

## Acknowledgments

**Funding**

Grant number NICHD-1R01HD072929-0 (SL).

## References

1. Finer NN, Barrington KJ. Nitric oxide for respiratory failure in infants born at or near term. Cochrane Database Syst Rev. 2006; (4):CD000399.doi: 10.1002/14651858.CD000399.pub2 [PubMed: 17054129]
2. Askie LM, Ballard RA, Cutter GR, et al. Meta-analysis of Preterm Patients on Inhaled Nitric Oxide Collaboration. Inhaled nitric oxide in preterm infants: an individual-patient data meta-analysis of randomized trials. Pediatrics. 2011; 128(4):729–739. [PubMed: 21930540]
3. Cole FS, Alleyne C, Barks JD, et al. NIH Consensus Development Conference statement: inhaled nitric-oxide therapy for premature infants. Pediatrics. 2011; 127(2):363–369. [PubMed: 21220405]
4. Kumar P. Committee on Fetus and Newborn; American Academy of Pediatrics. Use of inhaled nitric oxide in preterm infants. Pediatrics. 2014; 133(1):164–170. [PubMed: 24379225]
5. Ellsworth MA, Harris MN, Carey WA, Spitzer AR, Clark RH. Off-label use of inhaled nitric oxide after release of NIH consensus statement. Pediatrics. 2015; 135(4):643–648. [PubMed: 25755237]
6. Finer NN, Evans N. Inhaled nitric oxide for the preterm infant: evidence versus practice. Pediatrics. 2015; 135(4):754–756. [PubMed: 25755239]
7. Field D, Elbourne D, Truesdale A, et al. INNOVO Trial Collaborating Group. Neonatal Ventilation With Inhaled Nitric Oxide Versus Ventilatory Support Without Inhaled Nitric Oxide for Preterm Infants With Severe Respiratory Failure: the INNOVO multicentre randomised controlled trial (ISRCTN 17821339). Pediatrics. 2005; 115(4):926–936. [PubMed: 15805366]
8. Kinsella JP, Walsh WF, Bose CL, et al. Inhaled nitric oxide in premature neonates with severe hypoxaemic respiratory failure: a randomised controlled trial. Lancet. 1999; 354(9184):1061–1065. [PubMed: 10509496]
9. Subhedar NV, Ryan SW, Shaw NJ. Open randomised controlled trial of inhaled nitric oxide and early dexamethasone in high risk preterm infants. Arch Dis Child Fetal Neonatal Ed. 1997; 77(3):F185–F190. [PubMed: 9462187]
10. Van Meurs KP, Wright LL, Ehrenkranz RA, et al. Preemie Inhaled Nitric Oxide Study. Inhaled nitric oxide for premature infants with severe respiratory failure. N Engl J Med. 2005; 353(1):13–22. [PubMed: 16000352]
11. Chock VY, Van Meurs KP, Hintz SR, et al. NICHD Neonatal Research Network. Inhaled nitric oxide for preterm premature rupture of membranes, oligohydramnios, and pulmonary hypoplasia. Am J Perinatol. 2009; 26(4):317–322. [PubMed: 19067285]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

12. Geary C, Whitsett J. Inhaled nitric oxide for oligohydramnios-induced pulmonary hypoplasia: a report of two cases and review of the literature. J Perinatol. 2002; 22(1):82–85. [PubMed: 11840249]
13. Keszler M. Guidelies for rationnal and cost-effective use of iNO therapy in term and preterm infants. J Clin Neonatol. 2012; 1(2):59–63. [PubMed: 24027689]
14. Peliowski A, Finer NN, Etches PC, Tierney AJ, Ryan CA. Inhaled nitric oxide for premature infants after prolonged rupture of the membranes. J Pediatr. 1995; 126(3):450–453. [PubMed: 7869210]
15. Aikio O, Metsola J, Vuolteenaho R, Perhomaa M, Hallman M. 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. J Pediatr. 2012; 161(3):397–403. e1. [PubMed: 22554621]
16. Dani C. Does it exist pulmonary hypertension in the ELBW infants? Italian J Pediatrics. 2015; 41(Suppl 1):A6.doi: 10.1186/1824-7288-41-S1-A6
17. Cheng DR, Peart S, Tan K, Sehgal A. Nitric therapy in preterm infants: rationalised approach based on functional neonatal echocardiography. Acta Paediatr. 2016; 105(2):165–171. [PubMed: 26450016]
18. Rabinovitch M. Pulmonary hypertension: pathophysiology as a basis for clinical decision making. J Heart Lung Transplant. 1999; 18(11):1041–1053. [PubMed: 10598727]
19. Baker CD, Abman SH, Mourani PM. Pulmonary hypertension in preterm infants with bronchopulmonary dysplasia. Pediatr Allergy Immunol Pulmonol. 2014; 27(1):8–16. [PubMed: 24669351]
20. Murphy JD, Vawter GF, Reid LM. Pulmonary vascular disease in fatal meconium aspiration. J Pediatr. 1984; 104(5):758–762. [PubMed: 6716223]
21. Lakshminrusimha S, Keszler M. Persistent pulmonary hypertension of the newborn. Neoreviews. 2015; 16(12):e680–e692. [PubMed: 26783388]
22. Iyer NP, Mhanna MJ. Non-invasively derived respiratory severity score and oxygenation index in ventilated newborn infants. Pediatr Pulmonol. 2013; 48(4):364–369. [PubMed: 23359457]
23. Ballard RA, Truog WE, Cnaan A, et al. NO CLD Study Group. Inhaled nitric oxide in preterm infants undergoing mechanical ventilation. N Engl J Med. 2006; 355(4):343–353. [PubMed: 16870913]
24. Thibeault DW, Truog WE, Ekekezie II. Acinar arterial changes with chronic lung disease of prematurity in the surfactant era. Pediatr Pulmonol. 2003; 36(6):482–489. [PubMed: 14618639]
25. Thibeault DW, Kilbride HK. Increased acinar arterial wall muscle in preterm infants with PROM and pulmonary hypoplasia. Am J Perinatol. 1997; 14(8):457–460. [PubMed: 9376005]
26. Mourani PM, Abman SH. Pulmonary vascular disease in bronchopulmonary dysplasia: pulmonary hypertension and beyond. Curr Opin Pediatr. 2013; 25(3):329–337. [PubMed: 23615175]
27. Walther FJ, Benders MJ, Leighton JO. Persistent pulmonary hypertension in premature neonates with severe respiratory distress syndrome. Pediatrics. 1992; 90(6):899–904. [PubMed: 1437431]
28. Kumar VH, Hutchison AA, Lakshminrusimha S, Morin FC III, Wynn RJ, Ryan RM. Characteristics of pulmonary hypertension in pre-term neonates. J Perinatol. 2007; 27(4):214–219. [PubMed: 17330053]
29. Mirza H, Ziegler J, Ford S, Padbury J, Tucker R, Laptook A. Pulmonary hypertension in preterm infants: prevalence and association with bronchopulmonary dysplasia. J Pediatr. 2014; 165(5):909–14. e1. [PubMed: 25189821]
30. Mirza H, Ziegler J, Ford S, Padbury J, Tucker R, Laptook A. Temporal profile of early pulmonary hypertension in preterm infants. Am J Perinatol. 2016; 33(9):903–909. [PubMed: 27057770]
31. Desandes R, Desandes E, Droullé P, Didier F, Longrois D, Hascoët JM. Inhaled nitric oxide improves oxygenation in very premature infants with low pulmonary blood flow. Acta Paediatr. 2004; 93(1):66–69. [PubMed: 14989442]
32. Dani C, Bertini G, Pezzati M, Filippi L, Cecchi A, Rubaltelli FF. Inhaled nitric oxide in very preterm infants with severe respiratory distress syndrome. Acta Paediatr. 2006; 95(9):1116–1123. [PubMed: 16938760]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


33. Evans N. Towards rational use of inhaled Nitric Oxide in preterm babies. Acta Paediatr. 2016; 105(2):121–122. [PubMed: 26751417]
34. Arul N, Konduri GG. Inhaled nitric oxide for preterm neonates. Clin Perinatol. 2009; 36(1):43–61. [PubMed: 19161864]
35. Stutchfield P, Whitaker R, Russell I. Antenatal Steroids for Term Elective Caesarean Section (ASTECS) Research Team. Antenatal betamethasone and incidence of neonatal respiratory distress after elective caesarean section: pragmatic randomised trial. BMJ. 2005; 331(7518):662. [PubMed: 16115831]
36. Crossley KJ, Morley CJ, Allison BJ, et al. Blood gases and pulmonary blood flow during resuscitation of very preterm lambs treated with antenatal betamethasone and/or curosurf: effect of positive end-expiratory pressure. Pediatr Res. 2007; 62(1):37–42. [PubMed: 17515834]
37. Crossley KJ, Morley CJ, Allison BJ, et al. Antenatal corticosteroids increase fetal, but not postnatal, pulmonary blood flow in sheep. Pediatr Res. 2009; 66(3):283–288. [PubMed: 19542907]
38. Konduri GG, Bakhutashvili I, Eis A, Afolayan A. Antenatal betamethasone improves postnatal transition in late preterm lambs with persistent pulmonary hypertension of the newborn. Pediatr Res. 2013; 73(5):621–629. [PubMed: 23370411]
39. Gao Y, Tolsa JF, Shen H, Raj JU. A single dose of antenatal betamethasone enhances isoprenaline and prostaglandin E2-induced relaxation of preterm ovine pulmonary arteries. Biol Neonate. 1998; 73(3):182–189. [PubMed: 9535536]
40. Gao Y, Zhou H, Tolsa JF, Shen H, Raj JU. Antenatal betamethasone therapy augments isoproterenol and prostaglandin E2-mediated relaxation of preterm ovine pulmonary veins. Pediatr Res. 1997; 42(4):545–549. [PubMed: 9380451]
41. Zhou H, Gao Y, Raj JU. Antenatal betamethasone therapy augments nitric oxide-mediated relaxation of preterm ovine pulmonary veins. J Appl Physiol (1985). 1996; 80(2):390–396. [PubMed: 8929574]
42. Gao Y, Zhou H, Raj JU. Antenatal betamethasone therapy potentiates nitric oxide-mediated relaxation of preterm ovine coronary arteries. Am J Physiol. 1996; 270(2 Pt 2):H538–H544. [PubMed: 8779828]
43. Abman SH, Hansmann G, Archer SL, et al. American Heart Association Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation; Council on Clinical Cardiology; Council on Cardiovascular Disease in the Young; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Surgery and Anesthesia; and the American Thoracic Society. Pediatric Pulmonary Hypertension: Guidelines From the American Heart Association and American Thoracic Society. Circulation. 2015; 132(21):2037–2099. [PubMed: 26534956]
44. Konduri GG, Menzin J, Frean M, Lee T, Potenziano J, Singer J. Inhaled nitric oxide in term/late preterm neonates with hypoxic respiratory failure: estimating the financial impact of earlier use. J Med Econ. 2015; 18(8):612–618. [PubMed: 25853867]
45. Sharma V, Berkelhamer SK, Lakshminrusimha S. Persistent pulmonary hypertension of the newborn. Maternal Health, Neonatology and Perinatology BMC. 2015; 1(14):1–18.
46. Farrow KN, Lakshminrusimha S, Reda WJ, et al. Superoxide dis-mutase restores eNOS expression and function in resistance pulmonary arteries from neonatal lambs with persistent pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol. 2008; 295(6):L979–L987. [PubMed: 18790993]
47. Sheehy AM, Burson MA, Black SM. Nitric oxide exposure inhibits endothelial NOS activity but not gene expression: a role for superoxide. Am J Physiol. 1998; 274(5 Pt 1):L833–L841. [PubMed: 9612300]
48. Black SM, Heidersbach RS, McMullan DM, Bekker JM, Johengen MJ, Fineman JR. Inhaled nitric oxide inhibits NOS activity in lambs: potential mechanism for rebound pulmonary hypertension. Am J Physiol. 1999; 277(5 Pt 2):H1849–H1856. [PubMed: 10564139]
49. Ohara T, Ogata H, Tezuka F. Histological study of pulmonary vasculature in fatal cases of persistent pulmonary hypertension of the newborn. Tohoku J Exp Med. 1991; 164(1):59–66. [PubMed: 1926147]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

50. Austin ED, Loyd JE. Toward precision medicine in pulmonary arterial hypertension. Am J Respir Crit Care Med. 2015; 192(11):1272–1274. [PubMed: 26623685]

51. Kinsella JP, Steinhorn RH, Krishnan US, et al. Recommendations for the use of inhaled nitric oxide therapy in premature newborns with severe pulmonary hypertension. J Pediatr. 2016; 170:312–314. [PubMed: 26703869]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

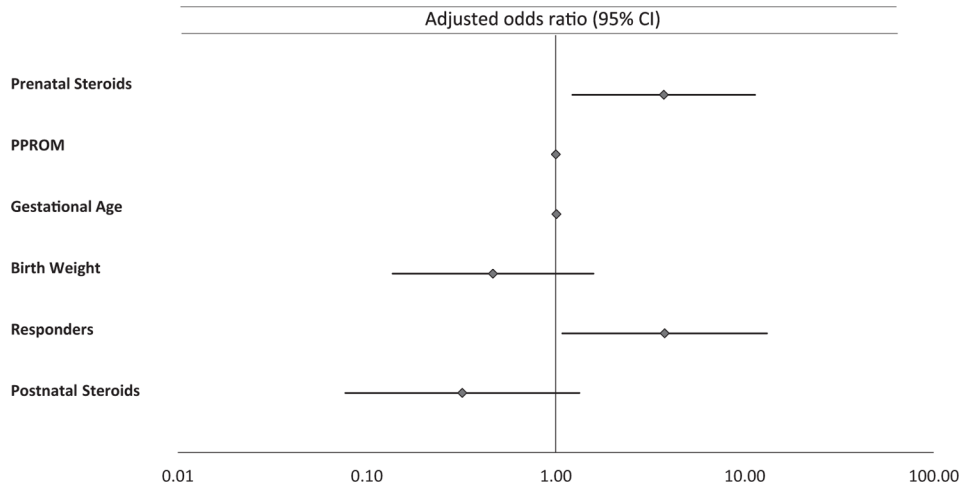


**Fig. 1.**

Multiple logistic regression of factors associated with survival in preterm infants treated with inhaled nitric oxide ($N = 93$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

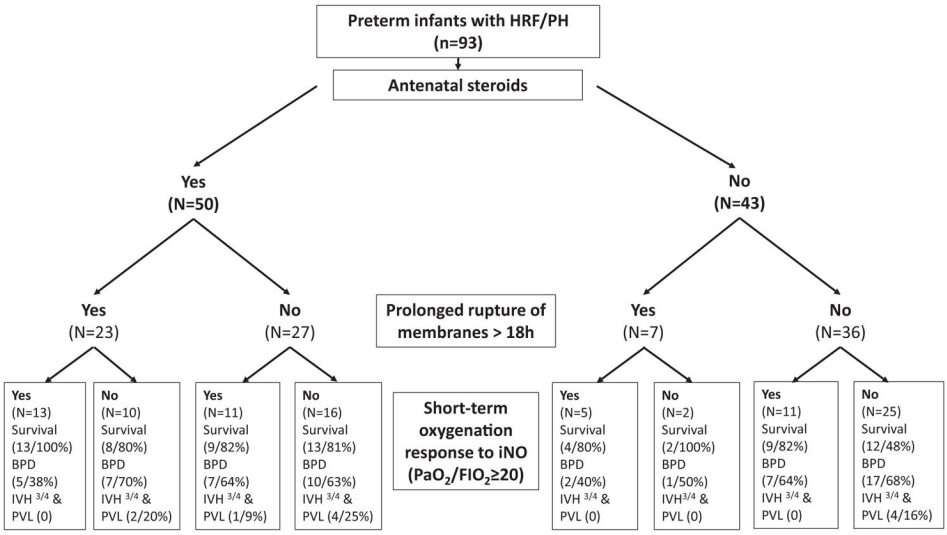


**Fig. 2.**

Flow sheet for preterm infants treated with inhaled nitric oxide (iNO) stratified for major outcome: mortality, bronchopulmonary dysplasia, intraventricular hemorrhage grade 3 and grade 4 and periventricular leukomalacia based on factors such as antenatal steroids, prolonged rupture of membrane, and short-term oxygenation response to iNO. Data represented as number of infants/percentage.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

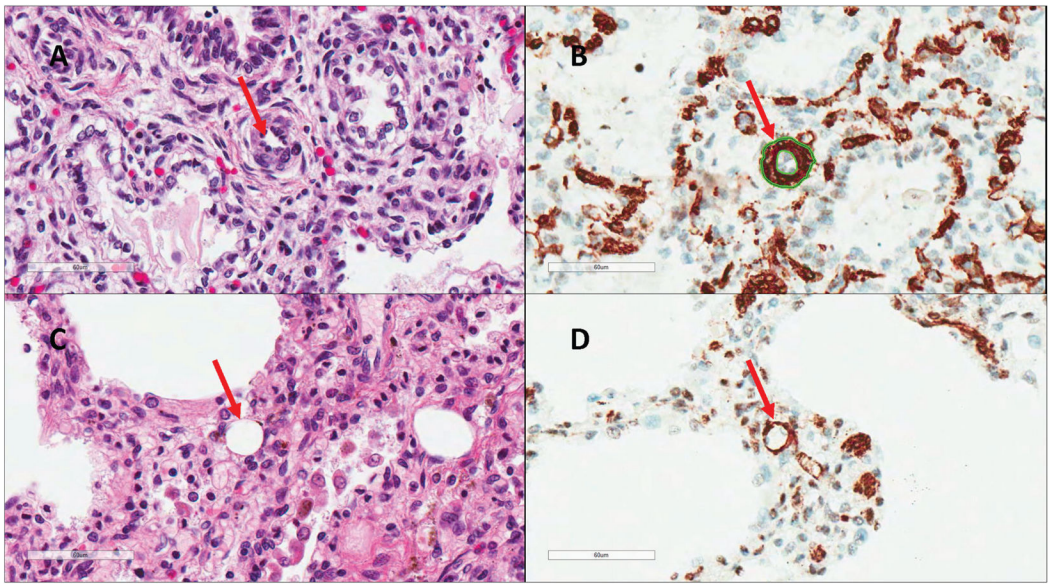


**Fig. 3.**

(**A**) Hematoxylin and eosin (H&E) stain showing intra-acinar arteries from a 25-week gestation infant with hypoxemic respiratory failure. (**B**) Smooth muscle actin (SMA) stain showing the smooth muscle area ratio (SMR) measurement (ratio of smooth muscle area shown between the two fluorescent green circles to total area of vessel). The SMR was high (0.80) suggestive of increased muscularization. (**C**) A 27-week gestation infant who died of gastrointestinal pathology showing normal appearance of intra-acinar arteries (H&E stain) with (**D**) incomplete or partial muscularization (SMA staining, fig. **D**).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

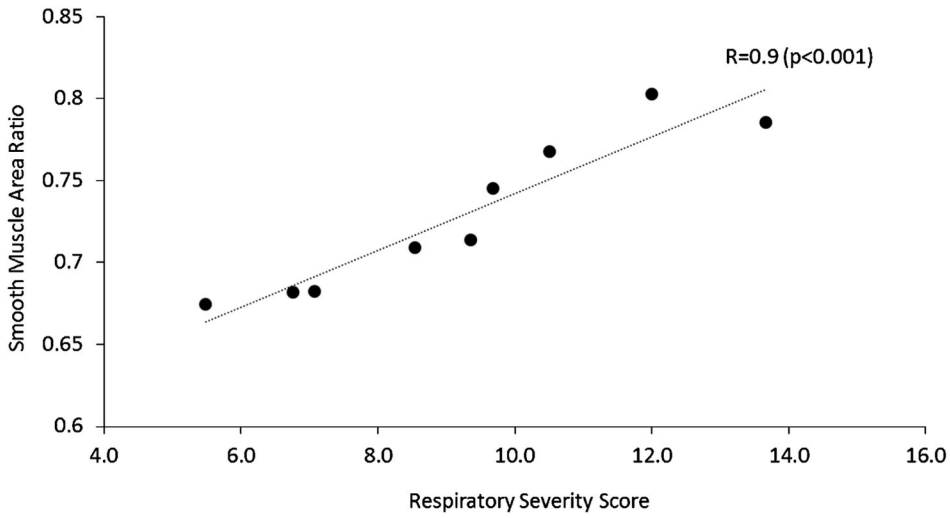


**Fig. 4.**

Scatter plot depicting the positive correlation between respiratory severity score and smooth muscle area ratio of the intra-acinar arteries.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

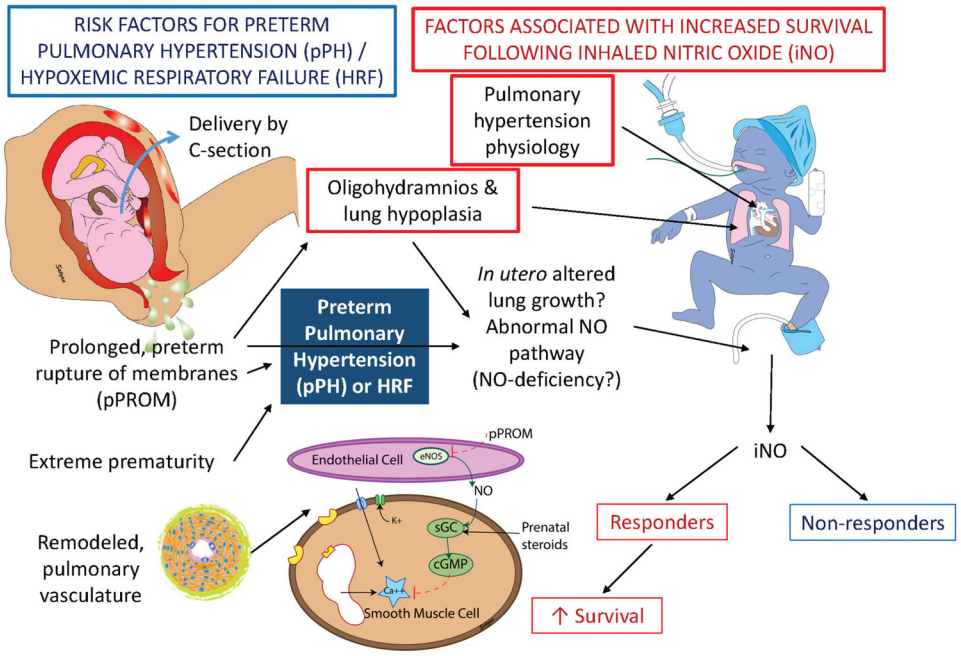


**Fig. 5.**

Risk factors associated with development of pulmonary hypertension (PH) and/or hypoxemic respiratory failure (HRF) in preterm infants include cesarean section delivery, extreme prematurity, and preterm prolonged rupture of membrane (pPROM). A subset of preterm infants with PH and/or HRF with prenatal steroids (possibly by enhancing the effect of soluble guanylyl cyclase), pPROM and PH physiology may respond to inhaled nitric oxide with improved survival.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

## Table 1

Baseline characteristics of the infants treated in the first 28 days of postnatal age with inhaled nitric oxide versus controls

| **Characteristics** | **Cases (*N* = 93)** | **Controls (*N* = 930)** | ***p*-value** |
|---|---|---|---|
| Birth weight, g (mean ± SD) | 912 ± 517 | 892 ± 243 | 0.51 |
| Gestational age, wk (mean ± SD) | 26 ± 4 | 26 ± 2 | 0.44 |
| Male, *n* (%) | 57 (61) | 539 (58) | 0.53 |
| Small for gestational age, *n* (%) | 13 (10) | 111 (12) | 0.56 |
| Duration of rupture of membrane, *n* (%) | | | |
| ≥ 18 h | 30 (32) | 178 (19) | 0.002 |
| ≥ 120 h | 24 (26) | 101 (11) | <0.001 |
| Chorioamnionitis, *n* (%) | 8 (9) | 120 (13) | 0.23 |
| Oligohydramnios, *n* (%) | 15 (16) | 54 (6) | <0.001 |
| Born outside, *n* (%) | 16 (22) | 198 (21) | 0.36 |
| Prenatal corticosteroids, *n* (%) | 50 (54) | 438 (47) | 0.23 |
| Delivery by cesarean section, *n* (%) | 76 (82) | 626 (67) | 0.004 |
| Apgar scores < 4 at 1 min, *n* (%) | 40 (43) | 344 (37) | 0.30 |
| Apgar scores < 4 at 5 min, *n* (%) | 11 (12) | 91 (10) | 0.53 |

Abbreviation: SD, standard deviation.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

## Table 2

Outcomes at discharge comparing cases treated with iNO and matched controls

| Characteristics | Cases (*N* = 93) | Controls (*N* = 930) | *p*-value |
|---|---|---|---|
| Ventilation, d (median [IQR]) | 49 (16–76) | 17 (38–63) | <0.001 |
| Invasive ventilation, d (median [IQR]) | 37 (13–61) | 17 (5–43) | <0.001 |
| Noninvasive ventilation, d (median [IQR]) | 5 (0–17) | 14 (3–26) | <0.001 |
| BPD, *n* (%) | 56 (80) | 328 (42) | <0.001 |
| Vasopressor use, *n* (%) | 60 (65) | 285 (31) | <0.001 |
| Postnatal steroid use, *n* (%) | 72 (77) | 410 (44) | <0.001 |
| NEC Stage II/III, *n* (%) | 18 (19) | 116 (15) | 0.22 |
| Sepsis (clinical/culture proven), *n* (%) | 16 (17) | 159 (12) | 1.0 |
| ROP stage III and above, *n* (%) | 16 (17) | 70 (8) | 0.005 |
| Grade 3 or 4 IVH and PVL, *n* (%) | 11 (12) | 70 (8) | 0.16 |
| Length of stay survivors, d (median [IQR]) | 98 (57–129) | 79 (57–108) | 0.005 |
| Mortality in the NICU, *n* (%) | 23 (25) | 141 (15) | 0.03 |

Abbreviations: BPD, bronchopulmonary dysplasia; iNO, inhaled nitric oxide; IQR, interquartile range; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; PVL, periventricular leukomalacia; ROP, retinopathy of prematurity.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## Table 3

Characteristics of the infants treated with inhaled nitric oxide in the first 28 days of postnatal age: responders versus nonresponders

| Characteristic | Responders (*N* = 40) | Nonresponders (*N* = 53) | *p*-value |
|---|---|---|---|
| Birth weight, g (mean ± SD) | 1076 ± 612 | 789 ± 388 | 0.007 |
| Gestational age, wk (mean ± SD) | 27 ± 3 | 26 ± 3 | 0.04 |
| Male, *n* (%) | 23 (58) | 34 (64) | 0.51 |
| Small for gestational age, *n* (%) | 2 (5) | 11 (21) | 0.03 |
| Duration of rupture of membrane, *n* (%) | | | |
| ≥ 18 h | 17 (43) | 12 (23) | 0.04 |
| ≥ 120 h | 14 (35) | 10 (19) | 0.07 |
| Chorioamnionitis, *n* (%) | 6 (15) | 2 (4) | 0.06 |
| Oligohydramnios, *n* (%) | 8 (20) | 7 (13) | 0.37 |
| Born outside, *n* (%) | 8 (20) | 8 (15) | 0.53 |
| Prenatal corticosteroids, *n* (%) | 24 (60) | 26 (49) | 0.29 |
| Delivery by cesarean section, *n* (%) | 29 (73) | 47 (89) | 0.05 |
| Apgar scores < 4 at 1 min, *n* (%) | 8 (20) | 17 (32) | 0.19 |
| Apgar scores < 4 at 5 min, *n* (%) | 1 (3) | 6 (11) | 0.23 |
| Echocardiographic evidence of pulmonary hypertension, *n* (%) | 14 (35) | 21 (40) | 0.64 |
| Postnatal age when iNO was initiated, d (median [IQR]) | 6 (1–12) | 9 (4–12) | 0.32 |
| Oxygenation index (mean ± SD) | | | |
| Preinhaled nitric oxide | 29 ± 20[a] | 34 ± 26[b] | 0.34 |
| Postinhaled nitric oxide | 13 ± 7[a] | 31 ± 17[b] | <0.001 |
| Survivors, *n* (%) | 35 (88) | 35 (70) | 0.03 |
| IVH grade III/IV and or PVL among survivors, *n* (%) | 1 (2.8) | 10 (28.5) | 0.01 |
| Duration of treatment among survivors, d (median [IQR]) | 40 (14–82) | 65 (17–86) | 1 |
| Duration of treatment among nonsurvivors, d (median [IQR]) | 3 (3–19) | 2 (1–8) | 1 |
| Time of death, d (median [IQR]) | 4 (3–13) | 14 (7–70) | 0.27 |

Abbreviations: iNO, inhaled nitric oxide; IQR, interquartile range; IVH, intraventricular hemorrhage; PVL, periventricular leukomalacia; SD, standard deviation.

[a]The oxygenation index pre- and postinhaled nitric oxide among the responders was significantly different ($p < 0.0001$).

[b]Oxygenation index pre- and postinhaled nitric oxide among nonresponders was not different ($p = 0.6$). Yates' correction was used for chi square test.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## Table 4

Characteristics of infants treated with iNO: survivors versus nonsurvivors

| Characteristic | Survived (*N* = 70) | Died (*N* = 23) | *p*-value |
|---|---|---|---|
| Birth weight, g (mean ± SD) | 898 ± 451 | 956 ± 677 | 0.64 |
| Gestational age, wk (mean ± SD) | 26 ± 3 | 27 ± 3 | 0.57 |
| Male, *n* (%) | 43 (61) | 14 (61) | 0.96 |
| Small for gestational age, *n* (%) | 7 (10) | 6 (26) | 0.05 |
| Duration of rupture of membrane *n* (%) | | | |
| ≥ 18 h | 26 (37) | 3 (13) | 0.03 |
| ≥ 120 h | 21 (30) | 3 (13) | 0.11 |
| Chorioamnionitis, *n* (%) | 7 (10) | 1 (4) | 0.68 |
| Oligohydramnios, *n* (%) | 12 (17) | 3 (13) | 0.64 |
| Born outside, *n* (%) | 12 (17) | 4 (17) | 0.77 |
| Prenatal corticosteroids, *n* (%) | 40 (57) | 5 (22) | 0.004 |
| Delivery by cesarean section, *n* (%) | 57 (81) | 19 (83) | 0.85 |
| Apgar scores < 4 at 1 min, *n* (%) | 16 (23) | 9 (39) | 0.12 |
| Apgar scores < 4 at 5 min, *n* (%) | 3 (4) | 4 (17) | 0.11 |
| Postnatal age when iNO was initiated, d (mean ± SD) | 8.5 ± 6.2 | 8.9 ± 6.7 | 0.14 |
| Oxygenation index (mean ± S.D; range) | | | |
| Preinhaled nitric oxide | 29 ± 19 (10–105) | 41 ± 31 (12–155) | 0.02 |
| Postinhaled nitric oxide | 20 ± 16 (3–94) | 39 ± 32 (11–67) | 0.001 |
| Response to inhaled nitric oxide, increase in $PaO_2/FiO_2$ | | | |
| > 20, *n* (%) | 35(50) | 5(22) | 0.02 |

Abbreviations: $FiO_2$, fraction of inspired oxygen; iNO, inhaled nitric oxide; $PaO_2$, partial pressure of oxygen in arterial blood; SD, standard deviation.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



**Table 5**

Outcomes based on gestational age among preterm infants exposed to iNO during the first 28 days of postnatal age

| GA | BPD | NEC II/III | Sepsis | ROP stage III and above | Grade 3/4 IVH and PVL | Survival |
|---|---|---|---|---|---|---|
| ≤24, 6/7 wk (*n* = 29) | 20 (90%) | 8 (28%) | 3 (10%) | 6 (21%) | 4 (14%) | 22 (76%) |
| 25–27, 6/7 wk (*n* = 40) | 28 (88%) | 8 (20%) | 10 (25%) | 6 (15%) | 6 (15%) | 32 (80%) |
| ≥28, wk (*n* = 24) | 8 (53%)[a] | 2 (8.3%) | 3 (13%) | 4 (17%) | 1 (4.2%) | 15 (63%) |

Abbreviations: BPD, bronchopulmonary dysplasia; GA, gestational age; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; PVL, periventricular leukomalacia; ROP, retinopathy of prematurity.

[a] *P*-value < 0.05: subgroup analysis by age group.

*Am J Perinatol*. Author manuscript; available in PMC 2017 August 27.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



**Table 6**

Characteristics of preterm infants with PH/HRF whose lung histology was evaluated

| S. No. | Antenatal steroid | GA | B.W. (kg) | Apgars | Growth | Echo | ROM (h) | Surfactant (no. of doses) | iNO therapy | Cause of death | Time of death (d) | RSS (mean ± SD) | SMR (mean ± SD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Incomplete | 24 wk 3 d | 0.52 | 1, 1, 2 | AGA | Pulmonary pressures, 40–50 mm Hg | 0 | 2 | Yes | PH and/ or HRF | 12 | 6.8 ± 2.5 | 0.68 ± 0.09 |
| 2 | Complete | 26 wk 5 d | 0.4 | 7, 8 | SGA | RVSP, 65–70 mm Hg | 0 | 2 | Yes | PH and/ or HRF | 4 | 9.4 ± 4.6 | 0.71 ± 0.12 |
| 3 | Incomplete | 29 wk 5 d | 0.928 | 4, 6, 8 | SGA | RVSP, 50 mm Hg | 0 | 2 | Yes | PH and/ or HRF | 23 | 10.5 ± 3.6 | 0.76 ± 0.06 |
| 4 | Incomplete | 25 wk 0 d | 0.505 | 1, 6, 8 | SGA | RVSP, 48 mm Hg | 0 | 2 | Yes | PH and/ or HRF | 23 | 8.6 ± 5.3 | 0.70 ± 0.08 |
| 5 | None | 23 wk 6 d | 0.66 | 1, 2, 2 | AGA | Trace TR | 0 | 4 | Yes | Sepsis/PH | 11 | 9.7 ± 4.5 | 0.74 ± 0.03 |
| 6 | Complete | 24 wk 0 d | 0.589 | 1, 4, 4 | AGA | Poor right and left ventricular function | 0 | 2 | No | HRF | 2 | 12 ± 0 | 0.80 ± 0.03 |
| 7 | Incomplete | 26 wk 0 d | 0.87 | 5, 6, 6 | AGA | RVSP,40–50 mm Hg | 0 | 2 | No | B/L IVH grade 4, withdrawal of support | 2 | 5.5 ± 1.2 | 0.67 ± 0.08 |
| 8 | Complete | 25 wk 1 d | 0.816 | 5, 6, 7 | AGA | PDA | 0 | 3 | No | HRF | 28 | 7.1 ± 2.4 | 0.68 ± 0.07 |
| 9 | Incomplete | 23 wk 0 d | 0.62 | 6, 8 | AGA | PDA | 0 | 2 | No | HRF/IVH 4 B/L withdrawal support | 3 | 13.7 ± 2.3 | 0.78 ± 0.03 |

Abbreviations: AGA, appropriate for gestational age; B.W, birth weight; GA, gestational age; HRF, hypoxemic respiratory failure; iNO, inhaled nitric oxide; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; PH, pulmonary hypertension; ROM, rupture of membrane; RSS, respiratory severity score; RVSP, right ventricular systolic pressure; SD; standard deviation; SGA, small for gestational age; SMR, smooth muscle area ratio; TR, tricuspid regurgitation.

*Am J Perinatol*. Author manuscript; available in PMC 2017 August 27.