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Original Article

Clinical Analysis of Inhaled Nitric Oxide Therapy in Preterm Infants at Different Gestational Ages: A National Retrospective Multicenter Study

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Abstract

Objective This study aimed to investigate clinical features of inhaled nitric oxide (iNO) in preterm infants with a gestational age (GA) < 34 weeks in China.

Study Design The clinical data of 434 preterm infants with GA < 34 weeks, treated with iNO in the neonatology departments of eight Class A tertiary hospitals in China over a 10-year period from January 2013 to December 2022, were included in this retrospective multicenter investigation. The infants were divided into three groups based on GA: 24 to 27 weeks (extremely preterm infants), 28 to 31 weeks (very preterm infants), and 32 to 33 weeks (moderate preterm infants). The use of iNO, perinatal data, incidence and mortality of indication for iNO treatment, therapeutic effects of iNO, incidence of short-term complications for iNO treatment, and mortality were compared among these three groups.

Results Over the past 10 years, the proportion of iNO use was highest in extremely preterm infants each year. The lower the GA, the higher the iNO use rate: 4.20% for GA 24 to 27 weeks, 1.54% for GA 28 to 31 weeks, and 0.85% for GA 32 to 33 weeks. There was no significant difference in the therapeutic effect of iNO among the three groups.

Keywords
- preterm infants
- inhaling nitric oxide
- clinical features
- gestational age

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The incidence of neonatal pulmonary hemorrhage, neonatal shock, late-onset diseases, retinopathy of prematurity requiring intervention, intracranial hemorrhage (grade 3 or 4), periventricular leukomalacia, neonatal necrotizing enterocolitis (≥stage II), and moderate to severe bronchopulmonary dysplasia was highest in extremely preterm infants and increased with decreasing GA. Mortality was negatively correlated with GA and birth weight. The highest rate of iNO treatment in 24 to 27 weeks' preterm infants was due to hypoxic respiratory failure (HRF), whereas the highest rate of iNO treatment in 32 to 33 weeks' preterm infants was due to documented persistent pulmonary hypertension of the newborn (PPHN). The rates of iNO treatment due to HRF and documented PPHN were 54.3 and 60.6%, respectively, in extremely preterm infants, significantly higher than in very preterm and moderate preterm infants (all $p < 0.05$). Within the same GA group, the proportion of preterm infants treated with iNO for HRF was lower than that for documented PPHN (all $p < 0.05$), but there was no statistically significant difference in mortality between HRF and documented PPHN treated with iNO (all $p > 0.05$).

Conclusion Among preterm infants with GA < 34 weeks, the rate of iNO usage was highest in extremely preterm infants. However, iNO failed to improve the clinical outcome of extremely preterm infants with refractory hypoxemia, and there was no significant difference in the therapeutic effect of iNO among preterm infants with different GAs.

In the 1990s, researchers discovered that nitric oxide is a vasodilator produced by the vascular endothelium. Inhaled nitric oxide (iNO) can selectively dilate the pulmonary vasculature and reduce pulmonary artery pressure, thereby increasing pulmonary blood flow and improving the ventilation–perfusion ratio in children. As a result, iNO is recommended for the treatment of hypoxic respiratory failure (HRF) and persistent pulmonary hypertension (PPHN) in term and near-term newborns in the United States, European Union, Japan, and other countries and regions.[1] Recent studies have demonstrated that preterm infants with HRF and PPHN born at a gestational age (GA) of less than 34 weeks' experience the same improvement in oxygenation from iNO treatment as those born at a GA of 34 weeks or more.[2] In a study comparing the effectiveness of iNO in newborns with GA < 34 weeks and ≥34 weeks, iNO treatment in GA < 34 weeks' preterm infants with HRF and pulmonary hypertension (PH) was found to be as effective as term and near-term newborn infants in improving oxygenation, without negatively affecting other complications or survival rate.[3] However, multiple guidelines continue to consider the use of iNO in preterm infants with GA < 34 weeks as off-label therapy.[4–6] In 2015, Ellsworth et al[4] reported that despite the U.S. National Institutes of Health's 2011 ban on the routine use of iNO in preterm infants with GA < 34 weeks, the use rate of iNO among 23 to 29 weeks' newborns in the United States still increased from 5.03 to 6.19% between 2009 and 2013. A multicenter cohort study in Japan in 2023 found that the use rate of iNO in extremely preterm infants increased from 0.3% in 2009 to 1.9% in 2016, even under the control of strict criteria.[5] Similarly, a 2021 retrospective analysis in England showed that the rate of iNO use in neonates admitted to neonatal intensive care units (NICUs) increased yearly from 2010 to 2015, with the rate of iNO use in preterm infants with GA < 29 weeks and GA 29 to 33 weeks rising from 4.9 to 15.9% and from 1.1 to 4.8%, respectively.[6] Although there are no guidelines at home or abroad to recommend the use of iNO in preterm infants with GA < 34 weeks, its off-label use has been increasing yearly. Currently, the efficacy and safety of iNO for preterm infants with GA < 34 weeks, particularly extremely preterm infants, have not been conclusively proven.[7,8] Additionally, literature reports indicate that the overall risk of adverse reactions to iNO tends to increase with decreasing GA.[9] Therefore, in order to better understand the use of iNO in preterm infants with GA < 34 weeks in China and evaluate its efficacy, a retrospective multicenter study based on GA (categorized into GA 32–33, 28–31, and 24–27 weeks) was conducted.

Materials and Methods

Study Subjects

This study was a secondary analysis of a retrospective multicentre epidemiological investigation, covering the period from January 2013 to December 2022. Data were collected from eight Class A tertiary hospitals across seven Chinese regions: the Northeast, North China, Northwest, Central China, East China, South China, and Southwest, and NICUs were above IIIB.[10] The children were divided into three groups based on GA: 24 to 27 weeks (extremely preterm infants), 28 to 31 weeks (very preterm infants), and 32 to 33 weeks (moderate preterm infants). The inclusion criteria

were (1) patients with GA between 24 and 33 weeks, (2) those who received invasive respiratory support, and (3) those who received iNO treatment for more than 3 hours. The exclusion criteria were (1) patients with congenital anomalies (except congenital diaphragmatic hernia) or inherited metabolic diseases, (2) those with complex congenital heart disease, (3) those with severe intracranial hemorrhage (IVH) before iNO treatment, and (4) patients who received iNO for preventing bronchopulmonary dysplasia (BPD). The study was registered with the Chinese Clinical Trial Registry (http://www.chictr.org.cn) under the registration number ChiCTR2200066935. The study protocol was approved by the Ethics Committee of the Affiliated Women and Children's Hospital, School of Medicine, Xiamen University (KY-2023-019-H01). Data collection and use were covered by blanket consent.

Data Collection

Using a standard questionnaire, the following data were collected for premature infants during hospitalization: (1) perinatal data: information on premature infants of different GA, including the use rate of iNO, primary morbidity, start time, duration, initial dose, and maximum dose of iNO treatment. (2) Direct causes and mortality: the direct causes for the application of iNO and the associated mortality rates. (3) Treatment effect: the effect of iNO treatment in preterm infants, categorized as complete effect, partial effect, or no effect. (4) Complication rate: the recent complication rate associated with the use of iNO. (5) Mortality: mortality rates among preterm infants with different GA and birth weights (BWs).

Data Management

Each quality control unit's data entry officers strictly adhered to the study protocol. The database was set up using EpiData, and the case report form data was entered in duplicate by two individuals to ensure accuracy. After data collection, each participating unit uploaded the data related to iNO treatment in preterm infants. The database was then locked after thoroughly checking for errors. Quality control personnel from the lead unit maintained close communication with all participating units throughout the study, regularly reviewing the case records and promptly addressing any issues that arose.

Definition of Related Diseases and Diagnostic Criteria

(1) Definition of iNO therapeutic effect: (i) complete effect: fraction of inspiration O_2 (FiO_2) decreased by $\geq$ 20% after 3 hours of iNO treatment; (ii) partial effect: FiO_2 decreased by 10 to 19% after 3 hours of iNO treatment; (iii) no effect: FiO_2 decreased by less than 10% after 3 hours of iNO treatment.[11–14] (2) Diagnostic criteria for related diseases: (i) HRF is defined as mechanical ventilation with an inhaled $FiO_2 \geq 0.6$, mean airway pressure (MAP) $> 10\ cmH_2O$, preductal arterial partial pressure of oxygen (PaO_2) < 50 mm Hg, percutaneous arterial oxygen saturation (SpO_2) $< 85\%$ or oxygenation index (OI) $\geq$ 10 [$OI = FiO_2 \times MAP\ (cmH_2O) \times 100/PaO_2$ (mm Hg) for $>$2 hours without any ultrasound evidence of PH][15]; (ii) the PPHN diagnostic criteria are based on the Expert Consensus on the Diagnosis and Treatment of Pulmonary Hypertension in the Neonate, 2017: either there is no ultrasound examination or there is a clinical manifestation of hypoxemia and ultrasound evidence of PH $>$ 30 mm Hg. After performing ultrasonography a difference of $\geq$5% in SpO_2 was observed pre–post ductal (right upper and right lower limbs)[16]; (iii) early-onset infection and late-onset infection diagnostic standards: early-onset infection diagnosed as one of early-onset sepsis (EOS), early-onset pneumonia, early-onset urinary tract infection, whereas late-onset infection is diagnosed as one of late-onset sepsis (LOS), late-onset pneumonia, late-onset urinary tract infection. The EOS and LOS diagnostic standards can be yielded from the Expert Consensus on the Diagnosis and Treatment of Neonatal Sepsis, 2019.[17] The Practice of Neonatology (5th edition) was consulted for the diagnosis of early-onset or late-onset neonatal pneumonia and urinary tract infections[18]; (iv) hemodynamically significant patent ductus arteriosus (hsPDA) is defined as follows: arterial ductal internal diameter $>$ 1.5 mm, left atrial internal diameter/aortic internal diameter $\geq$ 1.4, or left ventricular end-diastolic internal diameter/aortic internal diameter $\geq$ 2.1, and including at least one of the following clinical manifestations: cardiac murmur, tachycardia ($\geq$160 beats/minute), rapid breathing, enhanced pulse pressure ($>$25 mm Hg), hypotension, Corrigan's pulse, or cardiac enlargement[19]; (v) the Guidelines of Neonatologists Branch of Chinese Medical Doctor Association on Oxygen and Retinopathy in the Treatment of Premature Infants (revised edition) were used to diagnose retinopathy of prematurity (ROP)[20]; (vi) the diagnostic criteria of Bell's staging should be referred for diagnosing necrotizing enterocolitis (NEC) $\geq$ stage II[21]; (vii) moderate to severe BPD is defined as postnatal continuous oxygen dependence $\geq$ 28 days and graded as moderate: FiO_2 21 to 30% or severe: $FiO_2 \geq 30\%$ or need for positive-pressure or mechanical ventilation according to corrected GA of 36 weeks or oxygen demand at discharge;[22] (viii) the diagnosis of severe IVH (grade 3 or 4), periventricular leukomalacia (PVL), and meconium aspiration syndrome were established by referring to Practical Neonatology (5th edition).[18]

Statistical Analysis

The data were statistically analyzed using SPSS 26.0 software (IBM, Armonk, NY). Normally distributed data were expressed as mean $\pm$ standard deviation ($\bar{X} \pm S$), whereas the data with non-normal distributions were expressed as median (interquartile spacing) [M(P25, P75)]. Count data were expressed as a frequency or percentage. Comparisons between two groups of continuous variables were performed using the *t*-test for parametric data and the Mann–Whitney U test for nonparametric data. Fisher's exact test or the chi-square test was used for comparing categorical variables. A *p*-value of $<$0.05 was considered statistically significant. Survival analysis was used to compare the survival rates of preterm infants with different GA and BWs after iNO treatment. Spearman correlation

analysis was employed to assess the relationship between mortality and different GA and BWs in preterm infants.

Results

Inhaled Nitric Oxide Use Rates among Three Groups of Preterm Infants of Different Gestational Ages

The iNO use rates were 4.20% (112/2,666 cases) for GA 24 to 27 weeks, 1.54% (190/12344 cases) for GA 28 to 31 weeks, and 0.85% (132/15,483 cases) for GA 32 to 33 weeks (►Fig. 1). The difference was statistically significant ($\chi^2 = 183.747$, $p < 0.001$). The proportion of iNO use was highest in extremely preterm infants each year.

Perinatal Data and Primary Morbidity in Preterm Infants Treated with Inhaled Nitric Oxide in Different Gestational Age Groups

The incidence of in vitro fertilization, multiple pregnancies, neonatal pulmonary hemorrhage, and neonatal shock were the highest in the 24 to 27 weeks' group ($p < 0.05$), and the iNO start time is the latest in the 24 to 27 weeks' group ($p < 0.05$). The incidence of premature rupture of membranes was the highest in 28 to 31 weeks' group ($p < 0.05$). The incidence of cesarean section was the highest in 32 to 33 weeks' group ($p < 0.05$). There were no statistically significant differences in other perinatal data, the primary incidence of iNO, the duration of iNO treatment, the initial dose, and the highest dose (all $p > 0.05$; ►Table 1).

Mortality in Inhaled Nitric Oxide-Treated Preterm Infants by Gestational Age and Birth Weight among Three Groups

A comparison of mortality rates for preterm infants treated with iNO across different GA revealed that the highest mortality rate was among those born at 24 to 27 weeks (58.0%), with statistically significant differences among the three groups ($p < 0.05$). Similarly, the mortality rate for preterm infants weighing < 1,000 g (56.7%) after iNO treatment was the highest among the three BW groups, also showing statistical differences ($p < 0.05$; ►Table 2). The survival curve indicated that smaller GA and lower BW were associated with higher mortality rates, demonstrating a negative correlation (►Figs. 2 and 3). Spearman correlation analysis showed a GA correlation coefficient of −0.337 ($p < 0.001$) and a BW correlation coefficient of −0.329 ($p < 0.001$).

Inhaled Nitric Oxide Treatment Effect of Preterm Infants of Different Gestational Ages in the Three Groups

There was no statistical difference in the iNO treatment effect of preterm infants with different GA in three groups (all $p > 0.05$; ►Table 3).

Short-Term Complications in Preterm Infants Treated with Inhaled Nitric Oxide of Different Gestational Ages in Three Groups

The incidence of late-onset infection, ROP requiring intervention, IVH (grade 3 or 4), PVL, NEC (≥stage II), and BPD (moderate to severe) showed statistically significant differences among preterm infants in the three GA groups ($p < 0.05$). The highest incidence rates for these complications were observed in the 24 to 27 weeks' group. However, there was no statistically significant difference in the incidence of hsPDA among the three groups ($p > 0.05$; ►Table 4).

The Incidence and Mortality of Indication for Inhaled Nitric Oxide Treatment among Preterm Infants of Different Gestational Ages in Three Groups

The incidence and mortality in preterm infants aged 24 to 27 weeks were significantly higher than those in infants aged 28 to 31 weeks and 32 to 33 weeks in infants treated

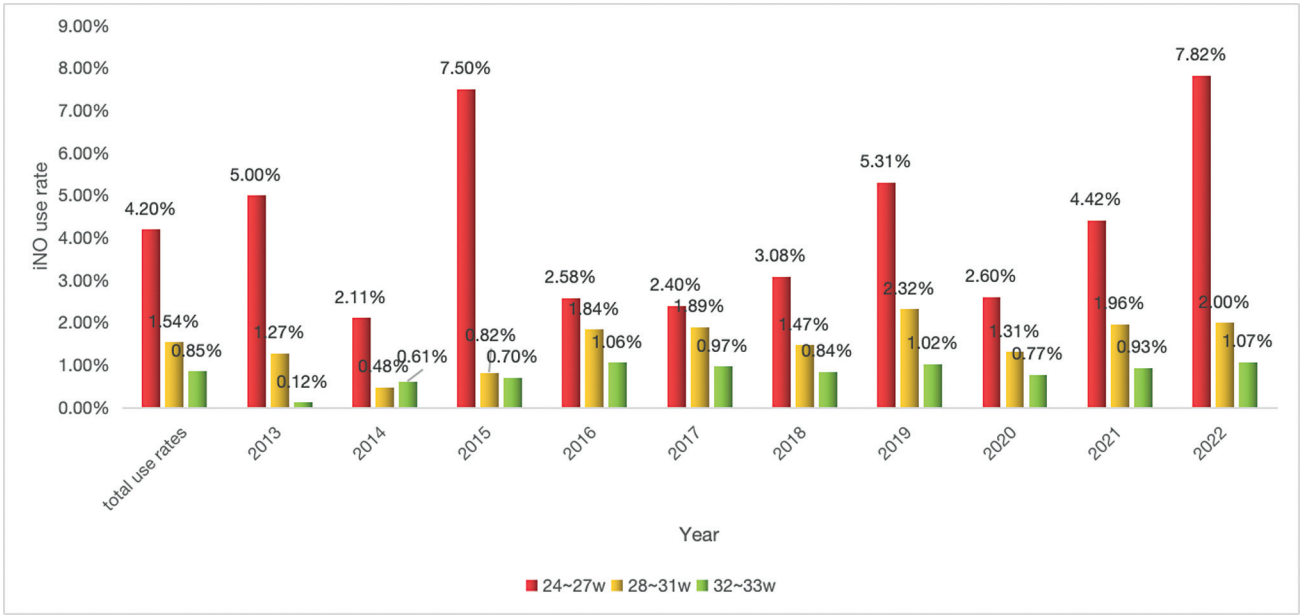

Fig. 1 Comparison of iNO use rates among three groups of preterm infants of different gestational ages. iNO, inhaled nitric oxide.

Table 1 Comparison of perinatal data and primary morbidity in preterm infants treated with inhaled nitric oxide in different gestational age groups

Groups	24–27 wk ($n = 112$)	28–31 wk ($n = 190$)	32–33 wk ($n = 132$)	χ^2/Z	p
Male	76 (67.9)	132 (69.6)	87 (65.9)	0.456	0.896
Cesarean section	31 (27.7)[a]	121 (63.9)[b]	99 (75.0)[c]	60.381	<0.001
SGA	8 (7.1)	7 (3.7)	2 (1.5)	5.147	0.076
Outborn	19 (17.0)	52 (27.2)	33 (25.0)	4.210	0.122
Antenatal steroid use	80 (71.4)	120 (63.3)	91 (68.9)	4.298	0.117
In vitro fertilization	39 (34.8)[a]	44 (23.0)[b]	14 (10.6)[c]	21.695	<0.001
Multiple pregnancies	48 (42.9)	63 (33.0)[b]	23 (17.4)[c]	19.188	<0.001
Premature rupture of membrane	30 (26.8)[a]	79 (41.9)	48 (36.4)	6.682	0.035
Chorioamnionitis	11 (9.8)	23 (12.0)	6 (4.5)	5.386	0.068
Oligoamnios	31 (27.7)	47 (25.1)	35 (26.5)	0.339	0.844
Pregnancy-induced hypertension syndrome	12 (10.7)	26 (13.6)	15 (11.4)	0.707	0.702
GDM	17 (15.2)	36 (20.4)	25 (18.9)	0.799	0.671
RDS	91 (81.3)	156 (81.7)	119 (90.2)	4.901	0.086
Early-onset infection	81 (72.3)	141 (74.3)	94 (71.2)	0.372	0.830
Neonatal pulmonary hemorrhage	32 (28.6)[a]	30 (15.7)	12 (9.1)[c]	16.637	<0.001
Neonatal shock	38 (33.9)[a]	35 (18.3)	17 (12.9)[c]	17.436	<0.001
severe asphyxia	11 (9.8)	22 (11.5)	8 (6.1)	2.797	0.247
MAS	1 (0.9)	4 (2.1)	3 (2.3)	0.766	0.682
Pulmonary surfactant use	102 (91.1)	166 (86.9)	114 (86.4)	1.409	0.494
Vasoactive drugs use	100 (89.3)	153 (80.6)	105 (79.5)	4.880	0.087
iNO start time [M (P_{25}, P_{75})] (h)	47 (13, 447)[a]	17 (8, 35)	19 (10, 28)[c]	22.707	<0.001
iNO duration [M (P_{25}, P_{75})] (h)	53 (21, 138)	54 (29, 83)	54 (27, 76)	0.047	0.997
iNO initial dose [M (P_{25}, P_{75})] (ppm)	10 (10, 20)	10 (6, 20)	10 (8, 20)	1.017	0.602
iNO highest dose [M (P_{25}, P_{75})] (ppm)	15 (10, 20)	15 (8, 20)	12 (8, 20)	1.124	0.570

Abbreviations: GDM, gestational diabetes mellitus; iNO, inhaled nitric oxide; MAS, meconium aspiration syndrome; RDS, respiratory distress syndrome; SGA, small for gestational age.
[a]The comparison between preterm infants in the 28 to 31 weeks' group and those in the 28 to 31 weeks' group was statistically significant.
[b]The comparison between preterm infants in the 28 to 31 weeks' group and those in the 32 to 33 weeks' group was statistically significant.
[c]The comparison between preterm infants in the 24 to 27 weeks' group and those in the 32 to 33 weeks' group was statistically significant.

Table 2 Comparison of mortality in inhaled nitric oxide-treated premature infants by gestational age and birth weight among three groups

Groups	n	Case fatality rate, n (%)	χ^2	p
Gestational age (wk)				
24–27[a]	112	65 (58.0)	44.445	<0.001
28–31[b]	190	63 (33.2)		
32–33[c]	132	23 (17.4)		
Birth weight (g)				
<1,000[a]	90	51 (56.7)	33.589	<0.001
1,000–1,499[b]	146	56 (38.4)		
≥1,500[c]	198	44 (22.2)		

[a]The comparison between preterm infants in the 28 to 31 weeks' group and those in the 28 to 31 weeks' group was statistically significant.
[b]The comparison between preterm infants in the 28 to 31 weeks' group and those in the 32 to 33 weeks' group was statistically significant.
[c]The comparison between preterm infants in the 24 to 27 weeks' group and those in the 32 to 33 weeks' group was statistically significant.

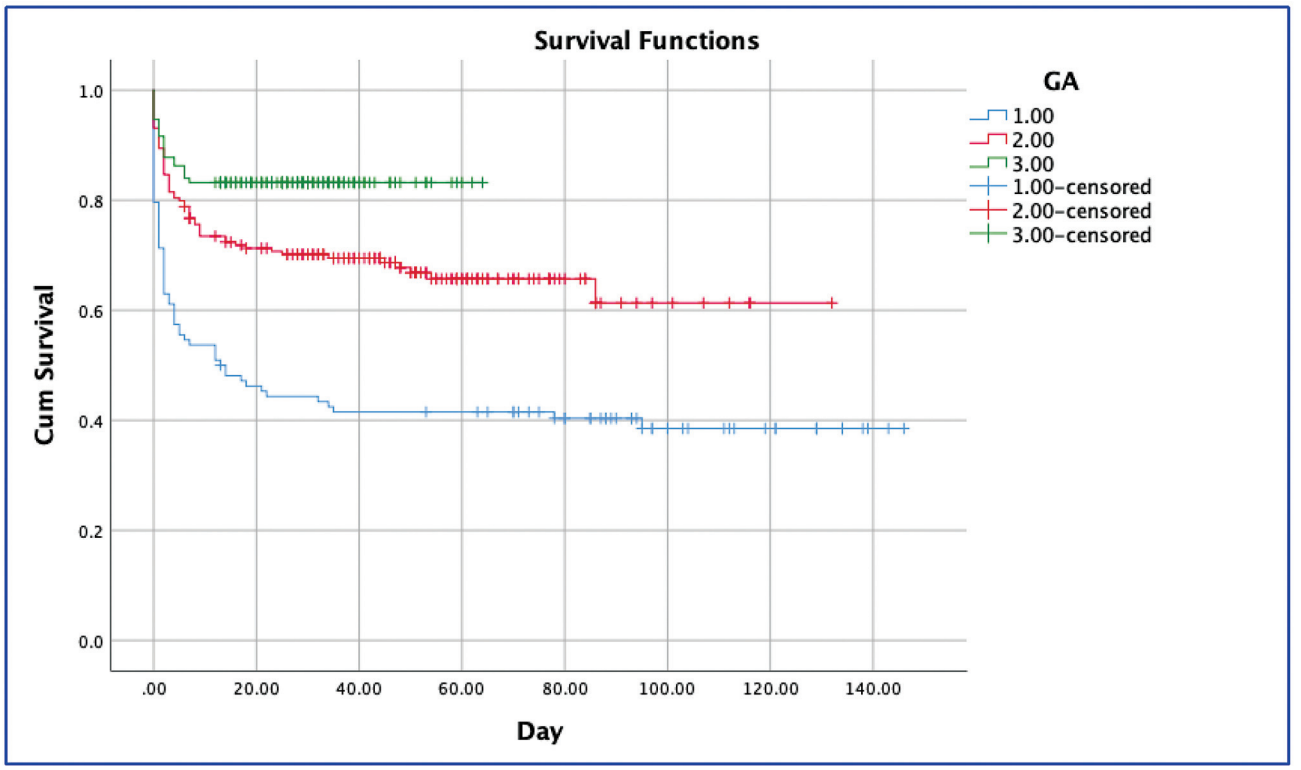

Fig. 2 Survival curves of preterm infants treated with iNO in the three groups at different gestational ages. GA, gestational age; iNO, inhaled nitric oxide.

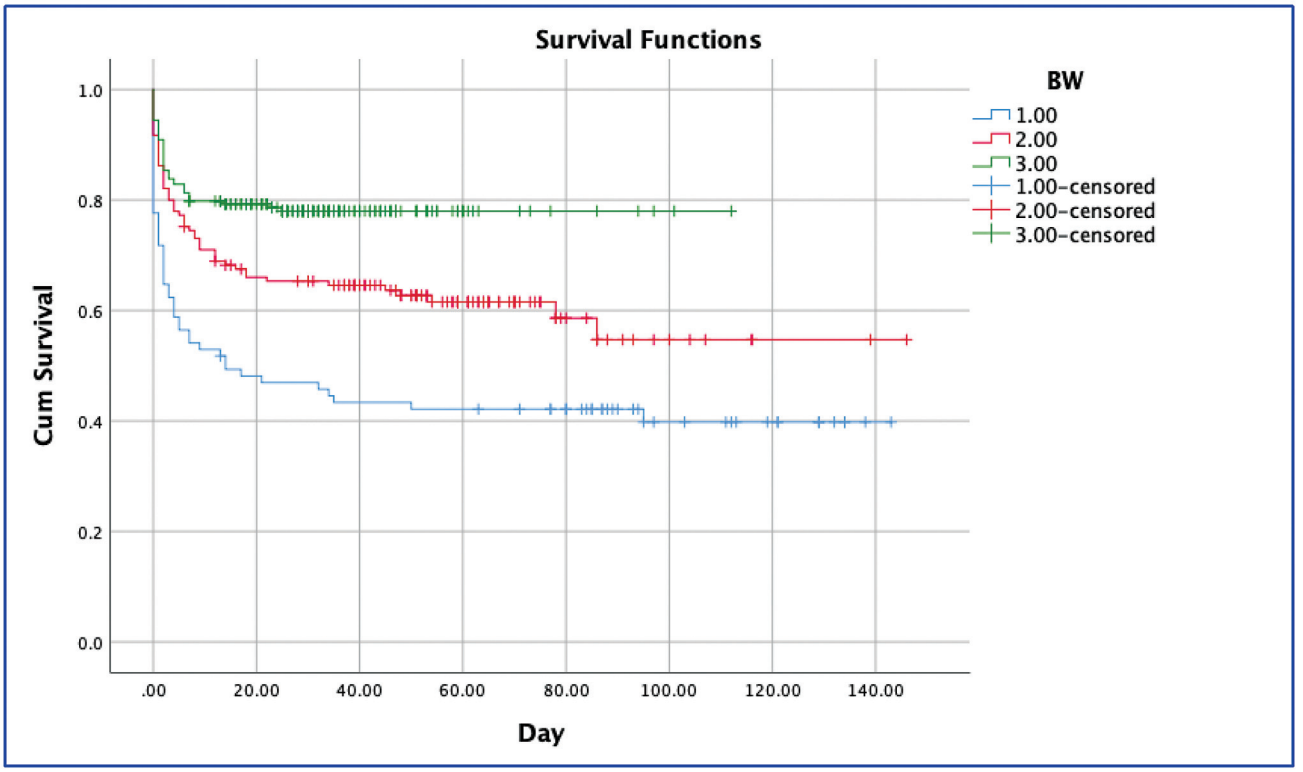

Fig. 3 Survival curves of preterm infants treated with iNO in the three groups at different birth weights. BW, birth weight; iNO, inhaled nitric oxide.

Table 3 Comparison of inhaled nitric oxide treatment effect of preterm infants of different gestational ages in three groups

Effect after 3 h of iNO treatment	24–27 wk ($n = 112$)	28–31 wk ($n = 190$)	32–33 wk ($n = 132$)	χ^2	p
Full effect	29 (25.9)	52 (27.4)	43 (32.6)	2.323	0.313
Partial effect	15 (13.4)	17 (8.9)	16 (12.1)	1.008	0.604
No effect	68 (60.7)	121 (63.7)	73 (55.3)	2.734	0.255

Table 4 Comparison of short-term complications in preterm infants treated with inhaled nitric oxide treatment of different gestational ages in three groups

Groups	24–27 wk ($n = 112$)	28–31 wk ($n = 190$)	32–33 wk ($n = 132$)	χ^2	p
Late-onset infection, *n* (%)	42 (37.5)[a]	30 (15.8)	15 (11.4)[c]	29.644	<0.001
ROP requiring for intervention, *n* (%)	14 (12.5)[a]	2 (1.1)	0 (0.0)[c]	27.028	<0.001
hsPDA, *n* (%)	24 (21.4)	36 (18.9)	18 (13.6)	2.713	0.258
IVH (grade 3 or 4), *n* (%)	28 (25.0)[a]	25 (13.2)[b]	8 (6.1)[c]	18.217	<0.001
PVL, *n* (%)	28 (25.0)[a]	21 (11.1)	14 (10.6)[c]	13.383	0.001
NEC (≥stage II), *n* (%)	15 (13.4)[a]	7 (3.7)[b]	0 (0.0)[c]	23.931	<0.001
Moderate and severe BPD, *n* (%)	44 (39.3)[a]	52 (27.4)[b]	4 (3.0)[c]	48.482	<0.001

Abbreviations: BPD, bronchopulmonary dysplasia; hsPDA, hemodynamically significant patent ductus arteriosus; IVH, intracranial hemorrhage; NEC, necrotizing enterocolitis; PVL, periventricular leukomalacia; ROP, retinopathy of prematurity.
[a]The comparison between preterm infants in the 28 to 31 weeks' group and those in the 28 to 31 weeks' group was statistically significant.
[b]The comparison between preterm infants in the 28 to 31 weeks' group and those in the 32 to 33 weeks' group was statistically significant.
[c]The comparison between preterm infants in the 24 to 27 weeks' group and those in the 32 to 33 weeks' group was statistically significant.

with iNO for HRF, with all differences being statistically significant ($p < 0.05$). For preterm infants treated with iNO for documented PPHN, the incidence rate was significantly lower in the 24 to 27 weeks' group compared with the 28 to 31 weeks and 32 to 33 weeks' groups; however, the mortality rate in the 24 to 27 weeks' group was significantly higher than in the other two groups, with statistical significance ($p < 0.05$). Within each GA group, the incidence of iNO treatment for HRF was lower than for documented PPHN ($p < 0.05$), whereas the difference in mortality between HRF and documented PPHN was not statistically significant ($p > 0.05$; ►Table 5).

Discussion

Due to differences in GA among preterm infants, lung tissue is at varying stages of development and may respond differently to iNO treatment.[23] In this study, we conducted a group

Table 5 Comparison of the incidence and mortality of indication for inhaled nitric oxide among preterm infants of different gestational ages in three groups

Groups	24–27 wk ($n = 112$)	28–31 wk ($n = 190$)	32–33 wk ($n = 132$)	χ^2	p^d	χ^2	p^e
	Incidence mortality	Incidence mortality	Incidence mortality				
HRF	46 (41.1) 25 (54.3)[a]	63 (33.2) 20 (31.7)	30 (22.7) 6 (20.0)[c]	9.564	0.008	10.435	0.005
Documented PPHN	66 (58.9) 40 (60.6)[a]	127 (66.8) 43 (33.9)[b]	102 (77.3) 17 (16.7)[c]			34.527	<0.001
χ^2	112.000 0.436	190.000 0.048	132.000 0.179	–	–	–	–
p^f	<0.001 0.509	<0.001 0.827	<0.001 0.672	–	–	–	–

Abbreviations: HRF, hypoxic respiratory failure; iNO, inhaled nitric oxide; PPHN, persistent pulmonary hypertension of the newborn.
[a]The comparison between preterm infants in the 28 to 31 weeks' group and those in the 28 to 31 weeks' group was statistically significant.
[b]The comparison between preterm infants in the 28 to 31 weeks' group and those in the 32 to 33 weeks' group was statistically significant.
[c]The comparison between preterm infants in the 24 to 27 weeks' group and those in the 32 to 33 weeks' group was statistically significant.
[d]*p* refers to the comparison of the incidence of direct causes of iNO application in preterm infants in the three groups.
[e]*p* refers to the comparison of the direct causes of mortality of iNO application in preterm infants in the three groups.
[f]*p* refers to the comparison between the incidence and mortality of iNO application in preterm infants due to HRF or documented PPHN.

analysis of preterm infants treated with off-label iNO therapy, focusing on those with a GA < 34 weeks. The results indicated that the iNO usage rate was highest in extremely preterm infants, followed by very preterm and moderate preterm infants, suggesting that iNO use is more prevalent in infants with lower GA. Over the past decade, the proportion of iNO cases was consistently highest among extremely preterm infants each year, similar to trends observed in developed countries such as the United Kingdom and the United States.[6,24] We also found that there was no statistically significant difference in the rates of complete effect, partial effect, and no effect of iNO usage. The treatment effect was generally poor across the GA strata. This may be due to alveolar dysplasia, as these preterm infants are in the canalicular and early saccular stages of lung development.[25] Additionally, the higher incidence of short-term complications in preterm infants with lower GA, coupled with suboptimal respiratory and circulatory support, may contribute to poor outcomes. Finally, the poor efficacy of iNO may be related to the absence of PPHN in preterm infants diagnosed with HRF.

This study demonstrated that the incidence of in vitro fertilization and multiple pregnancies among extremely preterm infants was significantly higher than in the other two groups. Mothers who used assisted reproductive technology often faced high-risk factors during pregnancy, and the presence of multiple pregnancies contributed to a higher incidence of extremely preterm births. Consequently, these infants had smaller GA and BW, leading to more severe clinical conditions. The incidence of neonatal pulmonary hemorrhage and neonatal shock was significantly higher in extremely preterm infants compared with the other groups, further indicating their critical condition. Treatment with inhaled iNO in extremely preterm infants began much later than in the other two groups. This delay suggests that clinicians were preferred to use iNO treatment as a rescue therapy after other treatments had failed. This approach aligns with the recommendations of the American Heart Association, the American Thoracic Society, and the Pediatric Pulmonary Hypertension Collaborative Network, which advocate for the selective use of iNO in preterm infants with PPHN when optimal respiratory and circulatory support proves ineffective.[26]

Clinicians often express concerns about the safety of using iNO beyond its indicated uses. Consistent with previous findings,[23,27,28] our study examined short-term complications in preterm infants treated with iNO and found the highest rates of late-onset infection, ROP required for intervention, IVH (grade 3 or 4), PVL, NEC (≥stage II), and BPD (moderate to severe) in extremely preterm infants. These rates increased as GA decreased. Van Meurs et al[27] studied 420 preterm infants (GA < 34 weeks, BW between 401 and 1,500g) with HRF and found that infants with BW > 1,000g appeared to benefit from iNO treatment, showing decreased mortality or BPD incidence and no increased IVH incidence. However, preterm infants with BW ≤ 1,000 g who received iNO had significantly increased mortality and higher IVH incidence. A multicenter randomized study by Kinsella et al[28] involving 793 preterm infants (GA ≤ 34 weeks) with HRF who required mechanical ventilation found that iNO did not reduce the composite outcome of mortality or BPD incidence in preterm infants weighing 500 to 1,250 g. Our study similarly showed a higher incidence of BPD (moderate to severe) in extremely preterm infants, consistent with Schmidt and Ramamoorthy's findings that lower GA is associated with higher BPD incidence.[29] Additionally, a multicenter randomized trial in the European Union confirmed that iNO could not prevent or reduce BPD occurrence.[30]

A 2019 Japanese study on extremely preterm infants revealed that iNO is primarily used as an early rescue treatment for echocardiographically diagnosed PH in these infants, which can reduce the case fatality rate and led to the development of a standardized protocol for iNO treatment in this population.[31] Our study found that the incidence of indication for iNO treatment for HRF was highest in extremely preterm infants, followed by very preterm and moderate preterm infants. The mortality of infants treated with iNO for HRF was significantly higher in extremely preterm infants than in very preterm and moderate preterm infants. Very preterm and moderate preterm infants treated with iNO were more likely to have documented PPHN compared with extremely preterm infants. Yet, the mortality of extremely preterm infants was significantly higher than that of very preterm and moderate preterm infants treated with iNO. The above results indicate that regardless of the indications for iNO treatment, the mortality rate for extremely preterm infants was over 50%, and mortality increased as GA decreased. In this study, survival curves demonstrated a negative correlation between mortality and both GA and BW. Since most extremely low BW infants are extremely preterm, and iNO use for documented PPHN was lowest among extremely preterm infants, they did not show a benefit from iNO for PH.

Among the preterm infants in the same GA group, the proportion of iNO use for documented PPHN was significantly higher than for HRF, whereas the mortality rate was not lower. This is inconsistent with previous studies suggesting iNO treatment might be effective for PPHN in preterm infants with GA < 34 weeks diagnosed by echocardiography.[32] Because iNO is an emergency treatment, some preterm infants this study did not actively undergo echocardiography before using iNO treatment and only clinically diagnosed PPHN. In addition, it is also possible that some cases of HRF without echocardiographic evaluation were actually complicated by undiagnosed PPHN. These factors may be the reason why there was no statistically significant difference in mortality rates between the two conditions. It cannot be denied that some HRF infants are primarily induced by PPHN and do not have lung disease, and these infants may have a good response to iNO. This study suggests that iNO failed to improve the clinical outcome of extremely preterm infants with refractory hypoxemia. This is consistent with the finding by Carey et al[11] that off-label prescription of iNO is not associated with reduced in-hospital mortality among extremely premature neonates with respiratory distress syndrome.

Limitations

This study has several limitations. First, the lack of GA < 34 weeks iNO treatment guidelines for preterm infants led to clinician discretion in iNO administration, causing selection bias toward treating more clinically severe cases. Secondly, as a retrospective study, it did not include a control group of preterm infants who did not receive iNO, affecting result analysis. Thirdly, the lack of active echocardiographic monitoring before iNO treatment meant some children were only clinically diagnosed with PPHN without ultrasound evidence, impacting the comparison between documented PPHN and HRF. Fourth, the study did not examine long-term complications of iNO, focusing only on short-term complications in infants treated with iNO and mortality, and did not follow up on long-term survival, lung function, and neurodevelopmental outcomes. Lastly, varying timing, dosage, and methods of iNO evacuation across centers affected the study results. Despite the aforementioned limitations, the strength of this study lies in its being the first multicenter study stratified by GA to describe the use of iNO therapy in preterm infants with GA < 34 weeks. The large sample size and robust data provide a relatively reliable evidence for further research on the application of iNO in preterm infants with GA < 34 weeks.

Conclusion

In conclusion, among the preterm infants with GA < 34 weeks, the rate of iNO treatment was the highest in extremely preterm infants, but iNO failed to improve the clinical outcome of extremely preterm infants with refractory hypoxemia. Future research should focus on prospective, multicenter studies of iNO treatment in preterm infants with GA < 34 weeks. This will provide evidence-based data and help develop standardized iNO treatment guidelines, ultimately improving clinical practice.

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Conflict of Interest

None declared.

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