Improved Oxygenation in a Randomized Trial of Inhaled Nitric Oxide for Persistent Pulmonary Hypertension of the Newborn

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ABSTRACT. *Objective.* **To determine the effect of inhaled nitric oxide (NO) on clinical outcome in newborns with persistent pulmonary hypertension (PPHN).**

Design. **A prospective, randomized trial of patients referred to a level 3 nursery in a single large center. Clinicians were not masked to group assignment. Crossover of patients from control to NO treatment was not permitted.**

Methods. **We randomized 49 mechanically ventilated newborns, transferred to our center with clinical and echocardiographic evidence of severe PPHN (arterial oxygen tension [Pa_{O_2}] <100; fractional inspired oxygen = 1) to treatment with or without NO. Patients with gestational age <34 weeks or with congenital heart disease or diaphragmatic hernia were excluded. High-frequency oscillatory ventilation was used but not allowed concomitantly with NO. Primary outcome variables were oxygenation, mortality, and use of extracorporeal membrane oxygenation (ECMO).**

Results. **Meconium aspiration syndrome and isolated PPHN were the most common diagnoses (32/49) and were distributed equally between groups. The median age at the time of entry into the study was similar between groups, 25 hours for control patients and 18 hours for NO patients. Median baseline oxygenation index (OI) was similar in 23 control (OI = 29) and 26 NO (OI = 30) patients. Mortality (8%), use of ECMO (33%), median days on mechanical ventilation (9 days), and duration of supplemental oxygen (13 days) were not different between treatment groups. Pa_{O_2}, oxygen saturation, and OI improved in the NO group compared with baseline and to control patients at 15 minutes. The median percent change in OI (−31%) in the NO group was significantly different from baseline and from the control group. The difference in oxygenation between treatment groups was still apparent 12 hours after baseline. Before cannulation for ECMO, oxygenation was better in the NO group compared with control patients. Among patients who were placed on ECMO, the median time from baseline to ECMO cannulation was 2.4 hours (range, 1 to 12 hours) among control patients and 3.3 hours (range, 2 to 68 hours) for those randomized to receive NO. There was a tendency to observe fewer adverse neurologic events (seizure and intracranial hemorrhage) in the NO group (4/26 vs 8/23). One child with alveolar capillary dysplasia confirmed by postmortem examination could not be weaned from 80 parts per million of NO and transiently developed methemoglobinemia (peak methemoglobin level = 17%). No other side effects were observed.**

Conclusions. **Although mortality and ECMO use were similar for both treatment groups using this study size and design, sustained improvement in oxygenation with NO and better oxygenation at initiation of ECMO may have important clinical benefits. We speculate that modification of treatment to include specific lung expansion strategies with NO treatment and recognition that early improvement of oxygenation may be sustained with NO may lead to reduced use of ECMO in NO treated patients compared with controls.** *Pediatrics* **1997; 100(5). URL: http://www.pediatrics.org/cgi/content/full/100/5/e7; *persistent fetal circulation, extracorporeal membrane oxygenation, high-frequency oscillatory ventilation, alveolar capillary dysplasia, methemoglobin.***

ABBREVIATIONS. PPHN, persistent pulmonary hypertension of the newborn; ECMO, extracorporeal membrane oxygenation; NO, nitric oxide; ppm, parts per million; Pa_{O_2}, arterial oxygen tension; F_{IO_2}, fractional inspired oxygen; Pa_{CO_2}, arterial carbon dioxide tension; OI, oxygenation index; HFOV, high-frequency oscillatory ventilation.

Persistent pulmonary hypertension of the newborn (PPHN) is a syndrome characterized by increased pulmonary vascular resistance, right to left shunting of blood, and severe hypoxemia.[1–3] PPHN is frequently associated with pulmonary parenchymal abnormalities, including meconium aspiration, pneumonia, sepsis, lung hypoplasia, and dysplastic alveolar capillary structure. In some instances, there is no evidence of pulmonary parenchymal disease and the etiology is unknown. Treatment strategies, including alkalinization, hyperventilation, and use of intravenous vasodilators are aimed at lowering pulmonary vascular resistance but are associated with adverse effects and are not always successful.[4] Extracorporeal membrane oxygenation (ECMO) has improved survival for neonates with refractory hypoxemia but may be associated with hemorrhagic, neurologic, and other complications.[5–7] Although survival for PPHN has improved, better treatment would further reduce mortality rates and morbid outcomes.

Inhaled nitric oxide (NO) is a selective pulmonary vasodilator.[8,9] Early investigations suggested that this drug improved oxygenation in patients with PPHN who were administered 6 to 80 parts per million (ppm) of NO with oxygen.[10,11] Although promising, these initial studies were small case series

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with physiologic rather than clinical outcomes and lacked a control group. Subsequent trials were informative but until recently were still limited by lack of controls, extensive treatment crossover designs, or inherent limitations of multicenter trials with varying definitions of standard clinical practice.[12–17] Although the efficacy of NO in the treatment of PPHN has been recently affirmed in multicenter randomized trials,[18,19] results of other studies may add to our understanding of this new therapy. We conducted a prospective, randomized trial of NO for treatment of PPHN among patients referred to a single large center. Our objective was to systematically introduce this investigational therapy in a randomized fashion to all patients with PPHN, allowing for an interim analysis and protocol modification, until we or others could demonstrate sustained improvement in oxygenation and superior outcome with NO.[20] Our primary hypothesis was that treatment with inhaled NO would improve oxygenation compared with controls and reduce mortality and utilization of ECMO.

METHODS

Patients

We screened all newborns with a clinical diagnosis of PPHN admitted to Children's Hospital between September 1, 1992 and September 1, 1994. Qualifying criteria for enrollment included gestational age ≥34 weeks and Pao_2 <100 mm Hg during mechanical ventilation on FIO_2 = 1 after optimization of ventilatory and pharmacologic strategies. Patients were sedated with narcotic and administered muscle relaxants, with efforts made to achieve moderate hyperventilation ($Paco_2$ = 30 to 40 mm Hg). Sodium bicarbonate was infused to correct metabolic acidosis and raise pH to 7.45 to 7.60. Systemic blood pressure was supported with colloid infusions, dopamine, and dobutamine. Intravenous vasodilators such as tolazoline or prostaglandin E1 were not used.

Echocardiographic evidence of pulmonary hypertension was required and included right to left or bidirectional shunting at the ductus arteriosus or foramen ovale. Evidence of systemic pressure in the pulmonary artery was inferred by Doppler assessment of tricuspid regurgitation or by ventricular septal position.

Patients were excluded from study if they had major anomalies including congenital heart disease or congenital diaphragmatic hernia, or if echocardiography demonstrated evidence of low pulmonary vascular resistance (eg, continuous left to right flow through a patent ductus arteriosus or isolated right ventricular dysfunction without pulmonary hypertension). Previous treatment with surfactant therapy or high-frequency oscillatory ventilation (HFOV) at the referring institution was permitted.

Patients were randomly assigned to control or NO treatment. Randomization schemes were developed using a permuted-blocks design with blocks of size 10. Primary outcome variables were oxygenation, mortality, and use of ECMO. The initial study design predicted that a reduction in ECMO utilization from 40% to 15% would require 50 patients in each treatment group to achieve 80% power. For continuous variables [oxygenation index (OI) and Pao_2], a 20% reduction would require 25 patients in each group. Additional outcomes included oxygenation before ECMO, duration of mechanical ventilation, duration of exposure to supplemental oxygen during hospitalization, and need for supplemental oxygen after discharge from the hospital. We recorded and analyzed continuous variables including oxygenation, airway pressures, heart rate, and systemic blood pressure during the 24 hours after baseline measurements were obtained. Measures of oxygenation were: Pao_2, Pao_2/FIO_2, OI ($OI = FIO_2 \times \text{mean airway pressure} \times 100 \div Pao_2$), and oxygen saturation by pulse oximetry. For patients who were supported with ECMO, we recorded the last Pao_2 before preparation for ECMO and the last oxygen saturation before initiation of ECMO. The clinical course was also noted for occurrence of seizures treated with anticonvulsants or for abnormalities on head ultrasound described as intracranial hemorrhage more severe than grade 1. Head ultrasounds were obtained at the discretion of the responsible clinician and for all patients before and after initiation of ECMO.

Protocol

We obtained informed consent from the parents of all patients using a protocol approved by the Clinical Investigation Committee of Children's Hospital with an investigational new drug number assigned by the United States Food and Drug Administration. Patients were randomized either to receive NO or to continue conventional therapy. Patients randomized to receive NO had the FIO_2 reduced to 0.97. After randomization, patients were continued in the study even though the baseline Pao_2 may have exceeded 100 mm Hg. Arterial blood gases, heart rate, blood pressure, pulse oximetry, and all ventilator settings were recorded at baseline and 15 minutes later. During this interval no change in pharmacologic or mechanical support was permitted except as a resuscitative maneuver. All clinical variables were again recorded and analyzed at 1, 2, 6, 12, and 24 hours after baseline and daily thereafter until hospital discharge.

All patient care decisions were made by the clinical care team according to standard practice guidelines and were not altered by the investigators. In both the treated and control groups attempts to wean mean airway pressure and FIO_2 were made after a stable Pao_2 >60 mm Hg had been achieved. Patients assigned to the NO group received a starting dose of 80 ppm. NO was weaned according to a preset protocol which lowered the NO dose from 80 ppm to 40 ppm after 1 hour. If tolerated, this dose was continued up to 12 hours and dose reductions to 5 ppm were attempted each morning. NO was discontinued when the dose could be successfully reduced to 5 ppm for at least 12 hours while Pao_2 was sustained >60 mm Hg with an $FIO_2 \leq 0.5$.

Alternatively, NO was discontinued when a patient was cannulated for ECMO or when the attending physician chose to convert from conventional mechanical ventilation to HFOV. The protocol permitted use of NO only during conventional mechanical ventilation. HFOV was allowed before randomization, or if NO treatment was discontinued in favor of HFOV, or at any time in patients randomized to control. Concomitant treatment with HFOV and NO was not permitted because of early theoretical concerns about toxicity when NO at 80 ppm was combined with HFOV. Criteria for initiation of ECMO included an OI >40 for at least 1 hour or hemodynamic instability despite inotropic support. If a patient failed to wean from an FIO_2 of 1.0 after persistent attempts during 2 to 3 days, ECMO was then utilized even though the OI was still just below 40.

We have described our NO delivery system previously.[21,22] We used NO gas (Scott Specialty Gases, Plumsteadville, PA and Ohmeda Pharmaceutical Division, Liberty Corner, NJ) of medical grade quality which conformed to United States Food and Drug Administration standards. The source tank concentration of NO was either 2200 ppm (45 patients) or 800 ppm (4 patients, later in the study). NO, nitrogen dioxide, and inspired oxygen were continuously monitored from a sampling port on the inspiratory limb of the ventilator circuit (Thermoenvironmental Instruments, chemiluminescence model 42H, Franklin, MA). Methemoglobin levels were measured by cooximetry (Ciba model 2500) in all patients receiving NO after the first 15 minutes of exposure and then every 12 hours.

Statistical Analysis

Data are represented by median values and ranges along with mean and standard error of the mean where appropriate. After a Friedman's analysis of variance by ranks, a paired nonparametric test (Wilcoxon signed rank test) was used to compare the difference between baseline hemodynamic variables and after 15 minutes of inhaled NO and five subsequent times up to 24 hours with correction for multiple comparisons. Comparison between patients in the control and NO treatment groups was made using the Mann-Whitney test. Binary variables were compared using Fisher's exact test.

RESULTS

We enrolled 51 patients. Two patients were promptly disqualified for study because on review of the echocardiogram shortly after enrollment 1 pa-

tient was noted to have total anomalous pulmonary venous connection; the other patient had an erroneously reported entry Pao_2. Neither patient received treatment under this protocol. Among the remaining 49 patients, 23 randomized to conventional treatment and 26 were assigned to receive NO. There were 3 departures from the intended protocol. One patient in the NO group received only conventional therapy. In 2 patients who randomized to NO, the drug was administered for only 15 minutes; conventional therapy was continued for 12 and 14 hours, respectively, before initiation of ECMO in both patients. Outcomes for these 3 patients were analyzed according to the intention to treat. There were no differences between groups for age at entry, gestational age, weight, or baseline Pao_2 (Table 1).

Associated conditions including meconium aspiration syndrome, isolated PPHN, pneumonia, sepsis, and rare patients with hydrops fetalis, respiratory distress syndrome, or pulmonary hemorrhage were similar between groups (Table 2). Surfactant therapy was permitted at any stage during hospitalization. Four patients received surfactant therapy including 1 after enrollment in the study.

Overall Outcome

Four (8%) of the 49 patients died, 2 in each group. Two had alveolar capillary dysplasia identified at a postmortem examination, and a third patient had clinical features consistent with alveolar capillary dysplasia but we were unable to obtain permission to perform an autopsy of this child. One child who died with alveolar capillary dysplasia while receiving NO had an intracranial (thalamic) hemorrhage which precluded use of ECMO. A fourth patient had poor left ventricular function and a right ventricular dependent circulation with echocardiographic evidence of a small left atrium and left atrial hypertension with continuous right to left ductal flow, but continuous left to right flow across the foramen ovale. Her clinical presentation and echocardiographic assessment were consistent with PPHN. Severe pulmonary hypertensive changes were identified microscopically during the autopsy. NO was administered to this patient for 15 minutes and then discontinued because of clinical deterioration. Hypoxemia and hypotension persisted with conventional therapy and ECMO was initiated. The patient died on ECMO with an intracranial hemorrhage 16 hours after baseline.

Sixteen (33%) of the 49 patients required ECMO, one half in each group (relative risk = 1). One-quarter of our patients had either seizures or intracranial hemorrhages more severe than grade 1. No patient was discharged home requiring supplemental oxygen (Table 3).

Differences Between Treatment Groups

There were no differences between groups with respect to death, use of ECMO, days on mechanical ventilation, or days receiving supplemental oxygen (Table 3). However, measures of oxygenation after baseline were markedly different between the two groups. The median percentage change in Pao_2/Fio_2 at 15 minutes compared with baseline for the control patients (−2%, range −37% to 249%; $P = .57$) compared with patients assigned to NO (+45%, range −33% to 539%; $P = .001$) was significant ($P = .03$ between groups, Fig 1).

Similarly, the percentage change in OI at 15 minutes compared with baseline was significant for the NO group and it had improved compared with control patients (Fig 2). Baseline OI was similar between the two groups but dropped dramatically in the treated NO patients compared with baseline (−31%, range −84 to 38%; $P = .003$) and also compared with the control population (5%, range −71 to 101%; $P = .39$) ($P = .009$ between groups). This observation was related to changes in oxygenation and not mean airway pressure or Fio_2; the median percentage change in Pao_2 was 43% (range, −35 to 539%; $P = .002$) for patients receiving NO and −2% for control patients (range, −37 to 247%; $P = .57$) ($P = .04$ between groups). The median change in mean airway pressure at 15 minutes compared with baseline was zero. Oxygen saturation by pulse oximetry increased by 4% (range, −9% to 21%; $P = .0003$) in NO

TABLE 1. Comparative Data at Baseline, Median (Range)

	Control (n = 23)	Nitric Oxide (n = 26)
Age at entry (hours)	25 (3–63)	18 (5–83)
Gestation (weeks)	40 (36–42)	40 (35–42)
Weight (kg)	3.4 (2.2–5.2)	3.5 (2.5–5.0)
Heart rate (beats per min)	165 (139–200)	175 (135–195)
Mean blood pressure (mm Hg)	54 (39–80)	53 (35–76)
Pao_2 (mm Hg)	64 (27–212)	47 (24–113)
pH	7.48 (7.23–7.62)	7.51 (7.14–7.66)
$Paco_2$ (mm Hg)	34 (18–58)	31 (13–75)
Mean airway pressure (cmH_2O)	15.2 (8.3–32)	16.3 (10.8–25.6)
Oxygenation index	29.4 (10.5–114)	30.4 (10.4–84.5)

TABLE 2. Diagnostic Categories and Associated Conditions

Diagnosis	All (n = 49)	Control (n = 23)	Nitric Oxide (n = 26)
Meconium aspiration syndrome	22 (45%)	9	13
Persistent pulmonary hypertension of the newborn	11 (23%)	7	4
Pneumonia	8 (16%)	3	5
Sepsis	4 (8%)	3	1
Hydrops fetalis	2 (4%)	1	1
Respiratory distress syndrome	1 (2%)	0	1
Pulmonary hemorrhage	1 (2%)	0	1

TABLE 3. Outcome

	All (n = 49)	Control (n = 23)	Nitric Oxide (n = 26)
Mortality	4 (8%)	2 (9%)	2 (8%)
Extracorporeal membrane oxygenation	16 (33%)	8 (35%)	8 (31%)
Seizure or intracranial hemorrhage	12 (25%)	8 (35%)	4 (15%)
Median days on ventilator	9	10	9
Median days on oxygen	13	12	13
Home oxygen	0	0	0

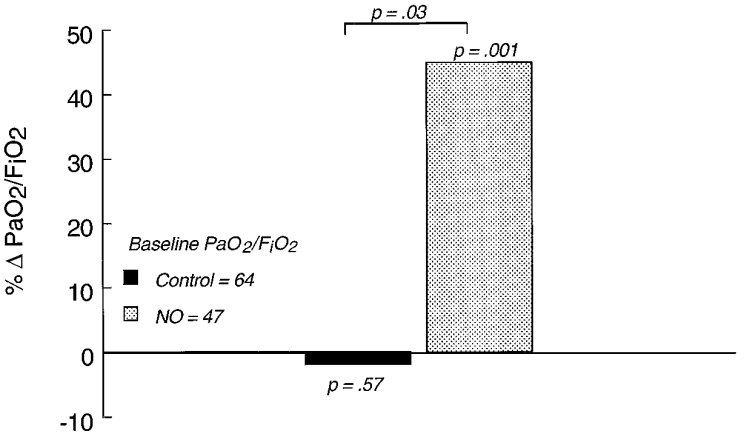

Fig 1. Median percentage change in Pao_2/Fio_2 at 15 minutes compared with baseline for control patients and patients treated with NO. Oxygenation significantly improved in NO patients compared with the control population ($P = .03$).

treated patients and 0 (range, −22 to 41%; $P = .97$) for control patients ($P = .006$ between groups). There was no change in heart rate or blood pressure within groups compared with baseline or between groups.

Fifteen patients who received NO increased their Pao_2 at 15 minutes by more than 20% from baseline. Only 2 of these patients were subsequently placed on ECMO. One of these patients, with the diagnosis of pulmonary hemorrhage, saw improvement in Pao_2 from 29 to 42 mm Hg at 15 minutes and to 55 mm Hg 2 hours later just before cannulation for ECMO (OI = 49). The second patient had improvement in Pao_2 from 43 to 54 mm Hg 15 minutes after NO was started. Pao_2 was sustained in the 60s in this patient. However, after 68 hours the Fio_2 still could not be weaned from 0.97 without reduction in the Pao_2 below 60 mm Hg; the child was placed on ECMO (Pao_2 = 63 mm Hg, OI = 28). Thus, no child had a positive response (more than 20% change) to NO followed by marked deterioration and need for ECMO.

The improvement in oxygenation among patients treated with NO was sustained. Figure 3 shows the median percentage change in OI for both treatment groups during the first 24 hours of study. The reduction in OI seen at 15 minutes with NO was sustained compared with baseline and was significantly different from controls at later time points. After 12 hours of treatment, the median percentage change in OI among NO treated patients was −50% (range, −86 to 30%; $P = .0007$) compared with control patients' change of −19% (range, −81 to 97%; $P = .20$) ($P = .03$ between groups). OI was excluded from analysis after patients were placed on ECMO. Because the number of patients treated with ECMO was the same in each group (n = 8), and because the number of patients treated with HFOV at any point in their treatment (n = 18, controls vs n = 15, NO) was not different between groups, the data suggest that the immediate and sustained improvement in oxygenation was attributable to NO inhalation. Analysis of oxygenation data with ECMO patients excluded at all times demonstrates similar findings, as does separate statistical analysis which excludes patients assigned to but not treated with NO. The median time receiving NO was 22.5 hours (range, 0.25 to 137 hours).

For those patients who were placed on ECMO the Pao_2 and oxygen saturation were higher in the NO group just before cannulation (Table 4). In control patients, the median value of the last recorded Pao_2 was 38 mm Hg, similar to the baseline value. Before ECMO the oxygen saturation by pulse oximetry had fallen from 86% to 82%. In contrast, in NO treated patients the median Pao_2 rose from 41 mm Hg at baseline to 55 mm Hg before ECMO ($P = .02$, between groups) and oxygen saturation rose from 87% to 91% ($P = .02$, between groups). The median time from baseline to ECMO cannulation was 2.4 hours (range, 1 to 12 hours; mean = 3.9 ± 1.3 hours) among control patients and for the NO group it was 3.3 hours (range, 2 to 68 hours; mean 17.7 ± 8.9 hours).

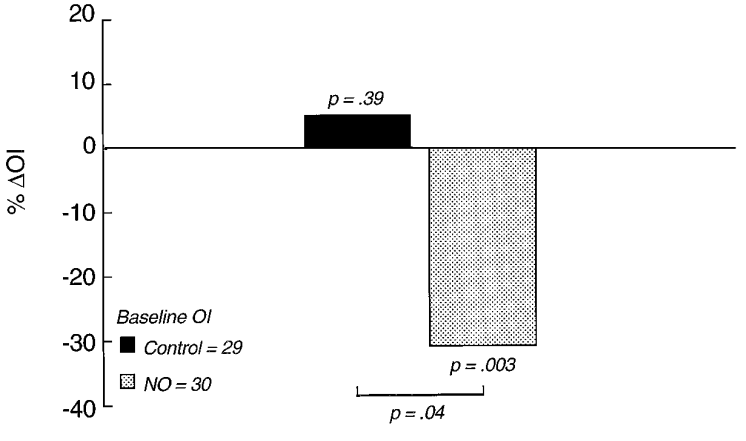

Fig 2. Median percentage change in OI at 15 minutes compared with baseline for control patients and for patients treated with NO. In NO treated patients, OI was reduced and was significantly different from control patients ($P = .04$).

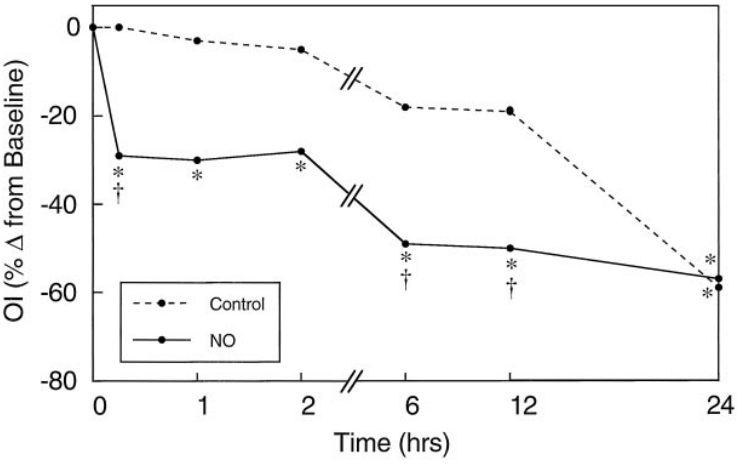

Fig 3. Median percentage change in OI during the first 24 hours of study. The reduction in OI during the first 15 minutes was sustained during subsequent times compared with baseline (*) or to control patients (†) ($P < .05$). OI data were not included in this figure after patients were cannulated for ECMO.

There was a tendency toward fewer neurologic complications in the NO treatment group. Eight of 23 control patients suffered from intracranial hemorrhage or seizures compared with 4 of 26 in the NO group including the incompletely treated patient with left atrial hypertension who died on ECMO ($P = .1$ by Fisher's exact test; Table 4).

Toxicity

The median peak methemoglobin level was 1.7% (range, 0.1 to 17%). One patient with subsequently documented alveolar capillary dysplasia could not be weaned to <80 ppm of NO and developed a peak methemoglobin level of 17% after 25 hours of treatment. The methemoglobin level was reduced below 8.0% with vitamin C therapy and transfusion with packed red blood cells. The patient died suddenly on 80 ppm of NO, 4 days after enrollment with a tension pneumothorax and intracranial hemorrhage.

Peak nitrogen dioxide levels of 1 ppm or less were recorded in 19 out of 26 patients who received NO. One patient had a spurious nitrogen dioxide level of 9 ppm which could not be subsequently confirmed using backup chemiluminescence devices. No other patient had nitrogen dioxide levels that exceeded 5 ppm.

DISCUSSION

This study showed that inhaled NO improved oxygenation in patients with PPHN compared with control patients. This confirms earlier reports from smaller uncontrolled trials of NO and supports the contention that improved oxygenation can be sustained with NO. The OI improved not only during the first 15 minutes of therapy, but was also reduced compared with control patients at 6 and 12 hours after initiation of therapy. Because the number of patients treated with ECMO or HFOV was not different between groups one cannot attribute these oxygenation differences to drop out of ECMO patients or artifact of mean airway pressure measurements during HFOV compared with conventional therapy.

However, sustained improvement in oxygenation was not sufficient in all cases to avoid treatment with ECMO. Thus, we could not demonstrate any difference in use of ECMO between the two treatment groups. Several possible reasons may account for this finding including: 1) lack of important clinical benefit of the drug, 2) insufficient sample size to detect clinical benefit, 3) poor patient selection for optimal NO effect, 4) physician preference to pursue strategies utilizing ECMO despite clinical improvement with NO, and 5) incomplete utilization of optimal ventilatory strategies to facilitate NO effect.

It seems unlikely that NO has no clinical benefit whatsoever other than a transient effect on Pao_2. Several studies, including ours, have shown sustained improvement in oxygenation with NO.[10–12,14] Severe hypoxemia is usually the main indication for ECMO. Along with cardiac output, oxygenation is the primary determinant of oxygen delivery and, therefore, of end organ function and clinical well being. If better oxygenation can be obtained without increased risk, it is likely to be desirable in PPHN. Use of NO did not prolong exposure to mechanical ventilation or supplemental oxygen. We did not increase the risk of intracranial hemorrhage and seizures. In fact, there was a tendency to observe fewer such events in patients treated with NO, although the number of patients affected was too small to predict improvement in neurologic outcome with confidence. We did not observe patients who had favorable transient responses, but then deteriorated to require ECMO support. This circumstance has been described more characteristically in patients with severe pulmonary parenchymal disease or lung hypoplasia rather than those predominantly affected by profound elevation in pulmonary vascular resistance.[13–16]

TABLE 4. Extracorporeal Membrane Oxygenation Patients (n = 16)

	Control (n = 8)		Nitric Oxide (n = 8)	
	Base	Pre-ECMO	Base	Pre-ECMO
Pao_2 (mm Hg)	38	38	41	55*
Oxygen saturations (%)	86	82	87	91*
Time to extracorporeal membrane oxygenation (hours)		2.4		3.3

* $P = .02$ compared to control patients.

Is it possible that within this study design, there was an observable effect on clinical outcome and we enrolled too few patients to reveal this effect? If we exclude patients who were randomized to receive NO, but who were prematurely withdrawn from NO therapy or never received the drug, then the differential use of ECMO (6/23 vs 8/23) still does not reach statistical significance. A 25% reduction in risk of ECMO at this rate of utilization would require 438 patients in each group to achieve a statistical power of 80%. Because many centers are now using NO, such a study design would have little chance of successful completion. It is unrealistic to assume that a larger enrollment with the same study design and clinical algorithms for care would have demonstrated differences in clinical outcome.

Exclusion of patients with congenital diaphragmatic hernia and selection of patients who had clinical and echocardiographic confirmation of high pulmonary vascular resistance should have optimized the likelihood of beneficial response to NO.[14] Nonetheless, this study included patients who retrospectively were thought to be unsuitable candidates for successful use of NO, including those with alveolar capillary dysplasia[23] and a patient with severe left ventricular dysfunction.[24] Better selection of patients may further enhance our ability to detect beneficial uses and effects of NO.

It is possible in this early limited experience with NO, clinicians were uncertain about the clinical course with the drug and were inclined to utilize ECMO despite improvement in oxygenation. We observed that the precannulation Pao_2 and oxygen saturation for patients who went to ECMO were better in the NO group. Pao_2 rose to the middle 50s in NO patients as they were directed to ECMO, but stayed between 30 and 40 mm Hg among control patients who went to ECMO. This improvement in oxygenation did not dissuade clinicians from utilizing ECMO during this phase of our NO experience. Our institution has reported a large ECMO experience with low mortality; new therapies may be accepted slowly.[25] It may be argued that although patients who were receiving NO and cannulated for ECMO were still receiving an FIo_2 of 0.97, the median Pao_2 of 55 mm Hg before ECMO was adequate to defer cannulation in at least one-half of the patients. This idea is supported by the short time between enrollment and cannulation for those patients who were supported by ECMO. It was also noted that NO patients had a tendency to undergo cannulation for ECMO at a slightly later time but generally within the first 24 hours of life. An aggressive mind set among clinicians toward utilization of ECMO may have hindered our evaluation of the clinical efficacy of NO but may also have accounted for the low incidence of chronic lung disease among our patients; no patient was discharged to home on supplemental oxygen.

It is most likely in our opinion that the study design of this investigation precluded the optimal effect of NO by excluding the concomitant use of HFOV with NO in any patient, including those with findings of pulmonary parenchymal disease and loss of lung volume on chest radiograph. As has been suggested by Abman and Kinsella[26] and others, lung recruitment strategies facilitated by HFOV ventilation may enhance the efficacy of NO. With this in mind, our protocol was reevaluated after 2 years of enrollment when this interim analysis was conducted. NO is now used in conjunction with HFOV when clinically indicated.

Limitations

Some limitations of the study have already been mentioned. The exclusion of HFOV and the low power to detect small differences in clinical outcomes are apparent.

The trial was unmasked which may introduce observer bias. Although it is possible that investigators may be biased toward overstating the benefits of the therapy, the timing of the first hemodynamic record and blood gas sampling was rigidly enforced at 15 minutes and 1 hour and then subsequently left to the execution of the bedside clinicians according to preset times. Thus the measures of oxygenation were objective and less susceptible to bias. On the other hand, the purported rapid onset of action of NO may lead more easily in an unmasked trial to premature and incorrect clinical assumption of treatment failure.[12,17] Investigators have recently suggested that the clinical benefit of NO may be manifest throughout several hours. An unmasked trial may permit clinicians to condemn slow responders to a category of failure to respond after a few minutes of NO therapy and therefore reinforce the perceived need for ECMO. Although indications for ECMO may be reasonably stated to include a sustained OI >40, many factors play a role in the timing and utilization of this resource.

We did not investigate the dose response relationship for NO nor did we establish the minimum effective dose of this drug. Based on earlier animal and human infant experience, we chose the initial dose of 80 ppm as the likeliest tolerable dose of NO able to achieve maximal pulmonary vasodilation.[8,27,28]

Methemoglobinemia was observed in 1 patient who was the only patient who could not be weaned from 80 ppm. This patient had postmortem evidence of alveolar capillary dysplasia. All other patients tolerated reduction in NO dose to 40 ppm. Although nitrogen dioxide levels did not exceed 3.5 ppm in any patient, these measurements were performed with chemiluminescence technique before our appreciation that quenching effects in high oxygen environments may contribute to falsely low (or even negative) measurements of nitrogen dioxide.[21] Modification of chemiluminescence technology for clinical use, along with improvements in electrochemical detection devices may be combined with the use of 40 ppm or lower doses of NO, to minimize toxicity without significant compromise of any potential therapeutic efficacy.[29] Nonetheless, the full range of potential toxicity of NO and its metabolites such as peroxynitrite, and the potential effects of adverse interaction with free radical scavenging among normal processes in immature and diseased lungs, have not been fully tested. This will require further study

with randomized trials which implement appropriate follow-up of patients and do not permit crossover of treatment.

Finally, we have seen that mortality for reversible causes of PPHN is low in an ECMO center. At most, 2 and probably only 1 patient in this series died with reversible pulmonary hypertension. This low event rate will make it unlikely that mortality is a realistic outcome variable for single-center randomized trials of the efficacy of NO in PPHN. Considering the potential to achieve zero mortality in this disease, centers without ECMO capability may need to re-evaluate the timing of patient referrals, especially if withdrawal of NO (during transport) may be associated with rebound pulmonary hypertension.[30]

The improvement in oxygenation and low incidence of identifiable side effects with inhaled NO in this study encouraged us to proceed with continued randomization in a second phase of the trial using lower NO doses and combined therapy with NO and HFOV when indicated. This phase has just been completed and confirms the value of HFOV. These and other studies will be required before one can conclude with certainty whether NO improves outcome in patients with PPHN.

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