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CLINICAL STUDY

Is inhaled nitric oxide a miraculous molecule? The analysis of nitric oxide use in newborns with persistent pulmonary hypertension of the newborn

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ABSTRACT

AIM: The aim of this study was to establish a set of hospitalised patients with persistent pulmonary hypertension of the newborn (PPHN) by using retrospective analysis according to gestational age, position during childbirth, type of childbirth, dosage and length of the treatment by inhaled nitric oxide (NO) and application of inotropic agents as well as interindividual specifics and the background of PPHN.

RESULTS: Our cohort consisted of 11 newborns who were hospitalised in Neonatal Department of Intensive Medicine between 1st January 2017 and 31st December 2019. Four of these patients were born prematurely. Only two out of eleven patients were born vaginally. Nine of the newborns were diagnosed with secondary PPHN, in three of these cases it was caused by infection. The highest dose of inhaled nitric oxide used was 40 ppm.

CONCLUSION: The focus of this paper was the therapeutic use of nitric oxide, its various applications and the effect of it on pulmonary circulation of the newborn. Inhaled NO is a selective pulmonary vasodilator used as a therapeutic agent for PPHN of the newborn. The conclusions of this paper can be beneficial in the development of better therapeutic strategies for patients with PPHN in the future *(Tab. 3, Fig. 1, Ref. 40)*.

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KEY WORDS: newborn, nitric oxide, persistent pulmonary hypertension of the newborn, inhaled nitric oxide.

Abbreviations: cGMP – cyclic guanosine monophosphate, ECMO – extracorporeal membrane oxygenation, iNO – inhaled nitric oxide, NDIM – Neonatal Department of Intensive Medicine, NO – nitric oxide, PDE3 – phosphodiesterase type 3, PH – pulmonary hypertension, PPHN – pulmonary hypertension of the newborn, PVR – pulmonary vascular resistance

Introduction

In small quantities, nitric oxide (NO) is a part of physiological processes as a signal molecule. These include regulation of blood flow (vasodilation), thrombocyte reactivity and neurotransmission. Higher concentration of NO has cytotoxic and cytostatic properties (1, 2). There is growing evidence that NO has an important role in the regulation of pulmonary function and pathophysiology of pulmonary diseases (3, 4). NO was detected in higher concentration in exhaled air of animals and people with various inflammatory diseases of the pulmonary system (5, 6).

The use of inhaled nitric oxide (iNO) to treat newborns with hypoxic respiratory failure associated with pulmonary hypertension (PH) was approved by the European Medicines Agency and Food and Drug Administration at the beginning of the 21st century due to numerous studies which demonstrated that treatment with iNO reduces the use of the extracorporeal membrane oxygenation (ECMO) in term and preterm babies (7).

Twenty years later, iNO is used for hypoxic respiratory failure in newborns with different aetiologies (8). In Slovakia, iNO is only used in patients with persistent pulmonary hypertension of the newborn (PPHN). The ability to influence pulmonary vascular tone without a decrease in systematic vascular tone is the reason why iNO is preferred in the treatment of PPHN (9). PPHN is a complex disease that can occur within a wide range of cardiopulmonary disorders, which are characterized by PH and altered vasoreactivity. It can lead to complications such as chronic lung disease, delayed psychomotor development and death (9, 10, 11). The main characteristic of these disorders is increased pulmonary vascular resistance (PVR) which causes right-to-left shunting of blood through the arterial duct and/or *foramen ovale*. The quick improvement in oxygenation after therapy with iNO is a result of selective decrease in PVR which leads to less mixing of venous and arterial blood through the shunts. Nonetheless, the oxy-

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genation can also improve in patients without right-to-left shunts (12, 13, 14).

When inhaled, NO binds to haemoglobin which is saturated with oxygen to 60 – 100 % in capillaries. If the saturation of haemoglobin is lower, NO is binding to deoxyhaemoglobin and creates nitrosylhaemoglobin which in the presence of oxygen changes to nitric oxides and methaemoglobin (15).

Methaemoglobin is toxic because it is unable to bind and release oxygen from the molecule. High concentration of methaemo globin has a negative effect on oxygen transport in the body (16). In newborns, the activity of methaemoglobin reductase is lowered compared to adults (17). It is advised to check the levels of methaemoglobin in the blood one hour after starting the therapy by spectrophotometric analyser, which is capable of distinguishing foetal haemoglobin from methaemoglobin. The physiological amount of methaemoglobin in blood is under 2 %. When the amount is higher, it is important to lower the dose of iNO or completely stop the administration of iNO. There is also the possibility to administer methylene blue to reduce methaemoglobin in circulation (15, 18).

The iNO is recommended for use for infants after 34 weeks of gestation with hypoxaemic respiratory failure associated with PH who have insufficient response to mechanical ventilation. The maximal starting dose is 20 ppm. It is important to observe preductal and postductal saturations if the gap is higher than 10 %, it means a significant right to left shunting is present. It is recommended to use high-frequency oscillatory ventilation if possible and also administer surfactant. It is important to rule out any congenital heart disease with an echocardiogram exam. Weaning of the therapy needs to be done cautiously because rebound PH can occur (9, 15, 19).

During the foetal period, foetal circulation has a high PVR because the lungs are not ventilated. The systemic vascular resistance in foetal circulation is lower than PVR because the placenta acts as a reservoir of blood. Gradient between systemic and pulmonary resistance is the reason why there is right-to-left shunting of blood through *ductus arteriosus* to be oxygenated in placenta (20, 21).

After being born and taking the first breath, the PVR quickly decreases due to distension of the lungs and endogenous chemical vasodilators such as prostaglandins and NO. Production of NO starts during birth and after birth, it is enhanced by the shear stress and the concentration of oxygen in the blood activates the enzyme endothelial NO-synthase which also helps with vasodilation after birth (22).

If the PVR remains high after birth, a resistance gradient like the one in foetal circulation causes right-to-left shunt of blood across the open *ductus arteriosus*. Therefore, some amount of blood is bypassing the pulmonary circulation without being oxygenated which leads to cyanosis. This is the basis for PPHN (11, 21).

Risk factors for PPHN include structural lung abnormalities caused by congenital diaphragmatic hernia (23, 24, 25). An American study which involved 12,000 newborns showed a 5-fold increase in the incidence of PPHN in neonates born by Caesarean section compared to those born vaginally. From the same study, also chorioamnionitis emerged as a significant risk factor, increasing the risk of PPHN three times (23, 26).

PPHN is most commonly diagnosed in term newborns but can also occur in premature infants. PPHN is manifested by respiratory failure and cyanosis, which occurs 6–12 hours after birth. Laboratory values for PPHN include hypoglycaemia, hypocalcaemia, polycythaemia, and thrombocytopaenia (12). Hypocalcaemia affects the functioning of endothelial NO-synthase, so its correction is important. The X-ray of the thorax finding is variable and depends on the primary disease associated with PPHN. In idiopathic PPHN, an X-ray shows reduced lung blood flow with mild hyperinflation without parenchymal infiltrations (22).

On echocardiography, we need to rule out other causes of hypoxaemia, such as structural changes in the heart. Although high pressure in the pulmonary arteries is a common finding in neonatal lung diseases, the diagnosis of PPHN is unclear until we have clear evidence of a bidirectional or dominant right-left shunt through the *ductus arteriosus patens* or *foramen ovale apertum* (27).

The management of a newborn with PPHN includes the treatment and prevention of hypothermia, hypoglycaemia, hypoxaemia, hypocalcaemia, anaemia, metabolic acidosis, infectious complications and hypovolemia. Proper nutrition, optimal ambient temperature, minimizing stress stimuli and, if necessary, administering analgesics and sedation (22, 27).

Less severe PPHN with minimal respiratory distress can be detected at screening for cardiac developmental defects after birth if a baby develops reduced oxygen saturation (28). The therapy in these newborns consists of supportive therapy and administration of oxygen. It is important that they are closely monitored for the risk of sudden deterioration of the disease and the need for administration of non-invasive ventilation or intubation (22). It is important to set the ventilation parameters to achieve an "ideal" ventilation volume, because both low and high ventilation volumes increase PVR. Adequate ventilation volume also prevents high pressure lung trauma. Lung tissue damage can lead to pulmonary oedema, decreased pulmonary compliance and increased sensitivity of lung tissue to inflammation due to increased cytokine production and neutrophil accumulation in the lungs (27, 29).

Today, PPHN therapy involves improving systemic haemodynamics by fluid repletion and cardiotonic therapy (dobutamine, dopamine), which has a positive effect on cardiac output and oxygen transport to the body. If there is no response to this therapy, ECMO is often required (30).

Low dose iNO therapy (5–20 ppm) improves oxygenation and reduces the need for ECMO in patients with various aetiologies of PPHN (13, 31). During the treatment, it is important to monitor the patient's oxygenation parameters and gradually reduce the dose of iNO as they improve. Discontinuation of iNO is possible only after a significant reduction in the patient's ventilatory support or after 96 hours of therapy. If we decide to stop the therapy, it is necessary to reduce the dose to 1 ppm for 30–60 minutes and then stop the administration of iNO and watch for signs of hypoxia in the newborns. If saturation drops by more than 20 %, therapy should be resumed at 5 ppm and the decision to discontinue therapy should be considered after 12 to 24 hours (15).

Milrinone and sildenafil are vasodilators which are used in severe iNO therapy resistant cases of PPHN. Milrinone inhibits cyclic guanosine monophosphate (cGMP) by degrading cGMP-specific phosphodiesterase type 5 and sildenafil inhibits cyclic adenosine monophosphate by degrading phosphodiesterase type 3 (PDE3) (32). Sildenafil administered orally in children with PPHN improved oxygenation and reduced mortality in studies in which iNO was not available (33). Intravenous administration of sildenafil, according to a study by Steinhorn et al, improves oxygenation in patients without prior iNO therapy (21). When administered systemically, there is a high risk of side effects such as hypotension due to systemic vasodilation. This risk can be reduced by slow intravenous administration of an initial dose (0.4 mg over 3 hours) followed by a maintenance dose (0.07 mg/kg/h). Administration of sildenafil reduces the intensity of the rebound phenomenon at the end of iNO administration (32).

Methods

data was collected from records of patients who were hospitalized at Neonatal Department of Intensive Medicine (NDIM) between January 1st, 2017 and December 31st, 2019 (included). These patients were selected based on the diagnosis of PPHN and therapy with iNO. The following data was collected: gestational age, birth weight and length, pathologies related to amniotic fluid, type of delivery, maximal dose of iNO, oxygenation index and type of PPHN. For growth assessment, we used the Fenton growth chart.

Results

The data was collected from 11 children. All of these patients were prenatally eutrophic and only 2 (18 %) were born vaginally. The rest of the patients were born by Caesarean delivery. Four of the newborns were born prematurely (36.3 %) and the rest of them (7 patients) were born in term. Four newborns were born without amniotic fluid volume pathology, two had anhydramnion, two had polyhydramnion, two had blood-stained amniotic fluid, and two had green-stained amniotic fluid (Tab. 1). The majority of patients had secondary PPHN. The most common reason was pulmonary hypoplasia, infection and meconium aspiration (Tab. 2).

The maximum dose of iNO used was 40 ppm in two patients (Patient 3; Patient 5). These two patients also had the longest duration of iNO use (Patient 3: 95 hours; Patient 5: 80 hours). Patient oxygenation indices ranged from 19.1 to 103 (Tab. 3). Two patients (patient 3; patient 7) were ventilated in synchronized intermittent mandatory ventilation mode, the other nine were ventilated in high-frequency oscillatory mode. Nine newborns were intubated within 24 hours of birth (patients: 1, 2, 4, 5, 7, 8, 9, 10, 11), four of them immediately after birth (patients: 2, 4, 8, 9). The shortest time to extubating was 82 hours, the longest 550 hours (Patient 6 and Patient 8 respectively). The surfactant was administered to three patients. Of these, one was born in gestational week 33 (Patient 8), one in week 41 (Patient 9) and one in week 42 (Patient 11). Sildenafil was administered to seven patients. Inotropic

Tab. 1. Foetal growth, amniotic fluid and type of delivery in hospitalized patients.

Patient	Gestational age (weeks+days)	Foetal growth	Amniotic fluid	Type of delivery
1	40+0	eutrophic	green	Caesarean delivery
2	33+5	eutrophic	anhydramnion	Caesarean delivery
3	35+0	eutrophic	polyhydramnion with blood	Caesarean delivery
4	37+2	eutrophic	anhydramnion with blood	Caesarean delivery
5	40+0	eutrophic	normal	vaginal delivery
6	38+0	eutrophic	normal	Caesarean delivery
7	34+0	eutrophic	normal	Caesarean delivery
8	32+1	eutrophic	polyhydramnion	Caesarean delivery
9	41+0	eutrophic	opalesque	vaginal delivery
10	38+0	eutrophic	normal	Caesarean delivery
11	41+3	eutrophic	green	Caesarean delivery

Tab. 2. Number of patients sorted by diagnosis and gestational age at delivery.

Diagnosis	All patients	Birth≥37 weeks	Birth<37 weeks
Primary PPHN	2	0	2
Secondary PPHN	9	7	2
Secondary PPHN due to			
Sepsis	1	1	0
Congenital heart problem	1	0	1
Pulmonary hypoplasia	3	2	1
Infection	2	2	0
Meconium aspiration	2	2	0

PPHN – persistent pulmonary hypertension of the newborn

Tab. 3. Specific parameters of therapy.

Patient	Duration of therapy (hours)	Maximal concentration of iNO (ppm)	Inotropic therapy	Use of sildenafil	Oxygenation index
1	44	20	DOP, ADR	yes	28.7
2	17	20	DOP, ADR	yes	60
3	95	40	ADR, DOB, NOR	yes	19.1
4	3	20	DOP, ADR, DOB	yes	103
5	80	40	ADR, DOB	yes	35
6	41	25	DOP, ADR, DOB	no	33.4
7	20	27	DOP	no	25
8	73	20	DOP, ADR	yes	28
9	5	20	DOP, ADR, DOB, NOR	no	32
10	21	20	DOP, ADR, NOR	no	41
11	68	15	DOP, ADR, DOB	yes	33

DOP – dopamine, ADR – adrenaline, DOB – dobutamine, NOR – noradrenaline, iNO – inhaled nitric oxide, ppm – parts per million

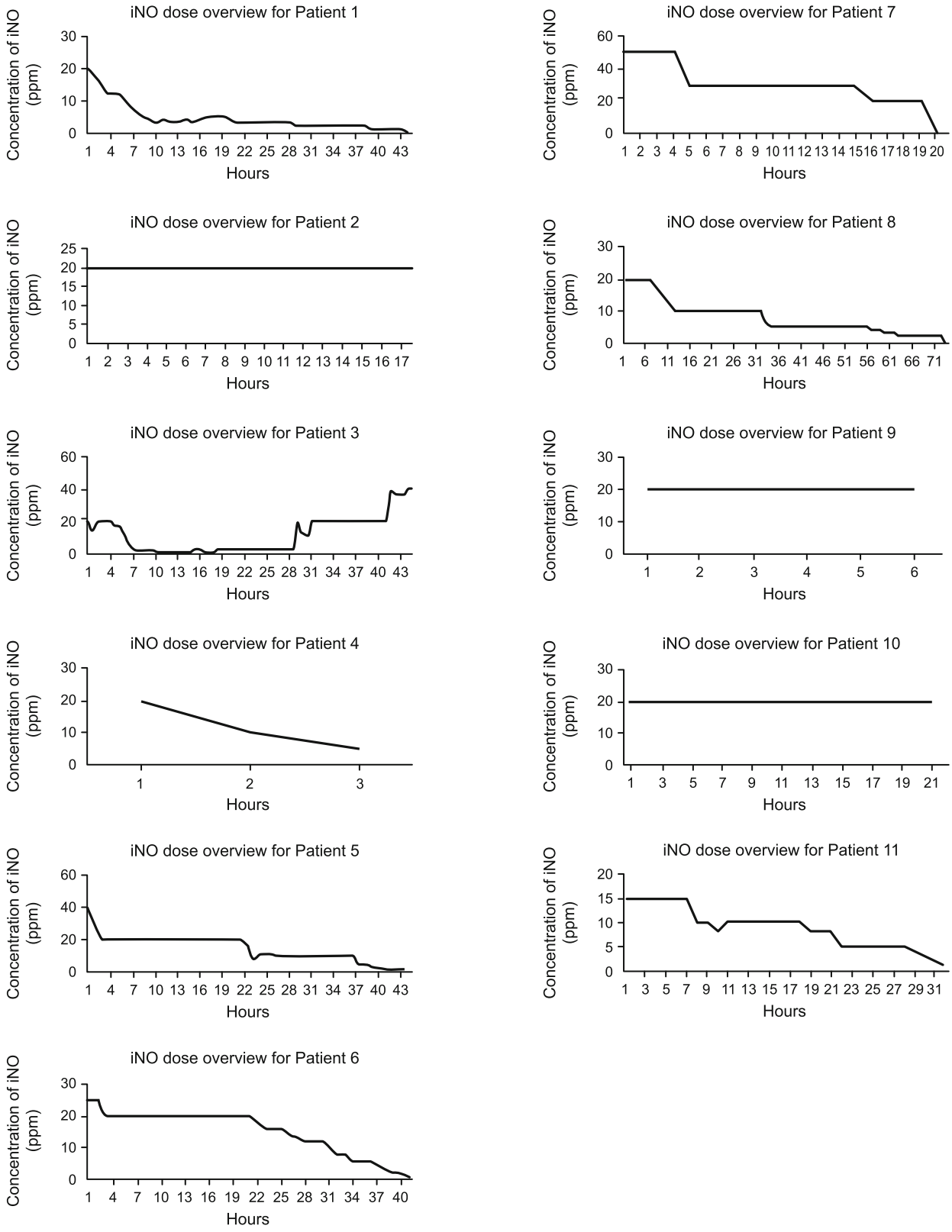

Fig. 1. Graphic representation of therapy course for patients 1-11. iNO – inhaled nitric oxide, ppm – parts per million.

support was required during therapy in all patients. Dopamine, dobutamine, noradrenaline and adrenaline were used.

Different courses of therapy with iNO in the patients in our study are presented in Figure 1. Patients 1, 5, 6, 8 and 11 display a positive trend in therapy with slow lowering of the doses and gradual weaning off the therapy, which prevents rebound PH. In the case of patient 3, we can see escalation of the therapy due to the worsening state of the patient. The longest duration of therapy was 95 hours in case of patient 3 and the shortest was 3 hours in case of patient 4.

Discussion

At birth, the human lung circulation undergoes a transformation from a high-resistance pulmonary circulation to a low-resistance circulation. In congenital lung diseases and congenital heart defects, this change can be slowed down or stopped. It is important that we are able to quickly and reliably diagnose the increased pressure in the pulmonary circulation and then try to identify the underlying cause of this condition. In the neonatal period, it can be PPHN, which can be caused by meconium aspiration, sepsis or pneumonia, congenital diaphragmatic hernia, congenital heart defects, pulmonary hypoplasia, and structural changes of the airways (34). In our cohort, most patients were diagnosed with secondary PPHN, which was caused by sepsis in one case (Patient 1), pneumonia in two cases (Patients 5 and 6) meconium aspiration in two cases (Patient 9 and 11), and congenital lung developmental defects in two cases (Patients 4 and 8).

PPHN is the cause of respiratory failure in newborns in almost 10 % of the cases. This complex disease can lead to many complications, even death (10, 35). Out of the eleven patients in this study, five patients died during the treatment.

Typical symptoms of PPHN are respiratory failure and cyanosis, which occur 6 to 12 hours after birth. It can often manifest as perinatal asphyxia, low Apgar score and amniotic fluid discoloration, but this is not specific. PPHN can occur even without any perinatal manifestations. In our cohort, based on the Apgar score, difficult postnatal adaptation can be seen in up to 9 out of the 11 patients. Amniotic fluid colour changes have been reported in 5 of the patients (27).

Therapy of the newborns with confirmed PPHN consists of supportive therapy aimed at improving systemic haemodynamics. Each patient in our cohort was treated with inotropic agents. In most cases, treatment with two or more substances was required, in one case a combination of up to 4 drugs (Patient 9 received dopamine, dobutamine, adrenaline, noradrenaline) (22, 30).

In our cohort, only two patients were born before the 34th week. In a study from 2006 to 2010, the newborns born before the 34th week required longer therapy than newborns born in the later gestational weeks. In our cohort, the longest therapy was required by a patient born at 35 weeks (Patient 3) and 32 weeks (Patient 8) (95 and 73 hours). Clark attributed these results to more comorbidities in preterm infants compared to full-term infants (36). In our cohort, 4 patients were born prematurely, two of them didn't survive. Although PPHN is more common in term patients, it is important to recognise early signs of preterm labour in mothers and apply adequate prophylaxis because the outcomes and the duration of the treatment are prolonged in preterm infants. There are several promising methods of early diagnosis of preterm labour such as detection of matrix metalloproteinase-8, interleukin-8 and heat shock protein 70 in vaginal samples of pregnant women in third semester (37).

Our findings regarding the management of childbirth correlate with the results of an American study that documented an increase in the incidence of PPHN syndrome in section-born infants compared to vaginal-born infants. Also, in our cohort, up to 9 children with PPHN requiring iNO therapy were born by section and only 2 vaginally (26).

Abman et al in their chapter mention that changes regarding the amount and colour of amniotic fluid can be associated with PPHN but are not universally applicable as a symptom of PPHN (27). In our cohort, four out of eleven patients had physiologic findings regarding amniotic fluid (Tab. 1). Two of them (Patients 1 and 11) had green stained amniotic fluid. In both cases it was associated with meconium aspiration. Both of these patients recovered, but prolonged therapy with iNO and inotropic agents was needed. Two of these patients (Patient 2 and 4), were diagnosed prenatally with anhydramnion, Patient 4 had prenatally diagnosed polycystic kidney disease which was associated with pulmonary hypoplasia and face anomalies – Potter sequence. Patients 3 and 8 had polyhydramnion diagnosed prenatally. After birth, oesophageal and duodenal atresia was confirmed in the case of patient 3. In the case of patient 8, pleural effusion was diagnosed and evacuated prenatally with pulmonary sequestration diagnosed postnatally. Out of 5 patients who died despite intensive therapy only one of them had physiological findings regarding amniotic fluid (Patient 10). Based on our cohort, pathologic findings regarding the amniotic fluid could be a negative prognostic factor in newborns with PPHN because it often indicates associated comorbidities.

The highest dose used in clinical studies was 20 ppm. Only in The Neonatal Inhaled Nitric Oxide Study, the dose was increased up to 80 ppm, if there was no response to treatment (38). In our cohort, doses from 15 ppm to 40 ppm were administered. The highest dose (40 ppm) was administered to two patients (Patients 3 and 5), one of them (Patient 3) didn't survive and one did (Patient 5). If we look at the gestational age of the patients, patient 3 was born in the 35th week and patient 5 in week 40. Patient 3 was born by Caesarean section which is a negative prognostic factor, however, patient 5 was born vaginally (26). The prognosis of the patient could be influenced by the dose, gestational age of the patient and the management of childbirth, although patient 3 also had complex congenital heart disease and atresia of oesophagus and duodenum which could be a risk factor.

The treatment courses for patients in our cohort varied. In graphical representations of treatments of patients 5, 6, 7, 8, and 11, the typical course of treatment with slow de-escalation of therapy to prevent rebound phenomenon is seen (Fig. 1) (39, 40). Out of 11 patients, 7 were given an initial dose of 20 ppm. Patient 6 was treated initially with a 25 ppm dose which could be lowered after 3 hours to 20 ppm which was sustained for nearly 20 hours

and then gradually lowered until the iNO could be discontinued. A similar course of treatment was given to patients 7 and 8, although each of these patients had different causes of PPHN. Patient 6 was diagnosed with perinatal infection, patient 7 with primary PPHN and patient 8 with pulmonary sequestration. Although diagnosed with primary PPHN, patient 7 received the lowest starting dose (5 ppm) which could be lowered slowly during the course of 20 hours. In the case of patient 1 the de-escalation of treatment was very successful in the first hours which led to a temporary rebound in the 17th hour of therapy which was managed by a slower decrease in iNO concentration. Graph of patients 2, 9, and 10 shows an inadequate response to iNO treatment. All three of these patients died within 21 hours after starting the treatment. Patient 2 and patient 10 were diagnosed with congenital pulmonary malformations which can potentially be the reason for lack of response to treatment. The highest dose (40 ppm) was used in two patients (Patient 3 and 5). Patient 5 received iNO in dose of 40 ppm at the beginning of the treatment which could be perceived as a negative prognostic marker. However, the concentration of iNO could be lowered over the course of 40 hours and the patient could maintain satisfactory saturation of oxygen without iNO. In the case of patient 3, the highest dose was administered during and after surgery (due to oesophageal atresia and perforation) because the patient didn't tolerate the anaesthesia during the procedure. Based on our cohort, the lower starting dose of iNO is not always associated with better outcomes and there are more factors which influence the outcome such as associated congenital defects of the lungs and heart and need for invasive treatment.

This work had its limitations, such as the lack of anamnestic data in the available documentation. Another limitation is the size of the group of patients, as iNO therapy is used in Slovakia in limited indications, it is not used off-label as in foreign studies. The results of this article could in the future serve as the basis for a study with a larger group of patients, whether prospective or retrospective.

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