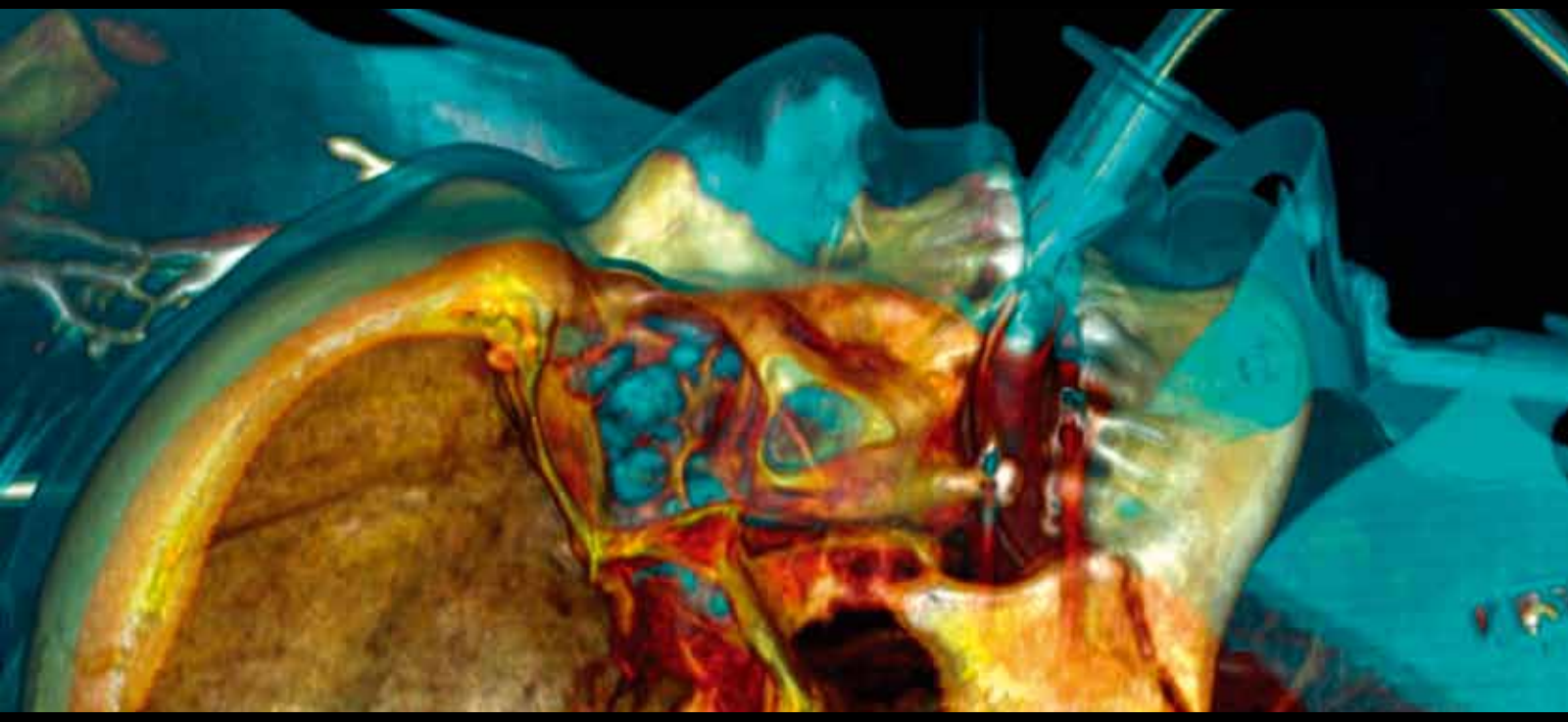
Critical Care Research and Practice

Neonatal Lung Disease and Respiratory Failure

Guest Editors: Hercília Guimarães, Anton van Kaam, Gustavo Rocha, and Manuel Sánchez Luna



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Editorial Board

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Editorial

Neonatal Lung Disease and Respiratory Failure

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Neonatal lung disease and respiratory failure are common in neonates. Causes of lung disease and respiratory failure are divers and are often associated with maternal pathology, prematurity, and congenital anomalies. Better knowledge and understanding of the pathophysiology of lung disease have led to the development of more effective and safe therapies for both acute and chronic disease.

This special issue includes research articles as well as review articles that will stimulate the continuing efforts to understand the neonatal lung, the pathophysiology of the lung diseases, the development of strategies to treat these conditions, and evaluation of outcomes.

Very preterm infants are commonly exposed to a chronic, often asymptomatic, chorioamnionitis that is usually diagnosed by the histological evaluation of the placenta, only after delivery. G. Rocha extensively reviewed and summarized the available literature on whether histological chorioamnionitis may be associated to lung injury of the preterm newborn. There is a strong evidence that histologic chorioamnionitis is associated with a reduction of incidence and severity of respiratory distress syndrome (RDS). Short-term maturational effects on the lungs of extremely premature infants seem to be, however, accompanied by a greater susceptibility of the lung, eventually contributing to an increased risk of bronchopulmonary dysplasia (BPD). Genetic susceptibility to BPD is an evolving area of research, and several studies have directly related the risk of BPD to genomic variants. There is a substantial heterogeneity across the studies in the magnitude of the association between chorioamnionitis and BPD, and whether or not the association is statistically significant. Recent studies generally seem to confirm the effect of chorioamnionitis on RDS incidence, while no effect on BPD is seen. Recent data have suggested susceptibility for subsequent asthma to be increased on long-term followup.

S. Gupta and S. M. Donn describe novel approaches to surfactant administration. Surfactant replacement therapy has been the mainstay of treatment for preterm infants with RDS for more than twenty years. Although tracheal instillation is still reputed as the classical method of surfactant delivery, alternative techniques have been investigated. In recent years, the growing interest in noninvasive ventilation has led to novel approaches of administration. These potential strategies include intra-amniotic instillation, pharyngeal instillation, administration via laryngeal mask airway, administration using a thin intratracheal catheter without IPPV, or aerosolized/nebulized surfactant administration in spontaneously breathing infants. Data from clinical trials of these novel techniques will need to evaluate long-term respiratory and neurodevelopmental outcomes and to assess the true cost effectiveness.

Survival and outcomes for preterm infants with RDS have improved over the past 30 years. F. Flor-de-Lima et al. report the changes in perinatal care and delivery room management at her center in 2005, when early nasal continuous positive airway pressure (NCPAP) and intubate surfactant extubate (INSURE) were introduced, and the positive impact on respiratory outcome and survival of very low birth weight newborns.

M. O'Reilly et al. focus the short- and intermediate-term outcomes of preterm infants receiving positive pressure ventilation in the delivery room. Although recent advances in neonatal care have improved survival rates, rates of BPD remain unchanged. Although neonatologists are increasingly applying gentle ventilation strategies in the neonatal intensive care unit, the same emphasis has not been applied immediately after birth. A lung-protective strategy should start with the first breath to help establish functional residual capacity, facilitate gas exchange, and reduce volutrauma and atelectotrauma. Ideally, a lung-protective strategy should start immediately after birth because the lungs of very preterm infants are uniquely susceptible to injury because they are structurally immature, surfactant deficient, fluid filled, and not supported by a stiff wall.

Flow-synchronized nasal intermittent positive pressure ventilation (SNIPPV) could be used to reduce endotracheal ventilation, increase successful extubation, decrease the rate of apnea of prematurity, and have better outcome indicated by fewer death and/or BPD in preterm and term newborn infants. C. Gizzi et al. also demonstrate that the introduction of the routine use of SNIPPV after INSURE technique in their NICU reduced the need for MV and favorably affected other short-term morbidities of premature infants <32-week gestation with RDS.

Vascular endothelial growth factor (VEGF), an angiogenic factor secreted by type II pneumocytes, could play a role in congenital diaphragmatic hernia (CDH) pathogenesis. Studies in rodents suggest that VEGF accelerates lung growth in hypoplastic lungs. E. Sanz-López et al. show the changes in the expression of VEGF after fetal tracheal occlusion (TO) in an experimental model of CDH. VEGF protein was significantly lower in fetuses with CDH. TO induced a significant increase in VEGF compared to the fetuses that did not undergo TO.

Patent ductus arteriosus (PDA) is a significant cause of morbidity and mortality in preterm infants. Many factors are associated with closure of ductus arteriosus in preterm infants. K. W. Olsson et al. show that a high ductal flow velocity is associated with successful pharmacological closure of PDA in 22–27-week gestational age infants during pharmacological treatment with cyclooxygenase inhibitors.

O. Carvalho and C. Gonçalves evaluated the lung retinoids content to study the possible difference between male and female mice during prenatal lung development, and to comprehend if the vitamin A metabolism is similar in both genders. They observed that there is a sexual dimorphism in the retinoids content during mice lung development, more evident in the last developmental days, as well as a difference in the retinoids metabolism.

Respiratory syncytial virus (RSV) lower respiratory tract infection is the most common viral respiratory infection in both term and preterm infants. Some studies have been done to determine which risk factors are the best predictors for severe RSV disease. A. Gonçalves et al. evaluated the chest radiographic pattern in RSV disease of the newborn and identified that newborns with a consolidation pattern on admission chest radiograph had a more severe disease course, with greater risk of respiratory support, invasive mechanical ventilation, supplemental oxygen, and prolonged hospitalization.

All of these chapters illustrate some important aspects of the contemporary respiratory neonatal medicine to be adopted in clinical practices and to stimulate experimental or clinical research.

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Research Article

Changes in the Expression of Vascular Endothelial Growth Factor after Fetal Tracheal Occlusion in an Experimental Model of Congenital Diaphragmatic Hernia

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Introduction. Vascular endothelial growth factor (VEGF), an angiogenic factor secreted by type II pneumocytes, could play a role in congenital diaphragmatic hernia (CDH) pathogenesis. Animal studies suggest that VEGF accelerates lung growth. *Aim*. To quantify VEGF on fetal lungs in a nitrofen rat model for CDH and to analyze the effect of tracheal occlusion (TO) in VEGF in fetal lung rats after nitrofen and in control rats not exposed to nitrofen. *Methods*. Pregnant rats received nitrofen on day 9.5 of gestation. Fetuses were divided into 2 groups: those that underwent TO on day 20 and those that did not. On day 21, fetuses were delivered, and the lungs were dissected for subsequent VEGF quantification. *Results*. CDH was detected in 43% of the fetuses that received nitrofen. Fetuses with CDH showed significantly reduced lung weight/fetal weight ratio and lower VEGF levels than the remainder. A higher VEGF value was observed after TO. *Conclusions*. VEGF protein was significantly lower in fetuses with CDH. TO induced a significant increase in VEGF compared to the fetuses that did not undergo TO. Although not statistically significant, we observed higher VEGF levels in fetuses with CDH and TO compared to fetuses with CDH and no further intervention.

1. Introduction

Congenital diaphragmatic hernia (CDH) is a malformation associated with incomplete closure of the pleuroperitoneal membrane, secondary herniation pushing the abdominal viscera into the thorax, and pulmonary hypoplasia. The patient usually experiences severe respiratory failure and pulmonary hypertension resulting from pulmonary hypoplasia. Consequently, morbidity and mortality are high. The prevalence of CDH is estimated in 1 case for every 3000 newborns [1], although it is difficult to measure given the high intrauterine mortality (spontaneous and induced). According to the Congenital Diaphragmatic Hernia Registry, which pools data from more than 50 centers, survival ranges from 50% to 67% depending on the series [2–4].

Current investigations are aimed at prevention and the search for an effective treatment for pulmonary hypoplasia. Several strategies have been proposed to improve growth of the hypoplastic lung before birth, the most outstanding being occlusion of the fetal trachea, which has been shown to stimulate growth of the fetal lung in an experimental model [5].

Adequate fetal pulmonary vascularization is an essential component to the development of a mature alveolus that is capable of performing effective gas exchange after birth [6]. Angiogenesis requires several growth factors, mainly vascular endothelial growth factor (VEGF), a potent angiogenic factor secreted by type II pneumocytes in the developing lung that mediates vasculogenesis and postnatal vascular remodelling. VEGF could play a role in the pathogenesis of CDH, and recent studies in rodents suggest that it could accelerate growth in prenatal nitrofen-induced hypoplastic lungs [6, 7].

2. Objectives

The objective of this paper was to measure VEGF in the lungs of fetuses with CDH induced by administration of nitrofen to the mother, and to analyze the effect in VEGF values after occlusion of the fetal trachea in an experimental model of CDH in rat fetuses.

3. Material and Methods

The study protocol was approved by the Animal Investigation Committee of Hospital Gregorio Marañón. All procedures were performed according to European legislation for the protection of animals used for scientific purposes (Directive 86/609/EEC).

3.1. Study Subjects. The study subjects were female Sprague-Dawley rats weighing 225–250 g and male rats of proven fertility. The animals were housed in custom facilities at the research laboratory at 55% humidity and 21°C. They were exposed to a 12/12-hour light-dark cycle and received special granulated feed and water on demand. Surgical procedures were performed in rooms equipped with the necessary specific equipment.

3.2. Experimental Model

3.2.1. Controlled Fertilization. After 24 hours' visual and olfactory contact, the females were cohoused at 20:00 hours with a male at a ratio of 3 : 1 so that they could be fertilized during the night. Day 0 started at 00:00 hours on the day cytology demonstrated the presence of a sperm plug in the vagina of the fertilized female.

3.2.2. Administration of Nitrofen. On day 9.5 of gestation, the pregnant rats received 100 mg of nitrofen (2, 4-dichlorophenyl 4-nitrophenyl ether diluted in 2 mL of olive oil) through an orogastric tube. The control animals received the same volume of olive oil without nitrofen.

3.2.3. Tracheal Occlusion. The trachea of the fetuses was occluded on day 20 of gestation. The pregnant rats were anesthetized with inhaled 3% isoflurane and 1.5% maintenance isoflurane. Intramuscular ketorolac was used as a postoperative analgesic. Rectal temperature was monitored constantly and maintained using a homeothermic blanket for rats. Access to the abdominal cavity was by medial minilaparotomy, which enabled the uterine horns to be visualized. Once the uterine wall was opened, the fetal head and neck were exposed (Figure 1), using a surgical microscope (Zeiss OPMI 99, Zeiss Inc. Oberkochen, Germany) to facilitate the maneuvers. The fetal neck was exposed and hyperextended, and a small transverse medial incision was done. The trachea was exposed, and a nonabsorbable 10/10 nylon ligament was tied to close the trachea (Figure 2). The occlusion was confirmed by direct visualization, and the fetus was returned to the uterus. Each mother underwent a number of hysterotomies that varied according to the number of fetuses, surgical time required, difficulty of the technique for each subject, and estimated time of temperature loss. In all cases, the objective was to perform 6 hysterotomies with tracheal occlusion in half of the fetuses and exposure with no manipulation of the trachea in the remainder (tracheal occlusion control group). The maternal laparotomy was closed on 2 planes (muscle aponeurosis and skin). Recovery was confirmed by evaluating normal activity and recovery of appetite and intestinal transit.

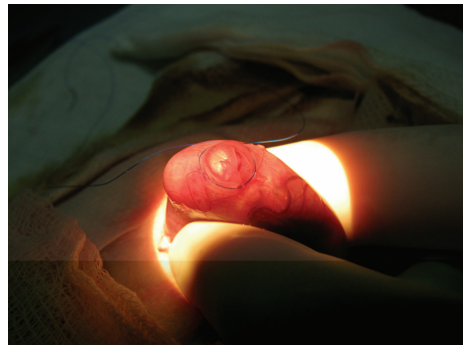

Figure 1: Exposure of the fetal head.

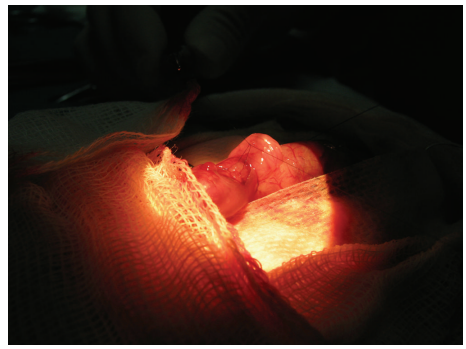

Figure 2: Intrauterine tracheal occlusion.

3.2.4. Extraction of the Fetal Lungs. The anesthetized rat underwent Cesarean section on day 21 (term was on day 22). The rat and her fetuses were then sacrificed. After weighing the fetus, the presence or absence of a diaphragmatic defect was confirmed, and 2 lung explants were taken and weighed before being frozen immediately in liquid nitrogen at −80°C for subsequent measurement of VEGF using immunoanalysis.

3.3. Statistical Analysis. All results are expressed as mean ± SD. The mean fetal weight, lung weight, lung weight/fetal weight ratio, and VEGF were calculated using SPSS 16.0 and compared between groups using Student's t-test. Statistical significance was defined as $P < 0.05$.

4. Results

137 fetuses were analyzed and divided into groups as follows: Group 1 comprised 62 control fetuses and Group 2 comprised

Table 1: Comparison of mean weight by group (control versus nitrofen).

	Control ($n = 62$)	Nitrofen ($n = 75$)	P
Fetal weight (mean)	5.16 g ± 0.41	4.42 g ± 0.71	**0.00**
Lung weight (mean)	0.057 g ± 0.03	0.049 g ± 0.02	**0.03**
Lung weight/fetal weight	0.011 ± 0.01	0.010 ± 0.02	0.96

Table 2: Group exposed to nitrofen (75 fetuses). Comparison of mean weights according to presence or not of congenital diaphragmatic hernia (CDH).

	No CDH ($n = 43$)	CDH ($n = 32$)	P
Fetal weight (mean)	4.44 g ± 0.70	4.36 g ± 0.73	0.58
Lung weight (mean)	0.053 g ± 0.05	0.039 g ± 0.02	**0.00**
Lung weight/fetal weight	0.0119 ± 0.01	0.0071 ± 0.01	**0.00**

Table 3: Fetal weight, lung weight, and lung-to-fetal ratio of fetuses with CDH divided into two groups—tracheal occlusion and no tracheal occlusion.

	Tracheal occlusion ($n = 6$)	No tracheal occlusion ($n = 26$)	P
Fetal weight (mean)	3.40 g ± 0.28	4.46 g ± 0.29	**0.01**
Lung weight (mean)	0.020 g ± 0.04	0.031 g ± 0.06	0.08
Lung weight/fetal weight	0.0061 ± 0.00	0.0072 ± 0.06	0.50

75 fetuses exposed to nitrofen. A defect in the diaphragm was observed in 32 of the fetuses exposed to nitrofen (42.6%). No cases of diaphragmatic hernia were observed in the control group.

Mean fetal weight, mean lung weight, and the ratio of lung weight to fetal weight were measured and compared between groups. The mean weights of the fetuses and lungs were significantly lower in Group 2, although no significant differences were found in the ratio of lung weight to fetal weight between the groups (Table 1).

In Group 2, fetuses who developed CDH had a significantly lower lung weight and lower ratio of lung weight to fetal weight ratio than those who did not (Table 2).

A third analysis of weight in the Group 2 fetuses with CDH (32 fetuses) compared the results to those fetuses that underwent tracheal occlusion (6 cases) and those that did not (26 fetuses) (Table 3). Statistically significant differences were found between the groups in mean fetal weight, but not in lung weight ($P = 0.08$) or in the ratio of lung weight to fetal weight.

The difference in the mean VEGF value between Group 1 and Group 2 was not statistically significant (4.12 ± 0.60 and 3.65 ± 0.74 pg/μg; $P = 0.157$) as seen in Figure 3. In Group 2, the VEGF value was significantly lower in the fetuses that had CDH than in those that did not (2.91 ± 0.59 pg/μg versus 3.85 ± 0.70 pg/μg; $P = 0.02$) (Figure 4).

Comparing the subgroup of fetuses who developed CDH with the remaining fetuses who did not develop CDH (Group 1 + Group 2 without CDH), the VEGF values were significantly lower in fetuses with CDH than in the other fetuses (2.91 ± 0.59 pg/μg versus 3.99 ± 0.73 pg/μg; $P = 0.03$) (Figure 5).

The mean VEGF value of the fetuses that underwent tracheal occlusion was 7.65 ± 0.92 pg/μg compared with

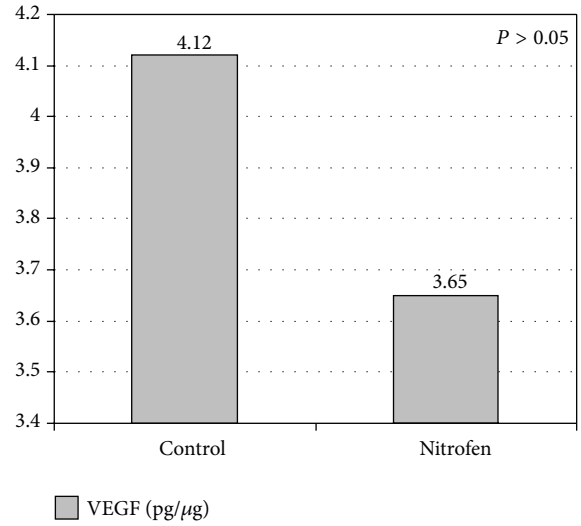

Figure 3: Vascular endothelial growth factor (VEGF) in Group 1 (controls) versus Group 2 (nitrofen). Values are expressed as mean (pg/μg).

3.39 ± 0.60 pg/μg in the total of fetuses that did not undergo tracheal occlusion ($P = 0.00$) (Figure 6).

Finally, we compared the VEGF values of fetuses with CDH that underwent tracheal occlusion with those that did not and found that the differences were not statistically significant (2.43 ± 0.66 pg/μg versus 2.20 ± 0.81 pg/μg; $P = 0.27$) (Figures 7 and 8).

5. Discussion

5.1. Experimental Model. Experimental induction of diaphragmatic defects in rats using nitrofen is a predictable

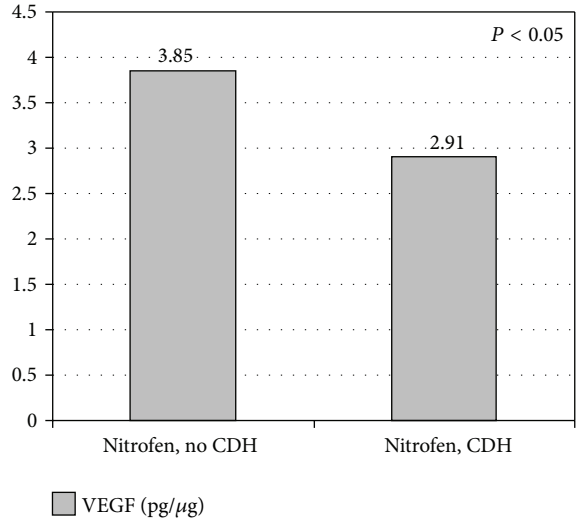

Figure 4: Vascular endothelial growth factor VEGF in Group 2 (nitrofen fetuses). Comparing fetuses with and without CDH. Values are expressed as mean in pg/μg.

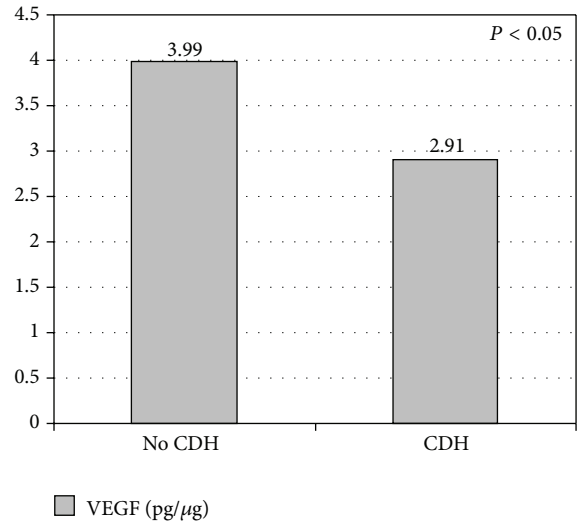

Figure 5: Vascular endothelial growth factor VEGF in fetuses with and without congenital diaphragmatic hernia. Values are expressed as mean in pg/μg.

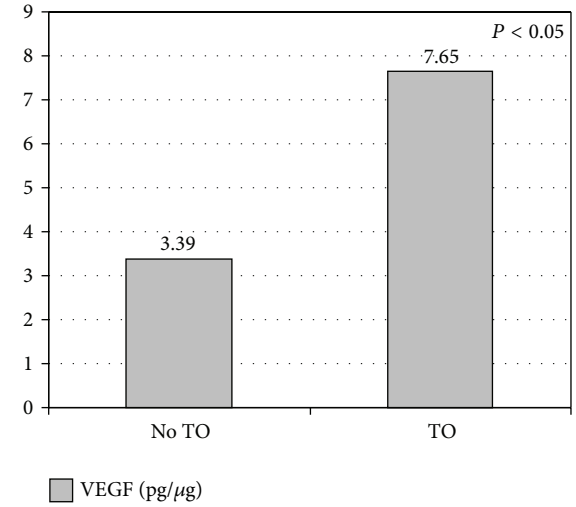

Figure 6: Vascular endothelial growth factor, VEGF, in fetuses with congenital diaphragmatic hernia. Comparison of tracheal occlusion versus no tracheal occlusion. Values are expressed as mean in pg/μg.

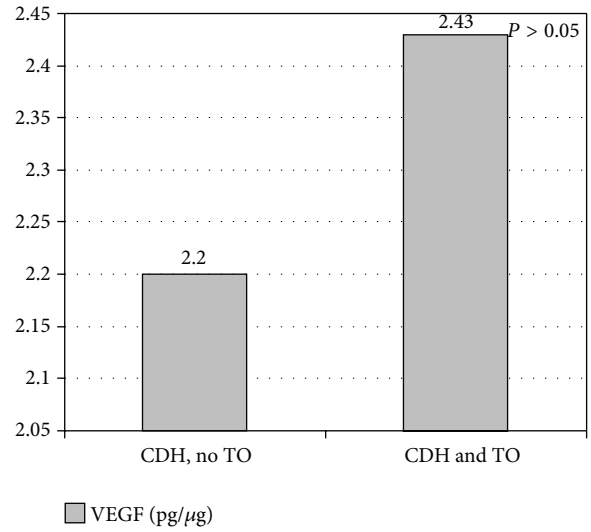

Figure 7: Vascular endothelial growth factor (VEGF) in fetuses with congenital diaphragmatic hernia (CDH). Comparison of tracheal occlusion (TO) versus no TO. Values are expressed as mean (pg/μg).

and easily reproducible approach for the study of CDH. The fetuses develop CDH at a specific point during gestation once the correct dose is administered [7]. Nitrofen-induced CDH is associated with malformations that are similar to the human CDH phenotype, for example, pulmonary hypoplasia, neural crest defects, cardiovascular defects, and other conditions [8–10]. Moreover, a phenotype similar to that of Fryns syndrome in humans has been reported in rats [11]. Experimental studies show that passage of the abdominal viscera into the thorax through a defect in the diaphragm is independent of the development of pulmonary hypoplasia. In the nitrofen model, pulmonary hypoplasia does not only result from diaphragmatic hernia and direct compression of the lung, since pulmonary hypoplasia is constant, whereas only 40–80% of fetuses develop CDH. These results were corroborated in our study, in which we found a lower lung weight in fetuses exposed to nitrofen than in control fetuses (Table 1). However, the ratio of lung weight to body weight was significantly lower in those fetuses that developed CDH. These findings could enable us to act against the mechanisms that govern lung development, regardless of the development of hernia.

5.2. Role of VEGF. VEGF is an angiogenic factor secreted by type II pneumocytes that induces growth in endothelial cells *in vitro*, angiogenesis *in vivo*, and proliferation of epithelial cells in the lungs. VEGF plays a crucial role in

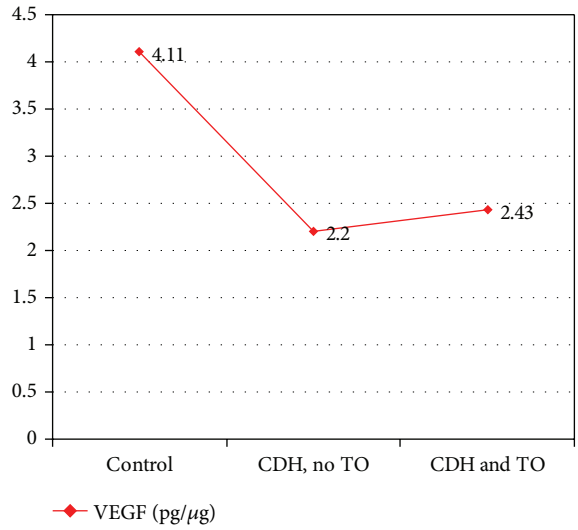

Figure 8: VEGF values in control rats, fetuses with congenital diaphragmatic hernia (CDH), and fetuses with CDH that underwent tracheal occlusion (TO).

the development of the human fetal lung. Expression increases between the canalicular and saccular phases, reaching a peak at week 31 of gestation. It subsequently decreases during the alveolar phase, thus acquiring a key role in alveolar development. Expression in the rat fetal lung peaks on day 16 of gestation, at the beginning of the saccular phase and before closure of the diaphragm [12, 13].

Various experimental findings suggest that VEGF plays an important role in pulmonary morphogenesis and in the pathogenesis of CDH [6, 14]; however, few data are available on the role of VEGF in the pathogenesis of CDH in humans. Increased expression of VEGF in small lung arteries and supernumerary arteries has been observed in newborns with pulmonary hypertension who died of CDH and may represent an attempt by the fetus to stimulate angiogenesis in lungs in which development has stopped [15]. These data differ from those obtained in experimental CDH models, in which the amount of VEGF is reduced, possibly because the method used to measure protein values varies between studies [16]. Our calculation of VEGF values in the fetal lung (measured using immunoanalysis and expressed as pg/μg) revealed no differences in VEGF levels between the fetuses that received nitrofen and the control group. In contrast, we did find a statistically significant difference in VEGF levels between fetuses with CDH and fetuses without it, as well as in the group of fetuses that received nitrofen. Consistent with the findings of other studies, our results confirm the importance of VEGF in lung morphogenesis and suggest a role for VEGF in the pathogenesis of CDH [6, 14].

The mechanical distension produced by tracheal occlusion accelerates growth and maturation of the lung; however, it delays differentiation of type II pneumocytes and formation of surfactant [17]. These mechanical factors seem to affect expression of VEGF in lung tissue. Figure 7 shows the trend toward recovery of VEGF levels in fetuses with CDH that underwent tracheal occlusion in our sample, although the differences were not statistically significant. Recent reports indicate that the increase in lung volume and the ratio of lung weight to fetal weight are greater when tracheal occlusion is prolonged [18, 19].

In summary, in this animal model of CDH in fetal rats induced by nitrofen administrated to pregnant rats, lung VEGF values were significantly lower in fetuses with CDH compared to those who did not develop CDH. Tracheal occlusion induced a significant increase in the mean VEGF compared to the total of fetuses that did not undergo tracheal occlusion, although differences were not statistically significant we did observe a trend toward reduced expression of this protein in fetuses with CDH. Tracheal occlusion could improve expression of VEGF in the lungs.

Conflict of Interests

The authors do not have any conflict of interests concerning this study.

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Review Article

Short- and Intermediate-Term Outcomes of Preterm Infants Receiving Positive Pressure Ventilation in the Delivery Room

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Although recent advances in neonatal care have improved survival rates, rates of bronchopulmonary dysplasia remain unchanged. Although neonatologists are increasingly applying gentle ventilation strategies in the neonatal intensive care unit, the same emphasis has not been applied immediately after birth. A lung-protective strategy should start with the first breath to help in the establishment of functional residual capacity, facilitate gas exchange, and reduce volutrauma and atelectotrauma. This paper will discuss techniques and equipment during breathing assistance in the delivery room.

1. Introduction

Approximately 20% of premature infants require breathing support at birth [1, 2]. An international consensus on resuscitation suggests equipment and techniques if infants fail to initiate breathing [3]. It is agreed that positive pressure ventilation (PPV) is the cornerstone of respiratory support at birth [3]. During the application of PPV in the delivery room (DR) the lungs of preterm infants are exposed to potentially injurious tidal volumes (V_T) [4, 5]. Although neonatologists are familiar with the concept of reducing lung injury and are increasingly careful in the neonatal intensive care unit (NICU) to apply PPV strategies that are gentle to the lung, the same gentle approach has not been translated into practice in the DR [6]. Ideally, a lung-protective strategy should start immediately after birth. At birth, the lungs of very preterm infants are uniquely susceptible to injury because they are structurally immature, surfactant-deficient, fluid-filled, not supported by a stiff chest wall, and are unable to generate adequate end expiratory pressure to maintain open alveoli [6]. To facilitate early development of functional residual capacity (FRC), reduce atelecto- and volutrauma, and improve oxygenation, sustained inflations (SIs), positive end expiratory pressure (PEEP), and continuous positive airway pressure (CPAP) have been advocated [7–15].

This paper summarizes the various methods available to clinicians for the provision of positive pressure ventilation to preterm infants in the DR, the impact on clinical outcomes, and potential areas for further research.

2. Search Strategy

The aim of this article was to review the available literature about delivery room interventions and their effect on outcomes in newborn infants. We reviewed books, resuscitation manuals and articles from 1960 to present with the search terms "Infant, Newborn," "Delivery Room," "Neonatal Resuscitation," "Intubation," "Surfactant," "Positive Pressure Respiration," and "Continuous Positive Airway Pressure."

3. Respiratory Support in the Delivery Room

The purpose of PPV is to establish FRC, deliver an adequate V_T to facilitate gas exchange, and stimulate breathing while minimizing lung injury [6]. The International Liaison Committee on Resuscitation and various national resuscitation guidelines recommend equipment and techniques for neonatal resuscitation [16–18].

3.1. Ventilation Devices during Respiratory Support in the Delivery Room. There is currently limited evidence to guide

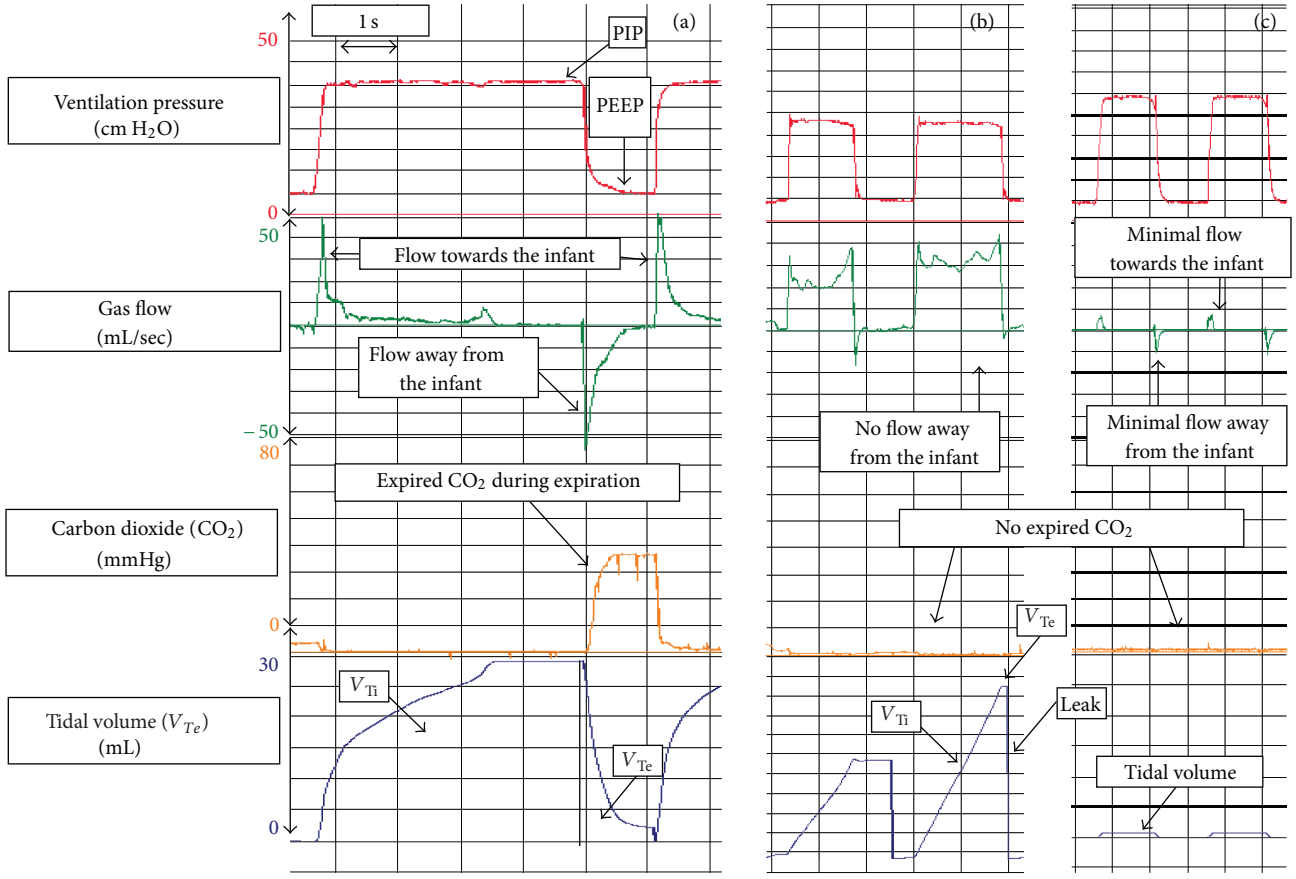

FIGURE 1: The figure shows how an RFM can help to optimize PPV in a 26-week preterm infant with 800 gram birth weight. In (a) during inflations the airway pressure increased form baseline (PEEP) to the set PIP. Similar gas flow towards and away from the infant indicates no leak around the mask. In addition the V_T wave returns to baseline indicating good mask ventilation. Expired CO_2 can be observed once the V_T wave returns to baseline. With the start of the next inflation expired CO_2 drops to zero. In (b) PEEP and PIP are achieved; however gas flow only moves towards the infant and only minimal gas flow away from the infant indicating mask leak. The V_T wave shows inspiratory V_T (V_{Ti}) but no expiratory V_T (V_{Te}). Mask leak indicated as a straight line in the V_T curve, and no expired CO_2 displayed. In (c) displays airway obstruction which can be identified by minimal or no gas flow movements, no expired CO_2, and no or minimal V_T waves.

clinicians' choice of device for providing PPV in the DR [19]. Self-inflating bags, flow-inflating bags, or T-piece devices may all be used for mask ventilation. A self-inflating bag, however, does not provide PEEP or CPAP [6, 20]. An attached PEEP-valve provides inconsistent PEEP and cannot deliver CPAP [21–24]. A flow-inflating bag provides variable- and operator-dependent PEEP [16, 25]. With a T-piece device a more consistent, predetermined level of PEEP and PIP can be delivered [5, 21, 22]. In addition, a T-piece device has been shown to be the most accurate device for delivering a sustained inflation breath [17, 22, 26, 27].

3.2. Respiratory Function Monitor. The use of respiratory function monitor (RFM) has been described during neonatal simulation [28], neonatal resuscitation [29, 30], and neonatal transport [31]. A Respiratory Function Monitor uses a small, low dead space flow sensor (~1 mL), which is placed between a ventilation device and a facemask or endotracheal tube [30]. The monitor can be set to continuously display airway pressure, gas flow, and tidal volume waves. It also measures and displays numerical values for peak airway pressure, PEEP, CPAP, V_T, respiratory rate, and expiratory minute ventilation [30]. Adverse events (e.g., mask leak or airway obstruction) can be identified by observing the displayed waveforms [30]. The leak between mask and face or around an endotracheal tube is expressed as a percentage of the inspired V_T. Leak is graphically presented as the difference in area under the flow curves above (inflation) and below (deflation) zero flow (Figure 1(b)) [30]. Several observational studies in the DR have reported on the advantages and disadvantages of an RFM during neonatal resuscitation. Recently, a randomized trial by Schmölzer et al. compared the additional use of an RFM with clinical assessment versus clinical assessment alone and reported significant reduction in mask leak, significant increase in CPAP use, and significant less intubation in the DR [29]. Although this is promising, further trials are warrant.

3.3. Mask Ventilation in the Delivery Room. Using airway maneuvers (e.g., jaw thrust or chin lift) to maintain airway

patency is a crucial step during mask ventilation in adults and children [32]. However in newborn and infants several factors can reduce the effectiveness of mask ventilation, including poor face mask technique resulting in leak or airway obstruction, spontaneous movements of the baby, movements by or distraction of the resuscitator, and procedures such as changing the wraps or fitting a hat [33, 34]. Delivery room studies have shown that mask leak and airway obstruction are common problems during PPV [5, 33, 34]. Both leak and obstruction are usually unrecognized unless expired CO_2 detectors or RFM (Figure 1) is used [33, 34].

3.4. Assessment of Mask Ventilation. If infants fail to initiate spontaneous breathing immediately after birth, PPV should be given [16]. A rapid increase in heart rate is the most important clinical sign for adequate mask ventilation [16, 35, 36]. If no heart rate increase is observed, chest wall movements should be assessed to gauge mask ventilation [16]. However, the current neonatal resuscitation guidelines do not describe how chest wall movement should be assessed [16]. Two observational studies in the DR compared clinical assessment with measurements of an RFM [4, 5]. Schmölzer et al. compared chest rise with V_T measurement during mask PPV in the DR [5]. Assessing chest wall movement during mask PPV whilst standing at the infant's head was difficult and unreliable [5]. However, limitations of this study were the inexperience of the resuscitators and the potential obstructed view of the resuscitators by the ventilation device [5]. Poulton et al. compared chest rise observed from two different angles (head view versus side view) and different level of experience (junior staff versus senior staff) [4]. Overall the accuracy of clinical assessment of chest wall movement was poor and did not appear to be influenced by either the observers' position or the level of experience. However, more resuscitators were unable to assess chest wall movements while performing PPV than those observing from the side [4]. These two studies demonstrate that resuscitators were unable to accurately assess chest wall movements during mask PPV. The additional use of an RFM to continuously measure and displays V_T delivery might improve the effectiveness of neonatal resuscitation. During mask PPV an RFM continuously display V_T wave forms which can be used to guide mask ventilation. The clinical team can identify mask leak or airway obstruction as well as high or low V_T delivery to guide ventilation. A recent randomized trial by Schmölzer et al. demonstrated that an RFM additional to clinical assessment demonstrated significant reduction in leak during mask PPV in preterm infants in the DR [29].

3.5. Mask Leak. Mannequin studies demonstrated large mask leaks during simulated mask ventilation, and operators were usually unaware of the extent of mask leak [37, 38]. Observational studies in the DR reported similar results with mask leak exceeding 75% in 50% of analyzed resuscitations [5, 34]. The leak between the mask and the face is an enemy of mask PPV, causing a reduction in tidal volume delivery and impairing resuscitation efforts. A mannequin study demonstrated that operators observing RFM graphics (Figure 1) were able to reduce mask leak during PPV [39]. A recent randomized controlled trial comparing mask PPV in the DR performed with either an RFM visible or masked showed similar results [29]. Observation of flow waves significantly reduced mask leak from 54% to 37% [29]. Furthermore, fewer infants were intubated or required oxygen at five minutes after birth, and more infants left the DR on CPAP [29]. Although no difference in any long-term outcomes was observed, the results of this study may indicate that flow wave guidance improves mask PPV and decreases short-term adverse outcomes [29].

3.6. Airway Obstruction. Current resuscitation manuals suggest that during mask PPV airway obstruction may be due to (i) manual compression of the soft tissues of the neck and tongue, (ii) hyperextension or flexion of the head, or (iii) the face mask being held on the face so tightly that it obstructs the mouth and nose [16, 33, 34].

Two observational studies in the DR reported on airway obstruction during resuscitation of preterm infants [33, 34]. Finer et al. used a colorimetric CO_2 detector to identify obstruction during mask PPV. They found airway obstruction in 75% of infants receiving PPV in the DR [33]. Although CO_2 detectors can be very useful to assess effective ventilation, they do not differentiate between an inadequate V_T, airway obstruction, or circulatory failure [40–43]. In contrast an RFM, which displays flow and tidal volume signals (Figure 1), may distinguish mask leak and airway obstruction, [30, 34]. A recent observational study in the DR showed that severe airway obstruction defined as a reduction in V_T of >75% occurs in 25% of infants receiving mask ventilation [34].

Several airway maneuvers, such as jaw thrust or chin lift, are recommended in children and adults to maintain airway patency during resuscitation [16]. An airway obstruction has been reported in 50% of cases when either chin lift or jaw thrust was applied during mask PPV, while using the combination of both, no airway obstruction was observed [32]. Similar studies are needed in newborn infants to clarify the best head and airway position.

3.7. Tidal Volume Delivery. During PPV a peak inflation pressure (PIP) is chosen with the assumption that this will deliver an adequate V_T [5]. However, the delivered V_T is rarely measured, and therefore airway pressure is not adjusted accordingly [4, 5]. A low V_T may be insufficient to achieve adequate gas exchange and may cause hypercapnia and atelectotrauma, whereas excessive V_T may cause hypocapnia and volutrauma [6]. Both low and excessive V_T delivery promote release of inflammatory mediators, which contribute to bronchopulmonary dysplasia (BPD) [44, 45]. In addition, clinicians struggle to achieve a balance between aerating the distal gas exchange units (alveoli) without overdistending the lung causing damage [6]. An animal study demonstrated that a few large manual inflations can damage the lungs [46]. Tidal volumes similar to this study have been reported during mask PPV of preterm infants [4, 5]. In addition, several animal studies demonstrated that PPV with high V_Ts contributes

to lung injury [45, 47]. Using a lung simulator Kattwinkel et al. demonstrated that operator adjusted to compliance changes faster when V_T was displayed compared to pressure [48, 49]. A recent randomized control trial compared V_T guidance with clinical assessment during mask PPV in the DR in infants <32 weeks of gestation [29]. Mask leak was significantly decreased in the RFM visible group; however V_T was similar in both groups [29]. Promisingly, the infants in the RFM visible group received less high V_T (>8 mL/kg) delivery compared to the masked group [29], which have been shown to contribute to lung injury [45, 50].

3.8. Sustained Inflation. In preterm infants a lung protective strategy should be started at birth to support lung fluid clearance and to establish FRC. Establishment of lung inflation in apneic newborn infants can be achieved with either shorter or longer inflation times [16]. In an experimental non-breathing rabbit model of neonatal resuscitation, a prolonged sustained inflation (SI) of 20 s coupled with PEEP resulted in a rapid increase in FRC as did PEEP alone when compared to PPV with or without PEEP [51]. Evidence in preterm infants comes from observational and randomized studies [7–9, 52]. Lindner et al. introduced a series of interventions in the DR that included giving a 15 s SI [8]. They observed a dramatic reduction in DR intubation rate from 84% to 40%, and the proportion of preterm infants never intubated during their admission at their institution increased from 7% to 25% [8]. Similarly, Lista et al. compared an initial 15-second SI in addition to PPV to control infants who did not receive SI [7]. They reported a reduction in surfactant (45% versus 61%) mechanical ventilation (51% versus 75%) and postnatal steroid (10% versus 25%) use. In addition, infants surviving without BPD increased from 7% to 25%, and mean duration of mechanical ventilation (5 versus 11 days) and oxygen therapy (21 versus 31 days) among infants <29 weeks was reduced [7]. Lindner et al. randomized 61 infants <29 weeks to receive a 15 second SI and PPV or PPV alone through a single nasal prong in the DR [8]. Although no difference in mortality, severe intraventricular hemorrhage, or BPD was observed, 30% to 40% of preterm infants were not intubated or mechanically ventilated within the first 48 hours after birth [8]. Harling et al. randomized 52 preterm infants to an initial 5-second SI and PPV compared to PPV alone and did not find a difference in cytokines measured in bronchoalveolar lavage fluid [52]. Te Pas and Walther randomized 207 preterm infants <33 weeks to an initial 10-second SI followed by nasal CPAP compared to mask PPV without PEEP [9]. Lower intubation rates in the DR (17% versus 36%), shorter duration of ventilatory support, and BPD (22% versus 34%) were observed in the infants randomized to SI/CPAP compared to those receiving mask PPV without PEEP [9]. Although these studies suggest that SI has the potential to reduce BPD, the results have to be interpreted with caution. Cohort studies are subject to confounders and can at best suggest an association between the use of an SI and improved outcomes. For example, infants in Te Pas and Walther's study were on average 500 g heavier compared to those in Lindner et al.'s study [8, 9]. Both Lindner et al.'s and Te Pas and Walther's studies reported more than one DR care change, with SI being just one element among [8, 9]. In addition, the randomized studies were not adequately powered to detect differences in important clinical outcomes [8, 9, 52]. Consequently, it is not possible to determine how many, if any, of the differences observed between the groups were related to the use of SI. Large randomized controlled studies of SI in preterm infants are urgently needed.

3.9. Continuous Positive Airway Pressure or Intubation. Observational studies have reported an association between decreased rates of BPD and increased use of early CPAP [53–56]. Avery et al. compared BPD rates in eight NICUs with one center having a significant lower BPD rate with much greater use of CPAP compared to the other centers [53]. Van Marter et al. reported that rates of BPD differed substantially between Columbia and Boston centers (4% versus 22%) [54]. Initial respiratory management was more likely to include mechanical ventilation (75% versus 29%) and surfactant (10% versus 45%) at Boston centers compared to Columbia, respectively [54]. A retrospective analysis of 261 preterm infants compared intubation and ventilation at birth with CPAP and reported lower mortality and rates of surfactant administration, BPD, or intraventricular hemorrhage in infants receiving CPAP [55]. Surprisingly, patent ductus arteriosus was more common among infants receiving CPAP [55]. Two randomized trials compared PEEP/CPAP with no PEEP in the DR [15, 21]. In a feasibility study Finer et al. randomized 104 extremely low birth weight infants to receive CPAP/PEEP or no CPAP/PEEP in the DR. The aim of the study was to sue CPAP/PEEP to avoid routine endotracheal intubation and to explore the CPAP or PEEP in the DR. Although no differences in rates of intubation, death, and BPD were reported, the use of CPAP/PEEP as initial respiratory management was feasible [15]. Dawson et al. randomized infants <29 weeks' gestation and reported no difference in oxygen saturation or heart rate at 5 min, mortality, rate of intubation, or BPD [21]. Two large trials randomized 1926 infants between 24 and 29 weeks of gestation to receive CPAP or endotracheal intubation at birth [12, 13]. The COIN trial reported fewer days of ventilation and reduction of surfactant use in infants receiving CPAP than those in infants endotracheally intubated at birth [13]. Worryingly, infants in the CPAP group had a significantly higher incidence of pneumothorax [13]. The SUPPORT trial randomized 1316 infants to receive CPAP or intubation and surfactant. Infants in the CPAP group had lower rates of postnatal steroids and had fewer days of mechanical ventilation than those in the latter group. However mortality and BPD rates were similar between groups in both trials [12]. Nonetheless, the results suggest that respiratory support in the DR should be started with CPAP before intubation and surfactant are considered.

3.10. Surfactant Administration. Surfactant deficiency is a contributing factor in the development of respiratory distress syndrome (RDS) and has become the standard of care for the treatment of RDS. Systematic reviews from randomized trials 15 years ago showed that prophylactic surfactant administration reduced mortality and initial inspired oxygen requirement for intubated infants <30 weeks' gestation or with birth

weight less than 1250 g [57]. This led many to advocate routine intubation and surfactant administration for infants at risk of RDS [58–60]. However, the care of very immature babies has changed considerably over the last decade, and early CPAP has become an accepted alternative to endotracheal intubation and surfactant treatment [12, 13, 61–65]. In a retrospective cohort study, selective intubation of ELBW infants resulted in a significantly reduced need for intubation, lower incidence of BPD, intraventricular hemorrhage, and decreased length of hospital stay as compared to routine intubation [8]. A recent Cochrane review summarized that early stabilization on CPAP with selective surfactant administration compared to prophylactic surfactant administration and mechanical ventilation lowers the risk of BPD or death [66]. Verder et al. described his "INSURE" technique "Intubation-Surfactant-Extubation" which aimed to intubate infants only for surfactant delivery while on nasal CPAP [61]. In a multicenter randomized trial the INSURE technique reduces the need for mechanical ventilation; however no difference in important long-term outcomes (e.g., BPD) was reported [61]. The major criticism of the INSURE technique was the necessity of analgesia and naloxone to reverse the potential respiratory depression because of opioids. Various techniques of minimally invasive surfactant therapy have been described. Kribs et al. described surfactant delivery in spontaneous breathing infants on CPAP [63]. Using a flexible feeding tube positioned in the trachea with Magill's forceps surfactant is delivered [63]. Compared to historical controls the rates of mortality, severe intraventricular hemorrhage, and pulmonary interstitial emphysema were significantly reduced [63]. Two further observational cohort studies by Kribs et al. showed similar results [64, 65]. In both studies the rates of mechanical ventilation, BPD, and death were significantly lower compared to infants receiving intubation and mechanical ventilation [64, 65]. A recent multicenter randomized control trial using the Kribs technique reported a decrease in need for mechanical ventilation in the group who received surfactant while one CPAP [67]. However, no differences in mortality, BPD or other serious adverse events were observed. Alternative Dargaville et al. described "The Hobart Method" were surfactant is in stilled using a 16 gauge vascular catheter. With this technique the catheter is inserted through the vocal cords and surfactant instilled [68]. The catheter is then immediately withdrawn and CPAP reinstituted. A recent observational study by Dargaville et al. reported a reduction in need for intubation <72 h in infants receiving minimally invasive surfactant therapy compared with controls [69]. Although infants receiving minimally invasive surfactant therapy had shorter duration of oxygen therapy, duration of ventilation and incidence of BPD were similar [69]. Currently a large RCT using "The Hobart Method" is underway.

4. Conclusion

Ideally, a lung-protective strategy should start immediately after birth. At birth, the lungs of very preterm infants are uniquely susceptible to injury because they are structurally immature, surfactant-deficient, fluid-filled, and not supported by a stiff chest wall. To facilitate early development of functional residual capacity, reduce atelecto- and volutrauma, and improve oxygenation, various methods have been advocated. However, randomized control trials are urgently needed to investigate short- and intermediate-term outcomes.

Abbreviations

PPV: Positive pressure ventilation
$V_T n$: Tidal volumes
NICU: Neonatal intensive care unit
DR: Delivery room
FRC: Functional residual capacity
SI: Sustained inflation
PEEP: Positive end expiratory pressure
CPAP: Continuous positive airway pressure
RFM: Respiratory function monitor
PIP: Peak inflation pressure
BPD: Bronchopulmonary dysplasia
RDS: Respiratory distress syndrome.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Research Article

Retinol and Retinyl Palmitate in Foetal Lung Mice: Sexual Dimorphism

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In this work, we evaluate the lung retinoids content to study the possible difference between male and female mice during prenatal development and to comprehend if the vitamin A metabolism is similar in both genders. The study occurred between developmental days E15 and E19, and the retinol and retinyl palmitate lung contents were determined by HPLC analysis. We established two main groups: the control, consisting of foetuses obtained from pregnant females without any manipulation, and vitamin A, composed of foetuses from pregnant females submitted to vitamin A administration on developmental day E14. Each of these groups was subdivided by gender, establishing the four final groups. In the lung of control group, retinol was undetected in both genders and retinyl palmitate levels exhibited a sexual dimorphism. In the vitamin A group, we detected retinol and retinyl palmitate in both genders, and we observed a more evident sexual dimorphism for both retinoids. Our study also indicates that, from developmental day E15 to E19, there is an increase in the retinoids content in foetal lung and a gender difference in the retinoids metabolism. In conclusion, there is a sexual dimorphism in the lung retinoids content and in its metabolism during mice development.

1. Introduction

Vitamin A has been regarded as a major contributor in the differentiation and maturation of the lung [1–3], and there is no doubt that retinoids, especially retinoic acid, are essential for the lung development [4].

Until now, little is known about the acquisition and use of stored retinoids, but functionally they are involved in lung differentiation and maturation [2, 3], surfactant production [5, 6], inducing the formation of alveolar septa [7–9], cell differentiation [1, 2, 10, 11], elastin synthesis and deposition [12–17], homeostasis and lung repair [18, 19], and alveolar regeneration capacity [20, 21].

Some studies showed the potential usefulness of retinoids in reducing the incidence of bronchopulmonary dysplasia (BPD) in newborns, because indirectly retinoic acid is able to inhibit the effect of glucocorticoids that are often used to treat this pathology [22, 23]. The administration of vitamin A to premature newborns with low birth weight subjected to mechanical ventilation promotes the regeneration of lung injury by reducing the morbidity associated with BPD [22, 23]. Administration of a vitamin A supplement 48 hours after birth significantly reduced the mortality of newborns during the first 3-4 months of life, and the greatest benefit occurs in children with low birth weight birth [24]. Kennedy and collaborators treated premature infants with vitamin A and observed a reduction in the incidence of bronchopulmonary diseases and a reduction in the mortality [25]. Retinoids also restricted inflammation by reducing the cell death and extracellular matrix degradation [26, 27].

During foetal development, lung accumulates retinoids, in particular retinol and retinyl esters [16, 28], but the biologically active molecule is retinoic acid (RA) [2, 3]. In fact, the retinoic acid deficiency during pregnancy causes severe defects in lung development, including lung hypoplasia and agenesis [29–31].

RA is generated by a series of oxidative reactions that convert retinol to retinaldehyde and ultimately to the active form retinoid acid [32]. The pleiotropic effects of retinoic acid are due to the variety of RA isoforms, polymorphism of the

receptors RAR and RXR, and to the possibility that RXR have to form heterodimers with other receptors [30, 32].

The retinoids metabolism and homeostasis is controlled by dietetic availability, but also by an accurate mechanism of absorption, transportation, and reserve mobilization [32–34]. About 75% to 95% of retinoids are in the liver stellate or Ito cells, but it can also be deposited in other organs such as kidney, intestine, lung, and eye, although in the adult this storage is minimal when compared to the total quantity in the liver [33].

In the plasma, retinol circulates bounded to a complex formed by two proteins, retinol binding protein (RBP) and transthyretin (TTR) [35, 36]. Inside the cell retinol is complexed with the cellular retinol binding protein—CRBP—type I or type II, and free retinol is almost undetectable [37]. The complexed retinol can have different targets, that is, transformed into AR; excreted to the extracellular medium, if there is no immediate need for retinoids; or accumulated in the form of retinyl esters [33, 34].

The lung cells that store retinol are similar to the liver Ito cells [38], but in the developing lung, these cells are the lipid-containing interstitial cells (LICs) [16, 39]. The LICs are present during alveogenesis [15, 40], period in which the number of this cells increased [26], and are the main producers of tropoelastin [15], which under the action of RA increase the synthesis and deposition of this protein [16].

In the embryonic stage of lung development, there is abundant synthesis and use of the RA by the primitive foregut, which demonstrates their direct involvement in the lung primordial bud formation [29, 41–43].

During the branching morphogenesis, retinoic acid signal remains low and the levels of enzyme RALDH-2 are locally controlled, being concentrated in the regions with less branching activity [43]. With the beginning of lateral buds, a RA proximal-distal gradient is established, with lower concentration in the distal mesenchyme near the sites of prospective budding [43]. The mesenchyme inactivation of retinoic acid signal allows the expression of FGF-10, which is the major responsible in the branching process [44, 45]. The inhibitory effect of RA on FGF-10 involves other molecules present in the epithelium and mesenchyme. RA activates the SHH protein, which inhibits the expression of Fgf-10 via PTCH pathway [44] or control the TGF-β activity, which acts in the local expression of FGF-10 [45].

At the alveolar stage, retinoic acid is functionally involved in several processes, already mentioned above, and during this stage we can observe an increase in expression of retinoic acid m-RNA, RARs receptors, and CBRP protein, which is consistent with the hypothesis that the endogenous RA contributes to the pulmonary development [2, 14, 15, 19].

Considering that retinoids are important in the lung morphogenesis mechanism and pulmonary function, that male children have a higher risk of neonatal death when compared to female children, and that the majority of diseases in neonatal life occur in the respiratory system, we thought it is pertinent and important to study possible existence of a sexual dimorphism in the lung retinoids content during prenatal life.

2. Material and Methods

2.1. Experimental Model. In our experimental model, adult (60–70 days) male and female CD1 mice from "Charles River Laboratories-Research Models and Services," were housed in the usual conditions, that is, 21°C temperature, 8/12 hours light/dark cycle, standard pellets, and water *ad libitum*. Mating was carried out under polygamous conditions, and in each male compartment we placed 3 females for a period of 16 hours. Developmental day 1 was determined based on the presence of a sperm plug and pregnancy was monitored (birth occurred between developmental days 19 and 20).

All procedures involving animals were approved by the scientific committee, supervised by a Federation of European Laboratory Animal Science Association- (FELASA-) trained scientist, and conformed to regulation of Portuguese law (Portaria 1005/92) based on European Union Laboratory Animal Experimentation Regulations.

We establish two main groups, control and the vitamin A foetus that were subdivided according to the gender, making a total of four experimental groups and a total of 600 foetus. The pregnant mice from group vitamin A were submitted to an injection of 150 μL of Aerovit (45000 UI) on the day E14 and no manipulation was made in the control pregnant mice.

The euthanasia of pregnant mice was performed with an intramuscular injection ketamine/xylazine solution, at a dose of 0.05 mg/g body weight. After sternotomy, 0.3 mL of a sodium heparin solution at a concentration of 5000 UI/mL was injected by an intracardiac catheter. After the spread of anticoagulant into the general circulation of the mother, we collected the foetuses by Caesarean section and immediately placed in a saline solution.

The lung samples were collected from developmental day E15 to day E19, and frozen at −80°C, until the high liquid pressure chromatography (HPLC) analysis was made, to quantify the retinol and retinyl palmitate levels.

The foetal sex was determined by light microscopy observation of the developmental gonads.

According to the developmental day, the number of lung samples was different due to the lung size difference, but the total mass of lung was similar in all developmental days and always obtained from at least four different litters.

All results were analysed with the program Statview 5.0, using the student t-test (paired) to compared groups, with a statistically significant value of $P < 0.0001$.

2.2. High-Performance Liquid Chromatography (HPLC)

2.2.1. Equipment. Lung retinol and retinyl palmitate were determined by HPLC, using a programmable liquid chromatographic system "Gilson, Unipoint, V1.9 system software." The UV/Vis detector was a Gilson, 151 equipment and the readings were done with a wavelength of 325 nm and with sensibility adjusted for 0.002 aufs. The HPLC column was a reversed-phase "Waters-Spherisorb, ODS2" stainless steel column (25 cm × 4.6 mm I.D.) from Waters associated, Inc., Milford, MA, USA.

In our system, 100% methanol was used as the mobile phase to separate retinol, retinyl palmitate, and retinyl acetate

(added to the sample as an internal standard). The flow rate was always 2.5 mL/min and the system was adjusted to elute retinol at 1.8 min and retinyl palmitate at 11 min.

2.2.2. Sample Extraction Method. 750 μL of the internal standard solution (2.5 μg/mL retinyl acetate, spiked with 1 μg/mL retinol and 4.5 μg/mL retinyl palmitate) and 3 mL of chloroform/methanol solution (2 : 1), containing 50 μg/mL of butylated hydroxy-toluene were added to the initial lung samples. After complete homogenization, the samples were centrifuged for 10 min (2000 g) to separate the layers. The top layer was removed and the residue was preserve at −20°C for total protein quantification. To 2 mL of the top layer we added 400 μL of potassium chloride (0.37% p/v) and centrifuged for 5 min (2000 g). The upper part was neglected and the lower layer was totally evaporated under an argon flow. The residue was dissolved in 1 mL chloroform/methanol solution (1 : 1), and after filtered (0.2 μm) was prepared for chromatographic analysis.

2.2.3. Total Protein Quantification. All these procedures were made using the protein kit, based on the Lowry's reaction, and also used to establish the calibration curve, with the final protein concentrations of 50, 100, 200, 300, and 400 μg/mL.

To begin, we diluted our sample in 1 mL distilled water, homogenized, and added 0.1 mL of sodium deoxycholate (0.15%) for 10 min. After that, 0.1 mL of trichloroacetic acid (70% p/v) were added and centrifuged for 8 min (10000 rpm). The supernatant was neglected and the "pellets" were dissolved in 0.1 mL of Lowry reagent for 20 min and then added Folin & Fenol Ciocalteu to give colour to the samples (30 min). All these procedures were done at room temperature.

The absorbance was determined using the spectrophotometer, in a wavelength of 750 nm, and using the calibration curve established in the beginning of this methodology.

2.3. Chemicals. All solvents used have high purity and were acquired from Sigma, Analar and Panreac. The retinol, retinyl palmitate, and retinyl acetate were acquired from Sigma and their reference were, respectively, R7632, R3375, and R4632.

The Micro-Lowry Peterson's kit used was acquired from Sigma P5656.

3. Results

During the five developmental days studied (E15 to E19), retinol was not detected in the lungs of males and females of the control group.

On the contrary, in female and male foetuses of the vitamin A group, retinol was quantified, and variations were observed throughout the developmental (Figure 1). We only detected lung retinol in females on developmental days E16, E17, and E19, whose values were 0.315 ng, 0.117 ng, and 0.684 ng, respectively. In the males, retinol was detected in all developmental days, observing that in the three initial days the values gradually increase, that is, 0.042 ng on day E15, 0.115 ng on day E16, and 0.188 ng on day E17. On day E18 values dropped to 0.082 ng, and increased on day E19 to 0.273 ng (Figure 1). Both genders had the highest retinol value on developmental day E19 (Figure 1).

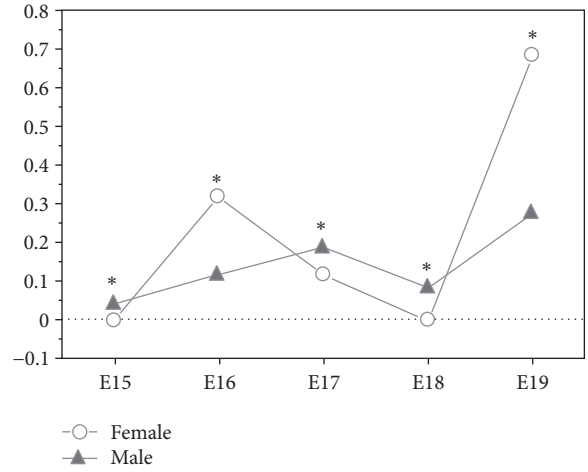

Figure 1: Lung retinol in the male and female foetuses of vitamin A group (ng/μg protein), from developmental days E15 to E19 (all SD values were ≤0.007 and SE ≤ 0.002; $^{*}P < 0.0001$ statistically significant).

The comparative study between males and females of vitamin A group showed statistically significant ($P < 0.0001$) differences in all developmental days (Figure 1). On developmental days E16 and E19, females had more retinol than males, but on the remaining three days, males showed a higher value than females (Figure 1).

From developmental days E15 to E19, retinyl palmitate was detected in the lungs of males and females of control group (Figure 2). Females retinyl palmitate values were 0.220 ng on day E15, 0.212 ng on day E16, increasing to 0.449 ng on E17. In the following days, retinyl palmitate value decreased to 0.430 ng on day E18 and to 0.359 ng on day E19. In the first three developmental days the male's retinyl palmitate values were always lower than females, with 0.167 ng on day E15, 0.126 ng on day E16, and 0.334 ng on day E17. On day E18 retinyl palmitate decreased to 0.266 ng and increased to 0.366 ng on day E19 (Figure 2). The highest retinyl palmitate value in the male foetuses was observed on developmental day E19 and in the female on day E17 (Figure 2).

The comparative study between male and female foetuses of control group evidence that from day E15 to E18, males had lower levels of retinyl palmitate than females, and that these differences were statistically significant ($P < 0.0001$) (Figure 2). On day E19 both genders had very similar retinyl palmitate content (Figure 2).

In the vitamin A group, we quantify retinyl palmitate between the developmental days E15 to E19 in both genders (Figure 3). In the females, values were 0.320 ng retinyl palmitate on day E15, 0.414 ng on day E16, decreasing to 0.377 ng on day E17. In the next day (E18) the value increased to 0.639 ng and decreased to 0.549 ng on day E19. For the male's foetuses, retinyl palmitate values were 0.154 ng on day E15, 0.386 ng on day E16 and 0.287 ng on day E17. On the following days, E18 and E19, the values increased to 0.507 ng

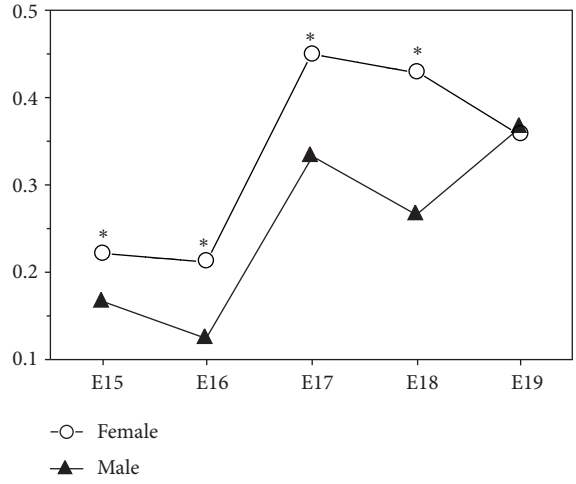

Figure 2: Lung retinyl palmitate in the male and female foetuses of control group (ng/μg protein), from developmental days E15 to E19 (all SD values were ≤0.003 and SE = 0.001; $^{*}P < 0.0001$ statistically significant).

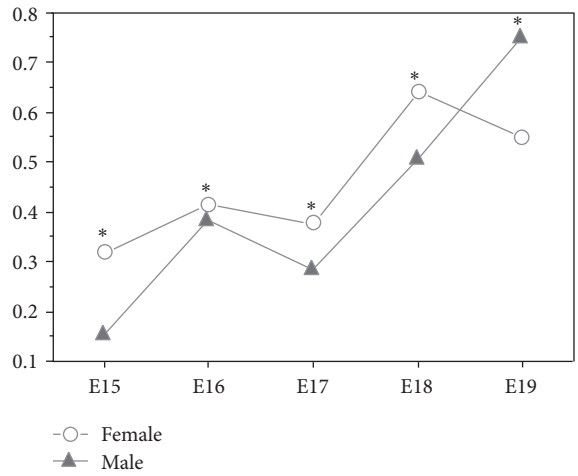

Figure 3: Lung retinyl palmitate in the male and female foetuses of vitamin A group (ng/μg protein), from developmental days E15 to E19 (all SD values were ≤0.003 and SE = 0.001; $^{*}P < 0.0001$ statistically significant).

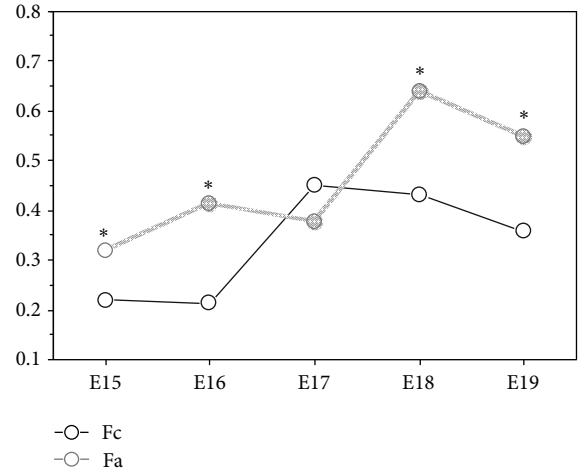

Figure 4: Lung retinyl palmitate in the female of control (Fc) and vitamin A (Fa) groups (ng/μg protein), from developmental days E15 to E19 ($^{*}P < 0.0001$ statistically significant).

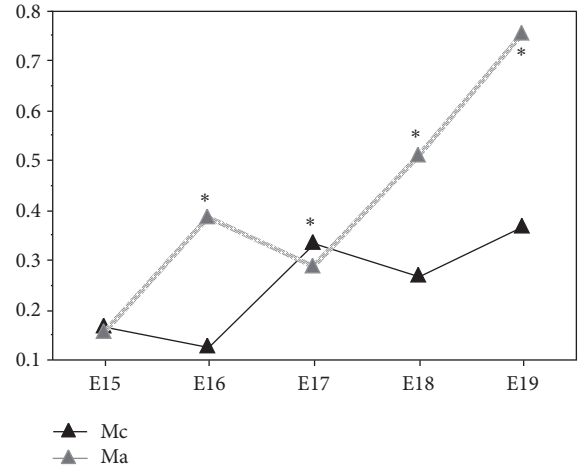

Figure 5: Lung retinyl palmitate in the male of control (Mc) and vitamin A (Ma) groups (ng/μg protein), from developmental days E15 to E19 ($^{*}P < 0.0001$ statistically significant).

and 0.753 ng, respectively (Figure 3). The highest retinyl palmitate value in the male foetuses was observed on developmental day E19 and in the females on day E18 (Figure 3).

In the comparative analysis between male and female foetuses of vitamin A group we observed that from developmental day E15 to E18, males had less retinyl palmitate than females (Figure 3). On day E19, male foetuses presented more retinyl palmitate than females. All these differences were statistically significant ($P < 0.0001$) (Figure 3).

When we compare the lung retinyl palmitate content between the control females and vitamin A females, we observed that on developmental days E15, E16, E18, and E19, control females have lower levels of retinyl palmitate, and that all these differences were statistically significant ($P < 0.0001$) (Figure 4).

On day E17, control females foetuses showed higher levels of retinyl palmitate, but this difference was not statistically significant (Figure 4).

The comparative analysis between males of control and vitamin A groups, evidence that on days E16, E18 and E19 the retinyl palmitate lung content was lower in the control group. On day E15 the retinyl palmitate value was very similar for both groups, but on day E17, the male foetuses of the control group had more retinyl palmitate (Figure 5).

The differences observed between groups were statistically significant on developmental days E16, E17, E18, and E19 ($P < 0.0001$) (Figure 5).

4. Discussion

In this work, retinol was not detected in the lung of male and female foetuses of control group, between developmental days E15 to E19. The results do not allow us to consider the complete absence of lung retinol, because it could be present in such a small amount that our methodology could not quantify.

Vitamin A group had lung retinol during the developmental days studied, but some content variations were observed according to foetal gender. Male foetuses accumulated retinol during all developmental days, while females only stored on developmental days E16, E17, and E19. The lung retinol amount in the male suffered fewer oscillations than female's, and with the exception of day E17, male accumulate retinol throughout developmental. The higher retinol value was observed on day E19 for both genders. These results evidence a gender difference throughout the studied days, which can be understood as a sexual dimorphism, more evident on developmental day E19, where females showed more than twice the value of males.

We also observed that during the developmental days E15 to E19, the retinol metabolism was different for each gender. The ability to metabolize and store retinol was not equal for both sexes, as well as the retinol quantity stored.

In the lungs of the control group, retinyl palmitate was quantified in both genders, with variations throughout developmental according with the foetal gender. Results showed that between developmental days E15 to E18, the variations in the retinyl palmitate content were similar in both gender, however, retinyl palmitate levels were very different between the two genders. From developmental days E15 to E18, females had more retinyl palmitate than males, but on day E19, this values became equivalent. The retinyl palmitate peak was on day E19 for males and on day E17 for females. We can conclude that during days E15 to E18, control group exhibit a sexual dimorphism, and that females have greater capacity to accumulate retinyl palmitate in the lung.

In the vitamin A group, retinyl palmitate was quantified, and variations throughout developmental were observed in accordance with the foetal gender. Although both genders have different retinyl palmitate lung content, we observed similar variations from developmental days E15 to E18. Females have higher retinyl palmitate levels than males, from days E15 to E18, and on day E19, the gender differences remained, but with male's displaying higher values when compared with females.

With these results we can conclude that the vitamin A group also exhibits a sexual dimorphism, that is more evident and occurs during all developmental days studied. This accentuated dimorphism probably results from the gender different capacity to metabolize lung retinoids.

Our results also showed that between developmental days E15 to E18, females of control and vitamin A groups, accumulate more retinyl palmitate than males from their respective groups. On day E19, control and vitamin A females decreased the level of lung retinyl palmitate, whereas the males from control and vitamin A groups increased this value. These data clearly demonstrate that female and male have different retinoids metabolism and that female are capable of accumulating more retinoids in the lung when compared with male.

Finally, our study also showed that from developmental days E15 to E19, there is a trend to increase the retinoids concentration in the foetal lung mice. Other studies with foetal lung rat, detected retinyl esters on the developmental day E14, and after day E15, their concentration increased rapidly, reaching a peak value around day E18, followed by a decline to reach the lowest value on the first days of postnatal life [14, 15, 39].

5. Conclusions

Understanding the lung morphogenesis and knowledge of lung structural differentiation process and action of certain factors during prenatal life, are of extreme importance to the lung, an organ that completes its structural and functional maturation in the postnatal life.

Several studies have shown that retinoids are important in the lung morphogenesis mechanism and in the expression of a number of components that are essential to the structure and pulmonary function.

Our study showed the existence of a sexual dimorphism in the lung retinoids contents during prenatal life in the mice. We observed that the administration of vitamin A during the developmental development, emphasize the differences between genders, fact that could be explained by the different ability to metabolize retinoids presented by the male and female foetuses.

Conflict of Interests

The authors declare that they do not have any conflict of interests in the submitted manuscript.

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Review Article

Chorioamnionitis and Lung Injury in Preterm Newborns

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There is a strong evidence that histologic chorioamnionitis is associated with a reduction of incidence and severity of respiratory distress syndrome (RDS). Short-term maturational effects on the lungs of extremely premature infants seem to be, however, accompanied by a greater susceptibility of the lung, eventually contributing to an increased risk of bronchopulmonary dysplasia (BPD). Genetic susceptibility to BPD is an evolving area of research and several studies have directly related the risk of BPD to genomic variants. There is a substantial heterogeneity across the studies in the magnitude of the association between chorioamnionitis and BPD, and whether or not the association is statistically significant. Considerable variation is largely dependent on differences of inclusion and exclusion criteria, as well as on clinical and histopathological definitions. The presence of significant publication bias may exaggerate the magnitude of the association. Controlling for publication bias may conduct to adjusted results that are no longer significant. Recent studies generally seem to confirm the effect of chorioamnionitis on RDS incidence, while no effect on BPD is seen. Recent data suggest susceptibility for subsequent asthma to be increased on long-term followup. Additional research on this field is needed.

1. Introduction

Watterberg and colleagues [1] were the first to report, in 1996, a decrease in the incidence of respiratory distress syndrome (RDS), while the incidence of chronic lung injury, marked by the presence of bronchopulmonary dysplasia (BPD), was increased in preterm newborns with histological chorioamnionitis. This paradoxical effect of prenatal inflammation on pulmonary outcome has been referred to as the "Watterberg effect." Since then, the effects of antenatal inflammation on both short- and long-term pulmonary outcome have received increasing attention, and many studies have been reported over the last years.

Chorioamnionitis is the leading cause of very preterm delivery and its incidence increases with decreasing gestational age [2–5].

BPD is one of the most frequent sequelae in very preterm infants and results in increased healthcare costs, prolonged hospital stays with frequent readmissions, and deleterious effects on subsequent growth and neurodevelopment [6, 7]. BPD is mostly multifactorial and a complex view of its pathogenesis ("multiple hits" hypothesis) has emerged, which includes antenatal exposure to a proinflammatory environment together with various postnatal inflammation-triggering events such as mechanical ventilation, sepsis, and patent ductus arteriosus [6, 8–10]. The "new" BPD results from an arrest of alveolar development with minimal fibrosis, associated with preterm birth, and is different from the "typical" BPD that follows severe RDS [6].

While the literature is consistent with the association between histological chorioamnionitis and a decreased incidence of RDS in the preterm newborn, many studies have found no association between chorioamnionitis and BPD [11, 12].

The purpose of this paper is to provide the actual evidence on neonatal pulmonary outcome after exposure to intrauterine inflammation.

2. Experimental Models of Inflammatory Neonatal Lung Injury

Animal models of intrauterine inflammation/infection provided evidence for altered lung development, with accelerated functional maturation and arrested alveolarization and vascular development. These models also demonstrated

the central role of the developing immune system in the pathogenesis of lung injury.

In the sheep model [9, 13], sterile chorioamnionitis caused by a single injection of *Escherichia coli* lipopolysaccharide stimulated pulmonary inflammation with increased expression of proinflammatory mediators as cytokines, chemokines, monocyte chemoattractant protein, recruitment of polymorphonuclear cells and monocytes, maturation of monocytes to alveolar macrophages, increased secretion of surfactant proteins and phospholipids, and arrested alveolar and microvascular development. This experimental model demonstrated both, a maturational response with increased pulmonary compliance, as well as aberrant structural changes on the developing lung. Injection of *Escherichia coli* lipopolysaccharide into the amniotic fluid of E15 BALB/cJ mice [14] increased the luminal volume density of fetal mouse lungs at embryonic day (E) 17 and E18. Lipopolysaccharide also increased luminal volume and decreased distal lung branching in fetal mouse lung explants. This effect required NF-kappaB activation and functional Toll-Like receptor 4. Airway branching may require fibronectin-dependent epithelial-mesenchymal interactions, representing a potential target for innate immune signaling. Antifibronectin antibodies and lipopolysaccharide both blocked distal lung branching. By immunofluorescence, fibronectin localized to the clefts between newly formed airways but was restricted to peripheral mesenchymal cells in lipopolysaccharide-exposed explants. These data suggest that lipopolysaccharide may alter the expression pattern of mesenchymal fibronectin, potentially disrupting epithelial-mesenchymal interactions and inhibiting distal airway branching and alveolarization. This mechanism may link innate immune signaling with defects in structural development of the fetal lung.

Two studies [15, 16] on a bitransgenic mouse model revealed that human cytokine-1 beta expression in the saccular stage was sufficient to cause a BPD-like illness in infant mice, whereas the lung was more resistant to cytokine-1 beta induced injury at later developmental stages. This is consistent with the clinical observation that extreme prematures (23–27 weeks, early saccular stage) are at the highest risk for inflammation-mediated BPD, whereas infants over 32 weeks (late saccular-alveolar stage) are at much lower risk.

3. Experimental Models of Ureaplasma Intrauterine Infection

The genital mycoplasmas *Ureaplasma urealyticum* and *Ureaplasma parvum* are the most common organisms isolated in the amniotic fluid in women with preterm labor and the rate of vertical transmission increases with the duration of membrane rupture [17, 18]. Although the ascending infection at or near the time of delivery has been pointed as the most common route of infection, *Ureaplasmas* have been detected in 13% of amniotic fluid samples at the time of genetic amniocentesis, at 16–20 weeks gestation, in asymptomatic women, indicating possible prolonged subclinical infection [17]. There is now considerable clinical and experimental evidence that these organisms contribute to chorioamnionitis, fetal inflammatory response, preterm birth, and neonatal morbidities including BPD, intraventricular hemorrhage, and necrotizing enterocolitis [13, 17, 19–28]. Pathologic changes in *Ureaplasma*-infected lungs of preterm infants are characterized by moderate-severe fibrosis, disordered elastin accumulation, myofibroblast accumulation, and chronic inflammation [29, 30].

Intrauterine *Ureaplasma* infection models in nonhuman primates [22, 26, 31], sheep [13], and mice [32] closely mimic the human exposure during early stages of lung development. In all intrauterine models, *Ureaplasma* organisms established a persistent infection in the intrauterine compartment, indicating limited capacity to clear these organisms. The findings of these studies suggest that *Ureaplasma* infection causes an imbalance of proinflammatory, profibrotic, and anti-inflammatory, antifibrotic factors in the fetal lung that may be augmented by postnatal exposure to hyperoxia and mechanical ventilation.

Humans are the specific host for *Ureaplasma parvum* and *Ureaplasma urealyticum*, so the organisms may elicit a less robust response in less related species. Alternatively, as shown in the mouse model, acute inflammation occurring during the saccular stage but not other stages of lung development results in arrested alveolarization, and airway remodelling typical of human BPD.

4. Genetic Susceptibility

Several studies have directly related the risk of BPD to genomic variants [33]. Polymorphisms of cytokines (IFNγ $T^{+874}A$), adhesion molecules (L-selectin-Prot213Ser), elements of rennin-angiotensin system (ACE-I/D), antioxidant enzymes (GST-P1 Val105lle), and surfactant proteins (SPA1, SPB intron4) have been identified as risk factors for BPD. Other studies investigated the role of genotype in BPD risk factors. Premature birth has been linked to polymorphisms with an impact on immune status (such as IL-6 $G^{-174}C$, MBL2 54G/A, VEGF $G^{+405}C$, and $HSP72A^{+1267}G$ genes) and matrix metalloproteases. Fetal inflammatory response syndrome, a major determinant of BPD, is also affected by genotype (including $LT\alpha A^{+250}G$) [33].

In our pilot study [34], HLA-A*68, -B*51, and -Cw*14 were the human major histocompatibility complex alleles associated with oxygen requirement at 36 weeks postmenstrual age in preterm neonates less than 32 weeks gestational age. The idea that an autoimmune process might be involved in BPD pathogenesis is novel and needs further investigation.

Twin concordance studies have suggested that the contribution of genetic risk to BPD is high, accounting for 35%–65% risk for the outcome [35, 36].

The challenges of enrolling adequate sample size in the preterm population, racial/ethnic heterogeneity in populations, variations in clinical practices, and the multifactorial pathogenesis of BPD make genetic studies difficult to be performed and may be responsible for the fact that some associations have not been replicated in subsequent studies.

5. Chorioamnionitis and Neonatal Respiratory Outcome in Preterm Infants

The primary process in the aetiology of intrauterine inflammation is believed to be ascending bacterial invasion from the cervicovaginal tract, although several other routes have been postulated [2, 37]. Extrauterine infections such as periodontitis, pneumonia, and urinary tract infections are risk factors for preterm birth [38, 39]. In the rodent model, maternal systemic inflammation resulted in prolonged pulmonary inflammation postnatally and a BPD phenotype [39].

Clinical chorioamnionitis does not correlate well with histological chorioamnionitis or culture positive amniotic fluid [40]. Histopathological examination of the placenta is the gold standard for evaluating antenatal inflammatory processes that might influence fetal development. Histological chorioamnionitis develops through a well-characterized stereotyped progression of maternal and fetal cellular stages that vary from patient to patient and are amenable to quantification. Increases in the intensity of these responses and their gradual transformation into a chronic phase are important variables that can adversely affect fetal physiology. Under recognised placental inflammatory lesions affecting the decidua, placental villi and fetal vessels are also potentially informative factors that should be taken into account in the studies of adverse pregnancy outcomes [37].

Most deliveries before 30 weeks gestation (saccular stage of lung development) are associated with histological chorioamnionitis, which is often clinically silent [2]. The more preterm the delivery, the more often the histological chorioamnionitis is detected [3]. Most infants delivered before 30 weeks gestation also have amniotic fluid that is culture positive for low pathogenic organisms such as *Ureaplasma* and *Mycoplasma* [2].

Lung inflammation is defined as increased inflammatory cells in the airspaces and lung tissue that are producing proinflammatory mediators such as hydrogen peroxide, interleukin 1, and interleukin 8 [40]. Lung inflammation starting before delivery with chorioamnionitis may continue as a result of routine care practices (ventilation, oxygen exposure), and adverse clinical events, such as nosocomial infection. The proinflammation is counterbalanced by anti-inflammatory effects of antenatal steroids, because about 80% or preterm infants are exposed to betamethasone or dexamethasone [40]. Postnatal steroids also acutely decrease indicators of inflammation. Both antenatal and postnatal steroids inhibit alveolar septation. Both proinflammatory and anti-inflammatory mediators disrupt alveolarization and to date no treatments are available to promote alveolarization [40].

Histological chorioamnionitis is defined by a maternal inflammatory response with neutrophilic infiltration of the membranes and/or chorionic plate. Fetal inflammation has been defined as either chorionic vasculitis [41], umbilical vasculitis [4], funisitis [42–45], "fetal response" [46], or subdivided into polymorphonuclear leukocyte infiltration of the chorionic plate or the umbilical cord [47]. Some studies showed that the RDS incidence was further decreased in infants with fetal involvement when compared to those with only maternal signs of inflammation [4, 41, 45, 47], an effect that appears to be additive to that of chorioamnionitis alone [4]. None of the studies found fetal inflammation to increase the risk of developing BPD, when compared to only maternal inflammation.

The studies where the association between chorioamnionitis and RDS has been assessed presented either similar [34, 43, 45, 48–52] or decreased [1, 4, 5, 47, 53, 54] RDS incidences. Today, it is generally accepted that there is enough evidence to consider that histological chorioamnionitis is associated with a reduction of incidence and severity of RDS [11, 12, 55].

Data on the need for respiratory support after chorioamnionitis differ greatly between studies. Often parallel to RDS incidence, chorioamnionitis has been reported to increase [49, 52] and decrease [53], as well not to affect the need for surfactant administration [42]. Moreover, while some report no effect on the need for mechanical ventilation [52, 53], time spent on the ventilator [48], and time on additional oxygen supplementation [48], others report increased need for ventilatory support or oxygen [51, 52].

The association between chorioamnionitis and BPD has been assessed in several studies, yielding inconsistent results. In order to clarify this issue, Hartling and colleagues [12] conducted a systematic review including an extensive and comprehensive search, duplicate screening, inclusion and data extraction to reduce bias, and metaregression to control for potential confounders. The authors identified 3,587 potentially relevant studies, of which 59 (15,295 patients) met the inclusion criteria. Studies were included if they had a comparison group, if they examined preterm or low birth weight infants, and reported primary data that could be used to measure the association between exposure to chorioamnionitis and the development of BPD. Studies classified chorioamnionitis as either clinical, histological, or microbiological. BPD was classified in the different studies as either (1) "Northway": X-ray abnormalities persisting beyond one month of age in patients who continued to require oxygen or respiratory support; (2) "Bancalari": need for oxygen on 28 of the first 28 days of life together with a compatible chest radiograph (or need for oxygen at 28 days of age, i.e., the National Institutes of Health (NIH) consensus definition of mild BPD); (3) "modern": need for additional oxygen at 36 weeks postmenstrual age (i.e., the NIH consensus definition of moderate or severe BPD). The evaluated studies included randomised trials, prospective cohort, retrospective cohort, and case control studies. The meta-analysis of unadjusted (OR 1.89) and adjusted (OR1.58) showed a significant association between chorioamnionitis and BPD. The limitations of this analyses were that (1) the comparison groups within the studies were other preterm infants likely to be affected by vascular and other placental pathologies, also associated with perinatal inflammation and neonatal morbidity such as BPD [56, 57]; (2) gestational age and birthweight were confounding factors; (3) there was substantial heterogeneity across studies in the magnitude of the association between chorioamnionitis and BPD, and whether or not the association was statistically significant; (4) the heterogeneity remained after grouping studies by

type of chorioamnionitis, definition of BPD, and whether the study included only very low birth weight infants or all preterm infants; (5) events such as exposure to antenatal steroids, postnatal sepsis, administration of surfactant, mode of ventilation, and patent ductus arteriosus were not known to be potential confounding factors at the time many of the studies were undertaken and therefore were not recorded; (6) the definition of chorioamnionitis was not consistent across the studies, and for instance, recent studies suggest that markers of fetal inflammatory response (leukocytosis or funisitis) are associated with the development of BPD, whereas chorioamnionitis alone is not [58, 59]; (7) the impact of ethnicity and the genetic background on the risk of BPD cannot be underestimated [36]; (8) the authors also found significant publication bias, and after controlling for publication bias for the adjusted data, the result was no longer significant; (9) also, the studies where the primary objective was not about the association between chorioamnionitis versus BPD showed no significant association, whereas studies reporting chorioamnionitis versus BPD as the primary objective were more likely to report a significant association; (10) the authors were restricted to data presented in the published reports and did not always have detailed information to control for important confounders, for instance, the gestational age; (11) the authors had to rely on the adjusted analyses as presented in the published reports and the variables which they included, which were not consistent across studies.

A study performed at our unit in order to assess the association between histological chorioamnionitis and BPD revealed a significant unadjusted association (OR 2.45 (95% CI: 1.16–5.18)), but when adjusted for gestational age and birthweight the association was no longer significant (OR 1.2 (95% CI: 0.51–2.95)) [60].

Been and Zimmermann [11] also reviewed the evidence of the association between intrauterine inflammation and pulmonary outcome. The authors also found numerous factors implicated in the interpretation of the results and that may explain, at least, part of the paradoxical findings among different studies. Inclusion criteria differ greatly among studies. Gestational age and birthweight differences were likely to affect results in some studies. Some studies selected their cohort by including only ventilated infants, or patients with premature prolonged rupture of membranes (PPROM), preterm labour, and suspected clinical chorioamnionitis. In contrast, cases of clinical chorioamnionitis or suspected maternal infection were excluded by others. Histopathological criteria and grading systems for diagnosing chorioamnionitis or fetal inflammation differ importantly between studies. The different definition of BPD complicates comparison of outcomes. Only a limited number of studies have adjusted the association for gestational age and birth weight. Some studies have performed multivariate models for other confounding factors without mentioning them, a fact that further complicates comparison of data.

Small for gestational age and intrauterine growth restriction are fetal conditions that increase the risk of BPD and prolong the duration of mechanical ventilation in preterm infants younger than 32 weeks of gestation [61–63]. The main mechanisms to explain how intrauterine growth restriction modulates the risk of a BPD have been described in animals. In experimentally growth restricted preterm lambs, impaired growth of terminal airways, gas exchange units, and blood vessels have been shown [64, 65]. Furthermore, growth restriction leads to reduced expression of surfactant protein mRNA and could induce increase inflammatory activation [64]. In a study performed at our center, we found a different profile of cytokines in venous cord blood of small for gestational age preterm newborns and the association of low levels of venous cord blood IL-6 and IL-10 and moderate/severe BPD in small for gestational age preterm newborns [66].

Racial differences in chorioamnionitis prevalence and the pulmonary response to intrauterine inflammation may account for additional inconsistencies between studies, as chorioamnionitis and vaginal bacterial colonisation are more prevalent in non-White [46, 67, 68], while White race has a higher prevalence of BPD [46, 69]. Another factor is that over the time period the cohort study data have been collected, important changes in general practice in neonatal intensive care have taken place. The widespread implementation of antenatal steroids is the most obvious and probably the most influential example [70]. Other practice changes include the increased use of exogenous surfactant [71] and noninvasive modes of ventilatory support such as continuous positive airway pressure (CPAP) [72]. These may reduce secondary lung injury in chorioamnionitis-exposed infants and partly account for the apparently diminishing association between chorioamnionitis and BPD over time [73].

Probably the most informative study concerning indirect evidence of a link between chorioamnionitis and BPD is that reported by Van Marter and colleagues [73]. In a case-control design, very low birth weight infants with BPD were matched with infants without BPD on gestational age, birth weight, and hospital specific treatment strategies. In these infants chorioamnionitis was associated with a decreased risk for BPD if infants were ventilated for less than seven days. However, when infants were exposed to mechanical ventilation for more than seven days or had sepsis, chorioamnionitis significantly increased BPD risk, the effect being the most prominent with all three risk factors present. This suggests that while antenatal exposure to inflammation in itself may reduce BPD risk, it increases the susceptibility of the lung to postnatal injurious events.

6. Chorioamnionitis and Long-Term Respiratory Outcome

The association between chorioamnionitis and pulmonary outcome beyond the neonatal period has not been extensively studied. No significant differences in the use of supplemental oxygen, bronchodilators, and systemic or inhaled steroids were reported between patients with and without chorioamnionitis at 18–22 months' corrected age in the hydrocortisone trial, irrespective of neonatal hydrocortisone treatment [74]. In the study of Kumar and colleagues [75], chorioamnionitis and prematurity were shown to have a joint predisposing effect on recurrent wheezing and physician-diagnosed asthma at a mean age of 2.2 years, mainly in

African-American children. Further investigation of the long-term effect of chorioamnionitis on respiratory outcome is warranted.

7. Conclusions

There is a strong evidence that histologic chorioamnionitis is associated with a reduction of incidence and severity of RDS. Short-term maturational effects on the lungs of extremely premature infants seem to be, however, accompanied by a greater susceptibility of the lung, eventually contributing to an increased risk of BPD. Genetic susceptibility to BPD is an evolving area of research and several studies have directly related the risk of BPD to genomic variants. There is a substantial heterogeneity across the studies in the magnitude of the association between chorioamnionitis and BPD, and whether or not the association is statistically significant. Considerable variation is largely depending on differences of inclusion and exclusion criteria, as well as on clinical and histopathological definitions. The presence of significant publication bias may exaggerate the magnitude of the association. Controlling for publication bias may conduct to adjusted results that are no longer significant. Recent studies generally seem to confirm the effect of chorioamnionitis on RDS incidence, while no effect on BPD is seen.

Additional research is needed to explore the effect of antenatal inflammation on the early and late pulmonary outcome of the extreme premature newborn.

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Research Article

Impact of Changes in Perinatal Care on Neonatal Respiratory Outcome and Survival of Preterm Newborns: An Overview of 15 Years

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Survival and outcomes for preterm infants with respiratory distress syndrome (RDS) have improved over the past 30 years. We conducted a study to assess the changes in perinatal care and delivery room management and their impact on respiratory outcome of very low birth weight newborns, over the last 15 years. A comparison between two epochs was performed, the periods before and after 2005, when early nasal continuous positive airway pressure (NCPAP) and Intubation-SURfactant-Extubation (INSURE) were introduced in our center. Three hundred ninety-five clinical records were assessed, 198 (50.1%) females, gestational age 29.1 weeks (22–36), and birth weight 1130 g (360–1498). RDS was diagnosed in 247 (62.5%) newborns and exogenous surfactant was administered to 217 (54.9%). Thirty-three (8.4%) developed bronchopulmonary dysplasia (BPD), and 92 (23%) were deceased. With the introduction of early NCPAP and INSURE, there was a decrease on the endotracheal intubation need and invasive ventilation ($P < 0.0001$), oxygen therapy ($P = 0.002$), and mortality ($P < 0.0001$). The multivariate model revealed a nonsignificant reduction in BPD between the two epochs (OR = 0.86; 95% CI 0.074–9.95; $P = 0.9$). The changes in perinatal care over the last 15 years were associated to an improvement of respiratory outcome and survival, despite a nonsignificant decrease in BPD rate.

1. Introduction

Respiratory distress syndrome (RDS), caused mainly by lung immaturity and surfactant deficiency [1] contributes to significant morbidity and some mortality in very low birth weight (VLBW) preterm newborns [2]. Changes in perinatal care, such as the use of antenatal steroids, exogenous surfactant administration, early nasal continuous positive airway pressure (NCPAP), and lung protective strategies of mechanical ventilation (patient-triggered modalities, volume controlled modes and high frequency oscillatory ventilation), have led to an improvement of survival and outcomes of infants with RDS over the past 30 years [1].

Exogenous surfactant reduces mortality and short-term respiratory morbidity in premature infants with RDS [3]; however, intubation and invasive mechanical ventilation are often required for its administration. In addition, prolonged invasive ventilation increases the risk of subsequent bronchopulmonary dysplasia (BPD) [4]. Early treatment with NCPAP helps the very preterm to rapidly achieve the functional residual capacity, stabilize the thoracic cage and airways, preserve endogenous surfactant, and reduce the need for invasive ventilation, and exogenous surfactant administration [5–8], but it may be an insufficient respiratory support for a significant number of infants born before 26 weeks of gestation [9]. Although there is a growing body of evidence to guide decision making, there is, not yet, consensus on the best treatment approach for acute RDS [1].

The aims of our study were to assess the changes in perinatal practices for VLBW preterm newborns admitted to our

neonatal intensive care unit (NICU) over the last 15 years and to perform a comparison of respiratory outcome between two epochs, the periods before 2005 and after, when early NCPAP and Intubation-SURfactant-Extubation (INSURE) were introduced in our center.

2. Material and Methods

A retrospective study, from 1997 to 2011, was performed at our center, a level III NICU, referral center for cardiac and surgical patients for the north of Portugal, with an average of 450 admissions per year, including about 50 VLBW infants. Preterms with birth weight less than 1500 g were included in the study. Newborns affected of major congenital anomalies, a TORCH infection, hydrops fetalis, and chromosomal anomalies, as well as the outborns and those transferred to other NICUs before 36 weeks of corrected gestational age, were excluded.

Clinical records were reviewed; demographics, histological chorioamnionitis and clinical data were assessed: gender, gestational age, birth weight, antenatal steroids pulses, delivery mode, respiratory support in the delivery room, Apgar score, the presence of RDS, the need for exogenous surfactant administration and mode of administration; respiratory support in the NICU, the need for oxygen therapy, the prevalence of BPD and other major morbidities (nosocomial sepsis, necrotizing enterocolitis, severe intraventricular hemorrhage, retinopathy of prematurity, patent ductus arteriosus, periventricular leukomalacia), and length of NICU stay and survival.

Antenatal steroid regimen was performed with dexamethasone (total dose of 24 mg, divided into two doses given intramuscularly every 12 hours) until 2003, and with betamethasone (24 mg, divided into two doses given intramuscularly 24 hours apart) thereafter, in pregnancies with threatened preterm labour below 35 weeks gestation.

Gestational age (in this study we considered the completed weeks) was assessed by menstrual age (women with regular menstrual cycles), ultrasound examination (when a discrepancy of two or more weeks existed between the age derived by menstrual dating and the age derived sonographically, or in the absence of a menstrual date) [10], or the New Ballard Score (in the absence of obstetrical indexes) [11]. Small for gestational age was defined as a birth weight below 10th centile of Lubchenco's fetal growth charts before 2003 [12] and a birth weight below 10th centile of Fenton's fetal growth charts after 2003 [13].

Early NCPAP is usually started immediately (first 15 minutes) after birth, although, in this study, it was considered when started in the first 30 minutes of life, once some patients were transported to the NICU, monitorized, and in spontaneous breathing. The first and 5th minute Apgar scores were dichotomized in two groups (≤ 7 and ≥ 8). RDS diagnosis was made on a combination of clinical and radiographic features according to the criteria of RDS of the Vermont Oxford Network: (1) $PaO_2 < 50$ mmHg in room air, central cyanosis in room air, a requirement for supplemental oxygen to maintain $PaO_2 > 50$ mmHg or a requirement for supplemental oxygen to maintain a pulse oximeter saturation over 85% within the first 24 h of life; and (2) a chest radiograph consistent with RDS (reticulogranular appearance to lung fields with or without low lung volumes and air bronchograms) within the first 24 h of life. The diagnosis of BPD was made in preterm newborns with gestational age 32 weeks or below, if the infant was chronically oxygen dependent at 36 weeks of corrected age and had a characteristic chest radiograph. In newborns above 32 weeks gestational age, BPD was considered if the baby was dependent on oxygen for 28 consecutive days [14]. Oxygen was used to maintain saturations given by pulse oximetry in the range of 88 to 94% for RDS and 90 to 95% for BPD. Exogenous surfactant was administered through the endotracheal tube in babies on invasive mechanical ventilation or by INSURE in babies off invasive mechanical ventilation [15].

Routine mechanical ventilation modes were patient-triggered modalities using a Babylog 8000+ (*Drager, Lubeck, Germany*), SIPPV (synchronized intermittent positive pressure ventilation) until 2000, and SIPPV + VG (volume guarantee) or PSV + VG (pressure support ventilation + volume guarantee) after 2000 [16]. High frequency oscillatory ventilation, at our unit, is used as a rescue ventilation using the Sensor Medics 3100 A (Sensor Medics Corporation, Yorba, Linda, CA, USA). Not intubated patients were placed on *InfantFlow* nasal CPAP (Care Fusion, Yorba Linda, USA) with nasal prongs or mask with a pressure of 5–7 cmH_2O, in prone position and started on caffeine, kept until 34 weeks of gestational age. Starting total fluid intake was 70 mL/kg/day and increased daily according to the hemodynamic status. Nasal CPAP (*InfantFlow*) was used with pressures of 5-6 cmH_2O in most cases but could be increased up to 7-8 in particular cases. Patients with apnoeas requiring stimulation were changed to NCPAP with synchronized pressure assistance (Infant Flow SiPAP, Viasys Health Care, Palm Spring, USA). Patients requiring $FiO_2 > 0.40$ with respiratory distress and/or arterial $PCO_2 > 65$ mmHg and $pH < 7.20$ were intubated for exogenous surfactant administration (poractant alfa). INSURE was routinely performed after an intravenous bolus of morphine (0.1 mg/kg). Naloxone (0.1 mg/kg, IV push) was used if needed, to reverse respiratory depression caused by morphine.

As early NCPAP and INSURE were introduced in our NICU in 2005, we compared demographic and clinical characteristics between two epochs (1997–2004 and 2005–2011, before and after their introduction, resp.). We, also, compared demographic and clinical characteristics between surviving preterm with and without BPD.

Histological chorioamnionitis was defined according to the Blanc classification [17]: stage I, intervillositis; stage II, chorionitis; stage III, chorioamnionitis; funisitis, polymorphonuclear leukocytes in the Wharton's jelly or umbilical vessel walls; vasculitis-polymorphonuclear leukocytes in chorionic or umbilical blood vessel walls. All stages of chorioamnionitis were considered together. Proven neonatal sepsis was defined as any systemic bacterial or fungal infection documented by a positive blood culture. The criteria of Bell were used for the diagnosis and staging of necrotizing enterocolitis [18]. Staging of retinopathy of prematurity was done according to the International Classification [19, 20].

Intraventricular haemorrhage was classified according to Papile et al. [21]. Periventricular leukomalacia was classified according to de Vries and Rennie [22]. Hemodynamically significant patent ductus arteriosus was diagnosed on the basis of the echocardiographic findings. The first evaluation is usually between 24 and 72 hours of life, with daily evaluation until closure of the ductus. The standard treatment is indomethacin.

The study protocol has been approved by our institute's committee on human research.

Continuous variables with symmetric distribution were characterized by mean (± standard deviation), those with asymmetric distribution by median (minimum–maximum values) and categorical variables were characterized by its absolute and relative frequencies. Mann-Whitney U test was used to compare two independent samples (asymmetric continuous variables) and chi-squared test or Fisher's exact test to compare categorical variables, the latest one for contingency tables 2×2 when expected values were less than 5. A multivariate analysis by logistic regression was performed to evaluate the outcome BPD. The results are presented by odds ratio (OR), 95% confidence interval (CI), and P value. The statistical analysis was performed using SPSS program v.19 (IBM, New York, USA) and a P value <0.05 was considered significant.

3. Results

Out of 735 clinical records, 395 were reviewed, with 198 (50.1%) females, mean gestational age 29.1 weeks (22–36), birth weight 1130 g (360–1498), and 95 (24.1%) small for gestational age. Three hundred and forty patients were excluded (outborns = 80, transferred before 36 weeks gestational age = 201, major congenital anomalies = 25, TORCH infection = 29, hydrops fetalis = 3, chromosomal anomalies = 2).

During the antenatal period, dexamethasone was used in 161 (49.5%) newborns and betamethasone in 164 (50.5%), with a full cycle in 213 (65.5%) cases (Table 1). The overall delivery room data and major morbidity are reported on Table 1.

Comparing both epochs (1997–2004 and 2005–2011), male gender was predominant (60% versus 39.5%) before 2005 ($P < 0.0001$). Betamethasone was administered to 163 (94.8%) newborns ($P < 0.0001$) and 139 (80.8%) had a full antenatal steroid cycle ($P < 0.0001$) after 2005. Also C-section was higher (69.2% versus 56.5%, $P = 0.009$) after 2005. After 2005, the need for endotracheal intubation decreased from 75% to 40.5% ($P < 0.0001$), the prevalence of RDS decreased from 66% to 59% ($P = 0.15$), the need of one single dose of surfactant increased from 27.7% to 48.6% ($P = 0.001$), and the need of two doses of surfactant decreased from 59.8% to 40% ($P = 0.001$). There was a delay in surfactant administration from 1–3 hours to 1–16 hours in the first dose ($P < 0.0001$) and from 6–16 hours to 3–96 hours in the second dose ($P = 0.03$). The need for invasive mechanical ventilation ($P < 0.0001$) and oxygen therapy ($P = 0.002$) decreased, along with a significant improvement of outcomes, less BPD ($P = 0.022$) and mortality ($P < 0.0001$) (Table 1). The causes of death were extreme immaturity/RDS ($n = 30$), sepsis ($n = 33$), severe intraventricular haemorrhage ($n = 18$), necrotizing enterocolitis ($n = 5$), and others ($n = 5$).

Demographics and clinical characteristics of surviving BPD patients are reported in Table 2.

The multivariate logistic regression, adjusted for gestational age, birth weight, complete antenatal steroid cycle, male gender, histological chorioamnionitis, early NCPAP use, 5th Apgar score, RDS, surfactant administration, patent ductus arteriosus, and sepsis, revealed a nonsignificant reduction in BPD between the two epochs (OR = 0.86; 95% CI 0.074–9.95; $P = 0.9$). Male gender (OR = 0.5; 95% CI 0.22–1.18; $P = 0.11$) and vaginal delivery (OR = 1.46; 95% CI 0.58–3.7; $P = 0.42$) were not associated to an increased risk for BPD.

4. Discussion

BPD affects thousands of preterm infants each year [23] and its cause is multifactorial. The pathogenesis has been linked to genetic background, lung tissue immaturity, baro and volutrauma from mechanical ventilation, oxidant injury, and proinflammatory mediators [4, 24–26]. The use of antenatal corticosteroids, postnatal surfactant therapy, and modern intensive care has modified BPD into a less-severe disease that is characterized by arrested lung development, the new BPD. As ventilator-induced lung injury is a major contributing factor to BPD [23], early respiratory management of preterm infants affects respiratory outcome.

Over the last 15 years, there were changes in perinatal care and delivery room management in our center. One of the chief advances has been the routine administration of antenatal steroids. In 1995, the National Institutes of Health and the American College of Obstetricians and Gynecologists formally issued a consensus statement advocating the use of antenatal steroids for induction of fetal maturation, establishing it as a standard of care for perinatal management of preterm deliveries 24 to 34 weeks' gestational age, with the goal of reducing the risks for RDS, intraventricular hemorrhage, and neonatal death [27]. According to Lee et al., there were notable trends for a reduced risk for adverse neonatal outcomes associated with betamethasone compared with dexamethasone for intraventricular hemorrhage and severe retinopathy of prematurity. Also, betamethasone reduces the risk for neonatal death [28]. In our study, according to the policy of the obstetrics department, dexamethasone was replaced by betamethasone in 2003 and the number of completed cycles of antenatal steroids increased since 2005. The use of antenatal steroids increased from the first to the second epoch, highlighting better obstetric practices.

Early NCPAP is an important first-line form of respiratory support in newborns and is used as an alternative to intubation and invasive mechanical ventilation in extremely preterm infants [5, 9]. Since its introduction by Keszler [29] in 1971, several studies in animals and human infants NCPAP have shown promising results in terms of reduction of lung injury, need for mechanical ventilation and incidence of BPD [30–32]. Since the introduction of early NCPAP in

Table 1: Demographics and clinical data.

	Total $N = 395$	1997–2004 $n = 200$	2005–2011 $n = 195$	P
Gender, *n* (%)				
Male	197 (49.9)	120 (60)	77 (39.5)	**<0.0001***
Female	198 (50.1)	80 (40)	118 (60.5)	
Gestational age (weeks), mean (±SD)	29.1 (2.9)	28.9 (3.2)	29.3 (2.6)	0.15¥
Birth weight (grams), median (min–max)	1130 (360–1498)	1120 (460–1495)	1130 (360–1498)	0.958¥
Small for gestational age, *n* (%)	95 (24.1)	42 (21)	53 (27.2)	0.15*
Less than 1000 g, *n* (%)	156 (40)	83 (41.4)	73 (37.4)	0.409*
Antenatal steroids, *n* (%)	325 (82.3)	153 (76.5)	172 (88.2)	
Dexamethasone	161 (49.5)	152 (99.3)	9 (5.2)	**<0.0001***
Betamethasone	164 (50.5)	1 (0.7)	163 (94.8)	
Full cycle	213 (65.5)	74 (48.4)	139 (80.8)	**<0.0001***
Incomplete cycle	112 (34.5)	79 (51.6)	33 (19.2)	
Histological chorioamnionitis, *n* (%)	111 (28)	53 (26.5)	58 (29.7)	0.364*
Delivery mode, *n* (%)				
Vaginal	147 (37.2)	87 (43.5)	60 (30.8)	**0.009***
C-section	248 (62.8)	113 (56.5)	135 (69.2)	
Apgar score, *n* (%) 1st minute				
≤7	283 (71.6)	170 (85)	113 (57.9)	**<0.0001***
≥8	112 (28.4)	30 (15)	82 (42.1)	
5th minute				
≤7	140 (35.4)	92 (46)	48 (24.6)	
≥8	225 (64.6)	108 (54)	147 (75.4)	
Respiratory management in the delivery room, *n* (%)				
Endotracheal intubation	229 (58)	150 (75)	79 (40.5)	**<0.0001***
Spontaneous ventilation	110 (27.8)	50 (25)	60 (30.8)	0.201*
Early NCPAP	56 (14.2)	0	56 (28.7)	**<0.0001***
Respiratory distress syndrome, *n* (%)	247 (62.5)	132 (66)	115 (59)	0.15*
Surfactant administration method, *n* (%)	217 (54.9)	112 (56)	105 (53.8)	0.667*
Endotracheal intubation with mechanical ventilation	202 (93)	112 (56)	90 (85.7)	
INSURE	14 (6.5)	0	14 (13.3)	**<0.0001**∞
Both methods	1 (0.5)	0	1 (1)	
Invasive mechanical ventilation, *n* (%)	250 (63.3)	149 (75.3)	101 (51.8)	**<0.0001***
Invasive mechanical ventilation, median day (min–max)	6 (1–184)	6 (1–184)	5 (1–140)	0.44¥
NCPAP, *n* (%)	221 (55.9)	67 (33.5)	154 (79.4)	**<0.0001***
NCPAP, median day (min–max)	12 (1–75)	10 (1–50)	13.5 (1–75)	**0.049**¥
Oxygen, *n* (%)	272 (68.9)	152 (76)	120 (61.5)	**0.002***
Oxygen, median day (min–max)	4 (1–184)	7 (1–184)	2 (1–147)	**<0.0001**¥
NICU stay, median day (min–max)	42 (1–184)	39 (1–184)	45 (1–160)	**0.006**¥
Bronchopulmonary dysplasia, *n* (%)	33 (8.4)	23 (11.5)	10 (5.1)	**0.022***
Patent ductus arteriosus (PDA), *n* (%)	115 (29.1)	60 (30)	55 (28)	0.340*
Surgical closure of PDA	15 (3.7)	8 (4)	7 (3.5)	0.257*
Nosocomial sepsis, *n* (%)	107 (27)	66 (33)	41 (21)	0.089*
Necrotizing enterocolitis ≥ 2A, *n* (%)	13 (3.2)	6 (3.0)	7 (3.5)	0.534*

Table 1: Continued.

	Total $N = 395$	1997–2004 $n = 200$	2005–2011 $n = 195$	P
Retinopathy of prematurity ≥ 2, *n* (%)	15 (3.7)	8 (4)	7 (3.6)	0.257*
Intraventricular hemorrhage III-IV, *n* (%)	22 (5.5)	6 (8.8)	16 (8.2)	0.736*
Cystic periventricular leukomalacia, *n* (%)	36 (9.1)	27 (13.5)	9 (4.6)	0.065*
Deceased, *n* (%)	91 (23)	70 (35)	21 (10.8)	<**0.0001***

* Chi-squared test; ¥ Mann-whitney *U* test; ∞ Fisher's exact test; SD: standard deviation; NCPAP: nasal continuous positive airway pressure; INSURE: intubation surfactant extubation; nicu: neonatal intensive care unit.

Table 2: Demographic and clinical characteristics of the 304 surviving newborns according to BPD.

	BPD $n = 23$	Non-BPD $n = 281$	P
Gender, *n* (%)			
Male	17 (73.9)	125 (45.5)	**0.007***
Female	6 (26.1)	156 (55.5)	
Gestational age (weeks), median (min–max)	27 (23–31)	30 (24–36)	<**0.0001**¥
Birth weight (grams), median (min–max)	875 (550–1270)	1220 (500–1500)	<**0.0001**¥
Small for gestational age, *n* (%)	2 (8.7)	83 (29.5)	0.05∞
Less than 1000 g, *n* (%)	16 (69.6)	67 (23.8)	<**0.0001**∞
Complete cycle of antenatal steroids, *n* (%)	10 (43.5)	177 (63)	0.064*
Histological chorioamnionitis, *n* (%)	3 (13.0)	15 (5.3)	**0.041**∞
Delivery mode, *n* (%)			
Vaginal	11 (47.8)	87 (31)	0.096*
C-section	12 (52.2)	194 (69)	
Respiratory support in the delivery room, *n* (%)			
Endotracheal intubation	22 (95.7)	120 (42.7)	<**0.0001**∞
Spontaneous	0	106 (37.7)	<**0.0001**∞
Early NCPAP	1 (4.3)	55 (19.6)	0.091∞
Apgar score, *n* (%)			
1st minute			
≤7	22 (95.7)	172 (61.2)	**0.001**∞
≥8	1 (4.3)	109 (38.8)	
5th minute			
≤7	15 (65.2)	64 (22.8)	<**0.0001***
≥8	8 (34.8)	217 (77.2)	
Respiratory distress syndrome, *n* (%)	22 (95.7)	137 (48.8)	<**0.0001**∞
Surfactant administration, *n* (%)	22 (95.7)	114 (40.6)	**0.008**∞
Invasive mechanical ventilation, *n* (%)	23 (100)	137 (48.8)	<**0.0001**∞
Invasive mechanical ventilation, median day (min–max)	35 (4–140)	4 (1–50)	<**0.0001**¥
Nosocomial sepsis, *n* (%)	15 (65.2)	59 (20.9)	<**0.001***
Necrotizing enterocolitis ≥ 2A, *n* (%)	1 (4.3)	7 (2.5)	0.175∞
Retinopathy of prematurity ≥ 2, *n* (%)	7 (30.4)	3 (1.0)	<**0.0001**∞
Intraventricular hemorrhage III-IV, *n* (%)	1 (4.3)	3 (1.0)	0.086∞
Cystic periventricular leukomalacia, *n* (%)	9 (39.1)	19 (6.7)	<**0.0001***
Patent ductus arteriosus	5 (21.7)	14 (4.9)	**0.001***
NICU stay, median day (min–max)	90 (49–147)	46 (1–160)	<**0.0001**¥

* Chi-squared test, ¥ Mann-whitney *U* test, ∞ Fisher's exact test; NCPAP: nasal continuous positive airway pressure; NICU: neonatal intensive care unit.

2005, at our center, there was a decrease on endotracheal intubation need. The significant change in delivery room management between the two studied epochs was also reflected by better Apgar scores at first and fifth minutes. Not only there was an increase in NCPAP associated to a decrease in invasiveness of ventilation, but there was also a decrease in both, oxygen therapy need and its duration.

Observational and cohort studies have shown that NCPAP followed by intubation, surfactant administration, and mechanical ventilation only if NCPAP failure criteria are reached reduces the need for mechanical ventilation. Furthermore, a reduced incidence of BPD without increased mortality has been reported [8, 33, 34]. Few randomized controlled trials have compared intubation and mechanical ventilation with early NCPAP or different types of NCPAP and did not highlight significant differences [35, 36]. Since the FDA (*Food and Drug Administration*) release of surfactant to treat RDS in 1989 [37], there has been a deeper understanding of surfactant physiology [38], as well as completion of multiple clinical studies to further delineate and refine the use of exogenous surfactant in RDS beyond the established evidence that surfactant administration reduces pneumothorax, pulmonary interstitial emphysema, and the combined outcome of BPD or death in preterm infants with surfactant deficiency [39, 40]. International guidelines have published recommendations on the optimal surfactant replacement strategy. According to van Kaam et al., with the exception of surfactant timing, these guidelines on surfactant replacement therapy seem to be implemented in daily clinical practice in European NICUs [41]. Since 2005, the use of surfactant in our unit was similar to the previous period; however, less doses were needed. Also there was a decrease on endotracheal intubation need, and on the prevalence of RDS and BPD. In addition, the length of invasive ventilation and oxygen therapy decreased. With these practices, the prevalence of BPD decreased as well as the overall mortality. The retrospective nature of this study does not allow reliable data on these figures. Male gender was predominant in the first period and in BPD group, however, when adjusting these variables in a logistic regression model there were no statistically significant association with the development of BPD. There were no differences in the prevalence of histological chorioamnionitis, nosocomial sepsis, necrotizing enterocolitis, retinopathy of prematurity, intraventricular hemorrhage, patent ductus arteriosus requiring surgical closure, and periventricular leukomalacia between the two compared epochs.

5. Conclusions

The changes in perinatal care introduced at our center over the last 15 years were associated to both, an improvement of respiratory outcome and survival. With the introduction of early NCPAP and INSURE there was a decrease on endotracheal intubation need and invasive ventilation and oxygen therapy, along with less mortality, but a nonsignificant reduction in BPD. The need of administration of one single dose of surfactant increased, and the need of two or more doses decreased.

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Research Article

A High Ductal Flow Velocity Is Associated with Successful Pharmacological Closure of Patent Ductus Arteriosus in Infants 22–27 Weeks Gestational Age

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Objective. To identify factors affecting closure of patent ductus arteriosus (PDA) in newborn infants born at 22–27 weeks gestational age (GA) during pharmacological treatment with cyclooxygenase inhibitors. *Method*. Infants born at 22–27 weeks of GA between January 2006 and December 2009 who had been treated pharmacologically for PDA were identified retrospectively. Medical records were assessed for clinical, ventilatory, and outcome parameters. Echocardiographic examinations during treatment were reviewed. *Results*. Fifty-six infants were included in the study. Overall success rate of ductal closure with pharmacological treatment was 52%. Infants whose PDA was successfully closed had a higher GA (25 + 4 weeks versus 24 + 3 weeks; $P = 0.047$), and a higher pretreatment left to right maximal ductal flow velocity (1.6 m/s versus 1.1 m/s; $P = 0.023$). Correcting for GA, preeclampsia, antenatal steroids, and age at start of treatment, a higher maximal ductal flow velocity was still associated with successful ductal closure (OR 3.04; $P = 0.049$). *Conclusion*. Maximal ductal flow velocity was independently associated with success of PDA treatment.

1. Introduction

Infants born before 28 gestational weeks have a high incidence of patent ductus arteriosus (PDA) [1, 2]. The postnatal presence of a haemodynamically significant left to right shunt through the duct is associated with a lower survival rate and an increased incidence of intraventricular haemorrhage (IVH), necrotizing enterocolitis (NEC), and bronchopulmonary dysplasia in preterm infants [3–9]. Inhibition of prostaglandin production with the cyclooxygenase inhibitors indomethacin or ibuprofen is the standard pharmacological treatment for PDA [10]. Nevertheless failure of pharmacologic treatment frequently occurs in extremely preterm infants and is associated with increased mortality [11–13].

Previous studies have identified low gestational age (GA), pregnancy-induced hypertension, antenatal indomethacin exposure, lack of antenatal glucocorticoid exposure, late indomethacin treatment, respiratory distress syndrome (RDS), use of high-frequency oscillatory ventilation (HFOV), large ductal diameter, and less ductal shunt velocity as independent risk factors for failure of pharmacological treatment of PDA [14–18].

Pulmonary factors such as prenatal steroid exposure and RDS thus appear to affect the closure of the ductus arteriosus, and parameters related to pulmonary circulation, for example, high PaO_2 and low blood pressure within the ductus arteriosus, relate to physiological ductal constriction in animal studies [19]. The objective of this retrospective study was to identify factors associated with closure of the ductus arteriosus during treatment with cyclooxygenase inhibitors in infants born at 22–27 weeks GA, with special focus on ventilatory and pulmonary circulatory factors.

2. Patients and Methods

2.1. Patients. Infants born at Uppsala University Children's Hospital between January 2006 and December 2009 at a GA of less than 28 weeks and pharmacologically treated for PDA

were included in this retrospective cohort study. Infants with any major congenital anomalies were excluded. The study was approved by the Swedish Central Ethical Review Board.

2.2. Pharmacological Treatment for Patent Ductus Arteriosus. All newborn infants born at a GA of less than 28 weeks were evaluated echocardiographically within the first days of life and echocardiographic examinations were repeated if indicated. Examinations were performed using an Acuson Sequoia Ultrasound System (Siemens AB, Upplands Väsby, Sweden) with a 10 MHz transducer and results were saved in digital format (Xcelera, Philips AB, Stockholm, Sweden).

A haemodynamically significant PDA was defined by one or more of the following echocardiographic parameters: ductus arteriosus diameter of >1.5 mm; left atrium to aortic root ratio (LA/Ao) of >1.5; reduced and reversed flow during diastole in the descending aorta in combination with clinical signs. Pharmacological treatment was initiated when a PDA of haemodynamic significance was found and none of the following contraindications were present: ductal-dependent heart defect; renal failure (serum creatinine >120 μmol/L or serum urea >12 mmol/L); thrombocytopenia (platelets $<50 \times 10^9$/L); IVH grade II–IV; or NEC. Indomethacin (Indocid, Merck & Co., Inc., West Point, Pennsylvania, USA) was administered in a three-dose regimen as an infusion (0.2 mg/mL, 0.2 mg/kg/dose) over at least 20 minutes per dose, the second dose 12 hours after the first, and the third dose 24 hours after the second. If echocardiography revealed a patent ductus after these three doses, one to three additional doses were administered at 24 hours intervals guided by echocardiographic examinations after each additional dose. No additional pharmacological ductus treatment was administered. Surgical ligation was carried out if signs of a hemodynamically significant ductus arteriosus persisted after pharmacological treatment. Before discharge a clinical assessment of ductus arteriosus was made and additional echocardiographic examination performed if indicated.

2.3. Concomitant Treatment. According to the policy at the unit, infants received mechanical ventilatory support immediately after birth and early surfactant (Curosurf, Nycomed International, Zürich, Switzerland) administration (100 mg/kg) if signs of respiratory insufficiency were detected. Otherwise early nasal continuous positive airway pressure (CPAP) therapy was instituted. HFOV was used as rescue treatment.

Guideline fluid volumes were 90–100 mL/kg during the first day of life, adding 10 mL/kg/day each day during the first week for infants with a GA of 24 weeks or less, and 80–90 mL/kg, adding 10 mL/kg/day for infants with a GA of 25 to 27 weeks. Fluid intake was further individually adjusted, guided by weight loss, urinary output, and serum sodium concentration. Volume substitution and inotropic drugs were used restrictively and only when combinations of low mean arterial blood pressure and low micturition were detected. Bacterial cultures were taken and intravenous administration of antibiotics initiated if clinical suspicion of bacterial septicemia arose.

2.4. Perinatal Characteristics and Outcome at Discharge. Medical records were retrospectively assessed for information regarding GA, birth weight, gender, preeclampsia, antenatal steroids, Caesarean section, surfactant administration and Apgar-scores, and death. Nursing flow charts from the neonatal intensive care unit were assessed for details about PDA treatment, including information about ventilatory settings and fluid administration.

2.5. Echocardiography. Each infant's last echocardiographic examination before treatment start was reassessed for this study by a single cardiologist (A. Jonzon), who was blinded to treatment results. Maximal ductal flow velocity was assessed from the parasternal short axis view with pulsed and continuous Doppler directly in line with the ductal flow and ductal diameter was measured at the ductus narrowest inner dimension from the same position with and without color Doppler. Left atrium to aortic root ratio was measured in M-mode from the parasternal long axis.

The first follow-up echocardiography after treatment defined successful (Closed group) or failed (Persistent group) ductal closure. The PDA was considered closed if no ductal flow could be found with color Doppler.

2.6. Statistical Analysis. Data for each group is presented below as median values and range or number and percentage. Statistical analyses were conducted with SPSS Statistics 18 for Windows (SPSS, Inc., Chicago, Illinois, USA). The Mann-Whitney test was used to compare non-parametric continuous data and the Fisher's exact test was used to compare categorical data. All P values presented are two-tailed and a $P < 0.05$ was considered statistically significant. Multivariable logistic regression was performed to assess the individual influence of predictive factors on the proportion of ductal closure. Factors previously found to affect ductal closure during treatment (GA, preeclampsia, antenatal glucocorticoid administration, time of treatment start) were included in the analyses together with maximal ductal shunt velocity adjusted for the second power of ductal diameter and the time of echocardiography. The adjustment for second power of ductal diameter was made to assess whether flow velocity was independent of ductal diameter according to the Hagen-Poiseuille equation.

3. Results

Between January 2006 and December 2009, a total of 130 infants were born at 22–27 weeks of GA at Uppsala University Children's Hospital. Fifty-six infants received pharmacological treatment for patency of the ductus arteriosus and 6 infants received primary surgical treatment because of contraindications for pharmacological treatment before discharge from the same neonatal unit. In 18 infants the PDA closed spontaneously without treatment, 13 died and 37 did not receive pharmacological treatment before discharge because of either contraindications for treatment or a PDA not considered haemodynamically significant. Out of the 56 pharmacologically treated infants, 29 (52%) showed

Table 1: Perinatal characteristics.

	Ductus closed ($n = 29$)	Ductus persistent ($n = 27$)	P
Gestational age, weeks (range)	25^{+5} (22^{+2}–27^{+4})	24^{+3} (22^{+3}–27^{+4})	**0.047**
Birth weight, grams (range)	718 (432–1217)	595 (440–1052)	0.363
Male gender (%)	20 (69)	14 (52)	0.274
Preeclampsia (%)	5 (17)	5 (19)	1.000
Antenatal steroids (%)	23 (79)	22 (81)	1.000
Cesarean section (%)	17 (59)	15 (56)	1.000
Surfactant (%)	28 (97)	26 (96)	1.000

successful PDA closure (Closed group) and 27 (48%) failed to close (Persistent group).

3.1. Perinatal Characteristics. All observed perinatal characteristics were similar in the two groups with the exception of GA, which was higher in the Closed group (Table 1). No infant had been exposed to antenatal indomethacin. Median Apgar-scores at one, five, and ten minutes were 5, 7, and 9 in the Closed group and 5, 8, and 9 in the Persistent group ($P = 0.980$, 0.807, and 0.773, resp.).

3.2. Echocardiography. All infants had a predominately left to right ductal flow. Besides a higher maximal ductal flow velocity in the Closed group compared to the Persistent group, no other differences in echocardiographic parameters or ventilator characteristics were observed at the time of the last echocardiographic examination before treatment (Table 2).

3.3. Pharmacological PDA and Concomitant Treatment Characteristics. No major differences in treatment characteristics or fluid intake during treatment were observed between the two groups studied (Table 3). All studied infants treated for PDA had been given indomethacin except eight, who received treatment with ibuprofen alone (Pedea, Orphan Europe SARL, Paris La Défense, France, 5 mg/mL, first dose 10 mg/kg/dose and 5 mg/kg/dose 24 and 48 hours after first dose) and two infants who received both indomethacin and ibuprofen due to shortage of indomethacin during part of the studied period. Two infants in each group received only two doses of indomethacin ($P = 1.000$) because of contraindications for treatment. In three infants in the Persistent group, treatment had been initiated late after birth at 20, 24, and 40 days, respectively.

Multivariate logistical regression analysis for factors previously found to affect ductal closure during pharmacological PDA closure and maximal ductal shunt flow velocity (adjusted for squared ductal diameter and for time of echocardiography) still showed an association between higher maximal ductal flow velocity and ductal closure (Table 4).

3.4. Outcome at Discharge. Three infants in the Closed group had not undergone post-treatment echocardiography before discharge, but were considered clinically closed. They were discharged in good condition and have not been subjected to any further examination or treatment for ductus arteriosus since then. In the Persistent group, eleven infants (41%) were subjected to surgery after follow up echocardiography, one infant (4%) received a second course of ibuprofen at a regional hospital, three infants (11%) died with an open ductus, five infants (19%) had spontaneous closure of their PDA at the time of echocardiographic examination before discharge, and seven infants (26%) were discharged with an open ductus. Three infants (10%) in the Closed group had reopened PDAs since the first echocardiographic examination after treatment when repeatedly examined at 10, 23, and 28 days after treatment, respectively.

Three infants (10%) in the Closed group and 4 (15%) in the Persistent group died ($P = 0.700$) at a median of 38 (range 22–42) and 17 (range 6–166) days ($P = 0.480$), but no death was related to patency of the ductus arteriosus.

Using the Swedish Perinatal Quality Register (a national register for quality control of neonatal care) and nursing flow charts from the neonatal intensive care unit, information regarding use of HFOV was collected. Medical records were retrospectively assessed for information regarding diagnosis of culture-proven episodes of sepsis in connection to PDA treatment, bronchopulmonary dysplasia (BPD, defined as a need for supplemental oxygen at 36 weeks GA), periventricular leukomalacia (PVL), IVH (including grade), retinopathy of prematurity (ROP), and NEC.

Eleven infants in the Closed group and 17 infants in the Persistent group were on ventilator treatment at the start of PDA treatment ($P = 0.108$). Infants in the Closed group received CPAP therapy for a median of 46 (range 3–95) days and were on ventilator treatment for a median of 8 (range 0–65 days) whereas infants in the Persistent group received CPAP therapy for a median of 42 (range 0–122) days and were on ventilator treatment for a median of 23 (range 0–100) days ($P = 0.533$ and 0.100, respectively). Four infants (14%) in the Closed group and seven infants (26%) in the Persistent group had undergone HFOV ($P = 0.322$), with a median of nine days (range 7–30) in the Closed group and four days (range 1–11) in the Persistent group ($P = 0.037$). None of the infants in the Closed group and two (7%) infants in the Persistent group had undergone HFOV before or during pharmacological treatment for PDA ($P = 0.228$). None of the infants in the Closed group and two (7%) infants in the Persistent group had undergone HFOV before or during pharmacological treatment for PDA ($P = 0.228$). One (3%) versus two (7sepsis in connection to PDA treatment ($P = 0.605$).

Twenty-nine (100%) versus 27 (100%) infants were diagnosed with RDS ($P = 1.000$), 15 (52%) versus 16 (59%)

Table 2: Characteristics at time of echocardiography.

	Ductus closed ($n = 29$)	Ductus persistent ($n = 27$)	P
Age at echocardiography, days (range)	2 (0–7)	2 (0–33)	0.079
Ventilator (%)	10 (34)	16 (59)	0.108
Ventilator MAP, cmH_2O (range)	8 (6–14)	9 (7–12)	0.220
CPAP (%)	19 (66)	11 (41)	0.108
CPAP pressure, cmH_2O (range)	5 (4–7)	5 (3–7)	0.618
Fraction of inspired oxygen, % (range)	25 (21–52)	27 (21–42)	0.848
Systolic blood pressure[1], mmHg (range)	47 (37–62)	47 (35–84)	0.987
Ductal diameter, mm (range)	1.7 (0.9–3.0)	1.8 (1.0–3.0)	0.399
Maximal ductal flow velocity, m/s (range)	1.6 (0.5–2.7)	1.1 (0.7–2.9)	**0.023**
LA/Ao (range)	1.5 (1.2–2.8)	1.7 (1.1–3.5)	0.198

[1] 21 versus 15 infants had an arterial catheter which enabled blood pressure measurements.

Table 3: Treatment characteristics.

	Ductus closed ($n = 29$)	Ductus persistent ($n = 27$)	P
Age at treatment start, days (range)	3 (1–8)	3 (1–40)	0.117
Indomethacin (%)	26 (90)	22 (82)	0.462
Ibuprofen[1] (%)	4 (14)	6 (22)	0.497
Change in weight, % (range)	1 (−10–9)	4 (−12–12)	0.090
Fluid intake, mL/kg/day (range)	134 (98–168)	139 (111–203)	0.354
Part IV, % (range)	59 (0–85)	43 (0–89)	0.468
Urine output, mL/kg/h (range)	2.1 (0.5–4.2)	2.2 (0.4–4.9)	0.594

[1] 1 versus 1 infants received treatment with both indomethacin and ibuprofen during the same course.

Table 4: Multivariable analysis for ductal closure.

	OR (95% CI)	P
Gestational age[1]	1.45 (0.93–2.25)	0.103
Preeclampsia	0.78 (0.11–5.60)	0.807
Antenatal steroids	0.83 (0.17–4.04)	0.817
Age at treatment start[2], days	0.82 (0.60–1.12)	0.213
Maximal ductal flow velocity[3], m/s	3.04 (1.01–9.22)	**0.049**

[1] OR for every 1 week increase.
[2] OR for every 1 day increase.
[3] OR for every 1 m/s increase, adjusted for age at echocardiography and squared ductal diameter.

with BPD ($P = 0.602$), 3 (10%) versus 4 (15%) with PVL ($P = 0.700$), 5 (17%) versus 7 (26%) with IVH ($P = 0.523$), 15 (52%) versus 15 (56%) with ROP ($P = 0.795$) and 1 (3%) versus 4 (15%) with NEC ($P = 0.185$) in the Closed and Persistent groups, respectively. One infant in each group had IVH grade III-IV ($P = 1.000$), and all others were grade I-II.

4. Discussion

Our study shows that higher gestational age and maximal shunt velocity is associated with successful pharmacological PDA treatment in infants born at 22–27 weeks GA. No other factor was found to differ between infants whose PDA closed and infants whose PDA did not close during treatment. In a multivariable logistic regression analysis, including the factors GA, preeclampsia, prenatal steroids, age at treatment start, and maximal ductal flow velocity adjusted for ductal diameter, only maximal ductal flow velocity was found to be independently associated with ductal closure. Furthermore, our study could not confirm any significant difference in outcome between infants whose PDA did or did not close during treatment.

Although the effect of ductal flow velocity on ductal closure has previously been noted in a more mature cohort of newborn infants, the mechanisms behind it are not extensively studied [14, 15, 20]. Assuming the ductus arteriosus to resemble a cylindrical pipe, the Hagen-Poiseuille equation states that the flow velocity in the ductus arteriosus is proportional to the pressure gradient between the systemic to pulmonary circulation and to the second power of the ductal diameter while it is inversely proportional to the blood viscosity and the ductal length [21]. In one previous study, the difference in maximal ductal flow velocity between infants whose ductus did or did not close has been suggested to be attributed to a difference in pulmonary arterial pressure [14]. In our study the influence of maximal ductal flow velocity was independent of the second power of the ductal diameter. Due to the retrospective design of our study we did not have the possibility to measure and adjust for blood viscosity or ductal length and we could only obtain data on the systolic blood pressure measured by arterial catheter at the time of echocardiography from 21 infants in the Closed group and 15 infants in the Persistent group. The uniformity in the measured systolic blood pressures between the two groups suggests however that a difference in the systemic

to pulmonary circulation pressure gradient likely reflects a higher pulmonary arterial pressure in the Persistent group.

Pulmonary vascular resistance normally decreases rapidly with the start of ventilation and oxygenation after birth, reversing the fetal right to left flow through the ductus arteriosus and foramen ovale [22]. The normal physiological ductus arteriosus closure occurs in two stages, where the initial contraction of the vessel is a response to the decrease in pulmonary vascular resistance and pressure, an increase in arterial oxygen pressure, a decrease in circulating prostaglandin E2 (PGE2), and a decrease in PGE2-receptors in the ductal wall [19, 23–25]. Besides the vascular effect of a higher pulmonary pressure on the ductus arteriosus, the resulting lower blood flow and lower oxygen exposure of the ductus would therefore prevent an effective closing with pharmacological treatment. The previous finding of RDS as a risk factor for failure of pharmacological treatment of PDA underlines the close connection between ventilation, pulmonary circulation and the existence of a PDA [16, 18]. The lack of any major difference in ventilatory parameters and FiO_2 between the Closed and Persistent groups in our study could indicate that ductal flow may be more sensitive to assess pulmonary vascular resistance at this early stage of life in extremely preterm infants.

Our study is limited by its retrospective design, the exclusion of a number of infants with PDA that were not treated before discharge, and the use of both indomethacin and ibuprofen during the study period. However, the two groups were well balanced and the study had the advantage of reflecting clinical treatment decisions based on strict guidelines.

5. Conclusion

In summary this study indicates that the maximal ductal flow velocity, independently of ductal diameter, is associated with successful treatment of PDA in extremely preterm infants. Pre-treatment echocardiographic maximal ductal flow velocity could possibly be used to assess the chances for treatment success in individual infants, but more information on the reliability of this parameter is needed.

Conflict of Interests

The authors have indicated that they have no conflict of interests relevant to this paper to disclose.

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Clinical Study

Value of Chest Radiographic Pattern in RSV Disease of the Newborn: A Multicenter Retrospective Cohort Study

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Respiratory syncytial virus (RSV) lower respiratory tract infection is the most common viral respiratory infection in infants. Several authors have sought to determine which risk factors are the best predictors for severe RSV disease. Our aim was to evaluate if a specific chest radiographic pattern in RSV disease can predict the disease severity. We conducted a multicenter retrospective cohort study in term and preterm neonates with confirmed lower respiratory tract RSV infection, admitted to neonatal intensive care units (NICU) from 2000 to 2010. To determine which factors independently predicted the outcomes, multivariate logistic regression analysis was performed. A total of 259 term and preterm neonates were enrolled. Patients with a consolidation pattern on the chest radiograph at admission ($n = 101$) had greater need for invasive mechanical ventilation (OR: 2.5; $P = .015$), respiratory support (OR: 2.3; $P = .005$), supplemental oxygen (OR: 3.0; $P = .008$), and prolonged stay in the NICU (>7 days) (OR: 1.8; $P = .025$). Newborns with a consolidation pattern on admission chest radiograph had a more severe disease course, with greater risk of invasive mechanical ventilation, respiratory support, supplemental oxygen, and prolonged hospitalization.

1. Introduction

Respiratory syncytial virus (RSV) lower respiratory tract infection is the most common viral respiratory infection in infants [1]. It is characterized by acute inflammation, edema, and necrosis of epithelial cells lining small airways, increased mucus production, and bronchospasm. Radiographically, RSV lower respiratory tract infection can present itself predominantly as bronchiolitis, a pulmonary obstructive disease with hyperinflation, or as pneumonitis, a restrictive parenchymal disease with diffuse consolidation areas [2, 3]. Although neonatal RSV infection is relatively infrequent [4], newborns have a significant risk for severe disease (i.e., need for mechanical ventilation and/or death) [5]. The ability to predict which neonates will have a more severe disease course could help in the selection of treatment facilities and guide management strategies. Several authors have sought to determine which risk factors are the best predictors for severe RSV disease. Younger age at presentation, lower birthweight, prematurity, congenital heart disease, chronic lung disease, and immunodeficiency have consistently been associated with greater chance for hospital admission, longer hospital stay, and need for mechanical ventilation in RSV-infected infants [6–11].

Several studies have sought to determine which radiographic findings are more frequently associated with RSV

infection [12–14]. Some authors have suggested that specific chest radiographic patterns in RSV-infected infants were related with disease course and severity [6, 15–17].

Our aim was to evaluate if a specific chest radiographic pattern (consolidation) in RSV infection can independently predict disease severity, namely, the need for supplemental oxygen, respiratory support, invasive mechanical ventilation, and prolonged length of hospitalization, in a newborn population.

2. Material and Methods

2.1. Study Design and Population. In order to establish the relative importance of chest radiographic patterns in RSV disease of the newborn, we conducted a multicenter retrospective cohort study, spanning an eleven-year period (2000–2010) by abstracting relevant data from clinical charts and birth files in eight level III-Neonatal intensive care units (NICU).

Term and preterm neonates, (≤28 days of life and/or ≤44 weeks corrected gestational age at time of diagnosis), with confirmed lower respiratory tract RSV infection (positive detection of viral RNA in respiratory secretions), admitted to a NICU were included.

The institutional ethics committee approval was obtained in all participant institutions.

2.2. Data Collection. Medical records were reviewed for (1) RSV diagnosis confirmed by viral diagnostic testing; (2) demographic characteristics including gender, birth gestational age, birthweigth, and corrected gestational age at time of diagnosis; (3) underlying medical conditions such as prematurity, congenital heart disease, and bronchopulmonary dysplasia (according to the National Institute of Health Consensus); (4) disease severity markers including length of stay in the NICU, need and duration of respiratory support (invasive mechanical ventilation (IMV) and/or continuous positive airway pressure (CPAP)), and requirement for supplemental oxygen therapy; (5) development of complications including pneumothorax, bacterial pneumonia, sepsis, and death; (6) chest radiographic findings grouped in two different categories: consolidation versus hyperinflation.

2.3. Chest Radiographic Patterns. Patients with alveolar infiltrates and/or opacities with bronchogram ("white lung") were considered as having a consolidation pattern (Figure 1). Patients with hyperinflated or normal radiograph ("black lung") were considered as having a hyperinflation pattern (Figure 2). Chest radiographic characterization was based on a chest radiograph taken within the first 24 hours after admission. When multiple chest radiographs were taken, the one with the most significant radiological findings was considered. Patients whose radiographs could not be clearly classified within those two categories, or had been taken ≥24 h after admission, were excluded from the analysis.

2.4. Statistical Analysis. Descriptive statistics of patient characteristics were performed and reported in terms of mean and standard deviation (SD) for the quantitative variables and absolute frequencies and percentages for the qualitative variables.

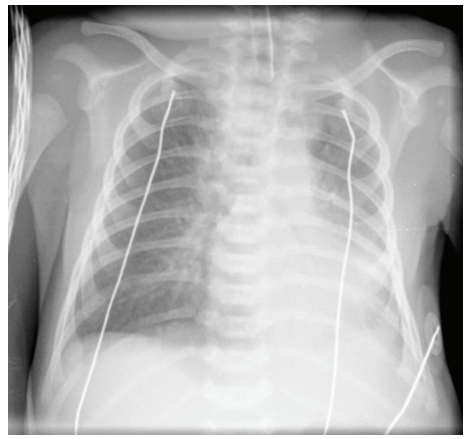

Figure 1: Consolidation pattern-chest radiograph showing pulmonary lower left lobe consolidation.

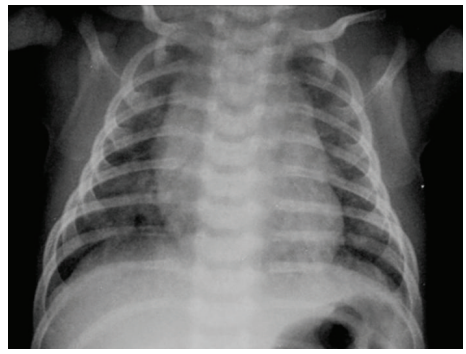

Figure 2: Hyperinflation pattern-chest radiograph showing pulmonary bilateral hyperinflation.

Demographic characteristics and risk factors were subjected to univariate analysis using the χ^2 test or Fisher's exact test for categorical variables and a 2-tailed Student's t-test or Mann-Whitney test for continuous variables, as appropriate.

As markers of severe disease, we selected the following primary outcomes: need for respiratory support need for invasive mechanical ventilation; supplemental oxygen requirement; length of stay in the NICU (dichotomized at >7 days using the median value of the variable).

To determine which factors independently predicted the outcomes, statistical models were built by using multivariate logistic regression analysis (backward stepwise). Variables statistically significant in the univariate analysis and/or considered clinically relevant for the outcome were entered in the model. Six potential independent predictors were considered: birthweight, gender, prematurity, chest radiographic pattern, congenital heart disease (CHD) and bronchopulmonary dysplasia (BPD).

Association of predictors with the primary outcomes was displayed using odds ratios (OR) and 95% CI's. Predictor variables with a P-value of <0.05 and multivariate odds

Table 1: Demographic characteristics and risk factors of newborns hospitalized in NICU with an RSV infection presenting a CPCR versus HPCR pattern.

	CPCR ($N = 101$)	HPCR ($N = 158$)	P
Demographic characteristics			
Gestational age, mean ± SD, wk	36.3 ± 3.0	37.6 ± 2.6	0.012[a]
Weight, mean ± SD, g	2828 ± 744	2980 ± 653	0.085
Gender, *n* (%)			0.036[a]
Male	46 (45.5)	93 (58.9)	
Female	55 (54.5)	65 (41.1)	
Corrected gestational age, mean ± SD, wk	39.6 ± 2.3	40.4 ± 2.2	0.006[a]
Postnatal infection, mean ± SD, d	23.7 ± 13.3	21.8 ± 14.2	0.285
Risk factors			
Prematurity, *n* (%)	33 (32.7)	38 (24.1)	0.129
Gestational age < 34 wk	12 (11.9)	12 (7.6)	
Gestational age 34–36^{+6} wk	21 (20.8)	26 (16.5)	
Congenital heart disease, *n* (%)	6 (5.9)	3 (1.9)	0.083
Bronchopulmonary dysplasia, *n* (%)	2 (2.0)	0 (0.0)	0.151

NICU: neonatal intensive care unit; RSV: respiratory syncytial virus; CPCR: consolidation pattern in chest radiography; HPCR: hyperinflation pattern in chest radiography; [a]significant differences.

Table 2: Disease characteristics in infants hospitalized in NICU with an RSV newborns presenting a CPCR versus HPCR pattern.

Markers of disease severity	CPCR ($N = 101$)	HPCR ($N = 158$)	P
Length of stay, median (IQR (25th–75th percentile)), d	8 (5–12)	7 (4–9)	0.005[a]
Supplemental oxygen			
Requirement, *n* (%)	93 (92.0)	124 (78.5)	0.004[a]
Duration, median (IQR (25th-75th percentile)), d	4 (3–7)	3 (2–5)	0.003[a]
O_2 maximum concentration, median (IQR (25th–75th percentile)), (%)	30 (28–50)	30 (27–38)	0.085
Respiratory support			
Requirement, *n* (%)	50 (49.5)	46 (29.1)	0.001[a]
Duration, median (IQR (25th–75th percentile)), d	3 (1–5)	2 (1–3)	0.184
Invasive mechanical ventilation			
Requirement, *n* (%)	23 (22.8)	17 (10.8)	0.009[a]
Duration, median (IQR (25th–75th percentile)), d	4 (2–6)	3 (1–4)	0.137
Maximum inspiratory pressure, median (IQR (25th–75th percentile)), mmHg	22 (20–28)	20 (20–23)	0.257

NICU: neonatal intensive care unit; RSV: respiratory syncytial virus; CPCR: consolidation pattern in chest radiography; HPCR: hyperinflation pattern in chest radiography; IQR: interquartile range; [a]significant differences.

ratios (and 95% CI's) that did not include 1 were considered significant. Statistical analyses were performed by using PASW statistics 18.0.

3. Results

From the 273 patients who met the inclusion criteria 14 (5.1%) were excluded: 12 (4.4%), due to inability to clearly classify the chest radiograph and 2(0.7%) due to missing data. Of the 259 remaining patients, 139 (53.7%) were male. The mean (±SD) gestational age was 37.3 ± 2.8 weeks, with 71 (27.4%) being preterm infants (<37 weeks). Corrected gestational age at time of diagnosis was 40.1 ± 2.3 weeks and the mean (±SD) birthweight was 2921 ± 692 grams.

3.1. Univariate Analysis. First we compared the baseline demographic characteristics and risk factors between patients with a consolidation pattern on chest radiography (CPCR) versus patients with a hyperinflation pattern on chest radiography (HPCR). Patients with CPCR were predominantly females and had lower birth and corrected gestational age. No significant differences were found between groups in any of the studied risk factors (Table 1).

Secondly, we compared differences in disease severity markers. The proportion of infants who required supplemental oxygen therapy, respiratory support, and invasive mechanical ventilation was significantly higher in the CPCR group with significantly longer median length of stay in the NICU and duration of supplemental oxygen therapy (Table 2).

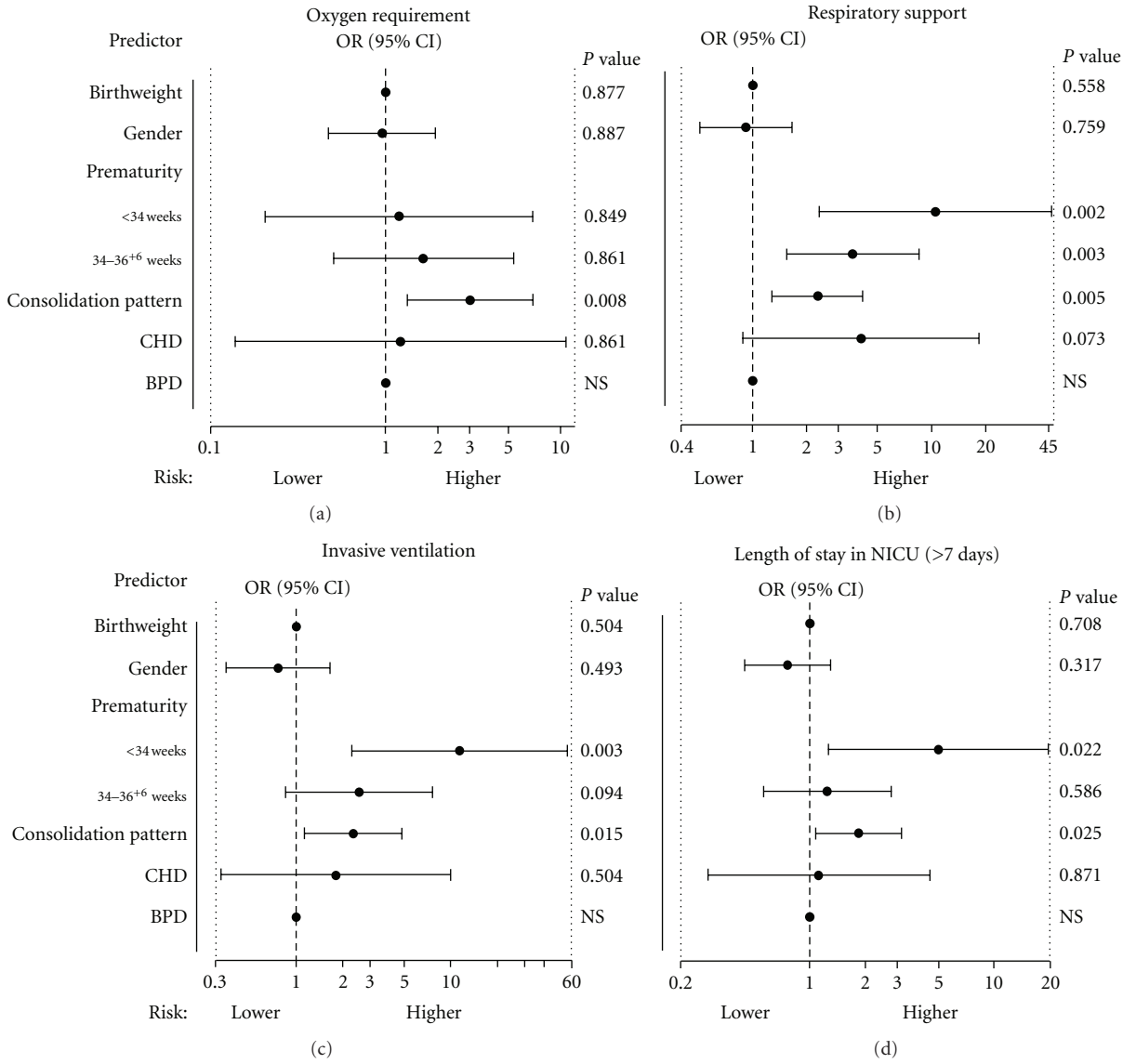

Figure 3: Odds ratios (ORs) for risk factors associated with disease severity in newborns with respiratory syncytial virus hospitalized in neonatal intensive care. According to multiple logistic regression analyses, the independent significant risk factors associated with disease severity, that is length of stay in NICU (≤7 versus >7 days), requirement of oxygen, respiratory support and invasive ventilation are those with a *P value* of <0.05. The reference category for gender is male, for prematurity (<34 and 34–36^{+6} weeks) is term (≥37 weeks) and for consolidation pattern is hyperinflation. NS indicates not significant.

Finally, we observed that CPCR patients were significantly more prone to develop complications with three times more cases of bacterial pneumonia when compared to HPCR patients (Table 3).

3.2. Multivariate Analysis. Of the considered predictors, prematurity (particularly in infants <34 weeks of gestational age) and chest radiograph pattern were independently associated with the need for respiratory support, need for invasive mechanical ventilation, and length of stay in the NICU > 7 days. Only the chest radiographic pattern was found to be an independent predictor for all four markers of disease severity (Figure 3).

4. Discussion

Several risk factors have been used to predict severe disease in RSV infected infants. Younger age at presentation, lower birthweight, prematurity, congenital heart disease, chronic lung disease, and immunodeficiency have consistently been associated with greater chance for hospital admission, longer hospital stay, and need for mechanical ventilation in RSV-infected infants [6–11]. However, currently existing models still fail to predict disease evolution in a considerable number of patients, suggesting that there are additional factors yet to be considered in risk stratification.

Our study showed that newborns with a consolidation chest pattern had more severe disease with greater

Table 3: Complications developed in newborns hospitalized in NICU with a RSV infection presenting a CPCR versus HPCR pattern.

	CPCR ($N = 101$)	HPCR ($N = 158$)	P
Pneumothorax, n (%)	0 (0)	1 (0.63)	0.610
Pneumonia, n (%)	42 (41.6)	21 (13.3)	<0.001[a]
Sepsis, n (%)	2 (2.0)	2 (1.3)	0.649
Death, n (%)	1 (1.0)	0 (0)	0.391

NICU: neonatal intensive care unit; RSV: respiratory syncytial virus; CPCR: consolidation pattern in chest radiography; HPCR: hyperinflation pattern in chest radiography; [a]Significant differences.

need for supplemental oxygen, respiratory support, invasive mechanical ventilation, and longer length of stay in the NICU. These observations support the relevance of chest radiographic pattern in RSV-infected newborns, as had been previously suggested [6, 15–17]. Indeed, it could serve as a surrogate marker of lower respiratory tract disease pattern in RSV disease. Prematurity (particularly those ≤34 weeks of gestational age) was also found to be an independent risk factor for severe disease in our population, with none of the other risk factors showing an independent effect.

Our study had a few limitations. Firstly, the sample size allowed only the detection of risk factors strongly associated with the primary outcomes and that were prevalent in our study population. The small numbers of congenital heart disease and bronchopulmonary dysplasia present in our sample could have underestimated their effect and results must be interpreted with caution. Low bronchopulmonary dysplasia (diagnosed according to the National Health Institute Consensus criteria) [18] prevalence relates to the low number of very premature newborns (<32 weeks) with RSV infection, which in turn could be explained by the universal use of anti-RSV human recombinant monoclonal antibody (palivizumab) in those patients [19].

Secondly, some patients cannot be clearly classified has having a consolidation or a hyperinflation pattern based on admission chest radiographs. Although such classification is possible in the vast majority of patients, some will have incipient or equivocal findings requiring chest radiograph repetition at a later time which falls beyond the scope of our study.

This study focused on a newborn population, for which there are few available data. We have shown that a consolidation pattern in RSV disease of the newborn is an independent predictor of disease severity and should be considered in clinical prediction rules. Better prediction of disease severity risk on admission will allow differential management strategies and more adequate resource allocation.

5. Conclusion

RSV-infected newborns with low gestational age (particularly those ≤34 weeks) and a consolidation pattern on admission (first 24 h) should be considered as high risk patients for a severe disease course, with greater risk of invasive mechanical ventilation, respiratory support, supplemental oxygen, and prolonged hospitalization.

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Review Article

Novel Approaches to Surfactant Administration

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Surfactant replacement therapy has been the mainstay of treatment for preterm infants with respiratory distress syndrome for more than twenty years. For the most part, surfactant is administered intratracheally, followed by mechanical ventilation. In recent years, the growing interest in noninvasive ventilation has led to novel approaches of administration. This paper will review these techniques and the associated clinical evidence.

1. Introduction

Respiratory distress syndrome (RDS) is the most common disease entity of premature infants. It is characterized by surfactant deficiency, immature airways, and lung parenchyma. With advances in the perinatal management, particularly antenatal corticosteroid therapy administered to the parturient, surfactant-deficient lung disease is now more prevalent among infants less than 29 weeks' gestation.

Surfactant is composed of phospholipids and associated proteins, produced by the type II pneumocytes that line the alveoli and smallest bronchioles. It reduces surface tension and stabilizes the air-liquid interface at the alveoli, thereby contributing to improvement in pulmonary compliance. While surfactant is necessary for normal lung function, adequate surfactant is not sufficient to assure normal gas exchange in the preterm infant. There are no simple ways to separate surfactant deficiency from other aspects of lung development, such as airway development, alveolarization, and the development of the pulmonary vasculature in the preterm infant. Babies born prematurely could be deficient in surfactant and also have underlying lung hypoplasia. Airway development also differs between infants of comparable gestational ages, as evidenced by susceptibility to the development of pulmonary interstitial emphysema. Nevertheless, surfactant treatment for RDS has been shown to dramatically improve survival of preterm infants.

The effects of surfactant therapy on RDS can be divided into pulmonary, cardiac, and radiologic. The immediate pulmonary effects include rapid improvement in oxygenation accompanied by increasing functional residual capacity, followed by a variable increase in lung compliance. The effects of surfactant administration on pulmonary artery pressure and pulmonary blood flow are not conclusive. Some studies suggest no changes in pulmonary flow after surfactant administration, while others suggest an increase in ductal shunt velocity and increased pulmonary blood flow. The radiologic changes reflect the recruitment of lung volume and decrease in atelectasis after surfactant treatment.

Administration of exogenous surfactant is the established treatment for RDS. It is the most widely studied drug in the last 25 years. The body of literature suggests that early or "prophylactic" administration of surfactant is more beneficial than late (rescue) therapy [1]. This has been a standard practice, and premature babies at risk of RDS often receive prophylactic surfactant in the delivery room during their initial stabilization. However, this approach is invasive, because it requires endotracheal intubation for administering the surfactant.

The complications of surfactant administration, which include bradycardia, hypoxia, and hypotension, and interest in noninvasive respiratory support, have highlighted the need to explore alternative forms of surfactant replacement

therapy. One study assessed the intubation times and number of attempts between neonatal consultants, neonatal fellows, and pediatric residents in Australia. The findings reflected the relationship between neonatal experience and ease of intubation [2]. Secondly, with the results of recent randomized clinical trials, many clinicians prefer to stabilize babies using noninvasive respiratory support initially without giving surfactant [3]. A modified approach, referred to as INSURE, requires endotracheal intubation, administration of surfactant, followed by rapid extubation to noninvasive support [4]. It still entails the attendant risks of intubation.

Reports of noninvasive approaches to stabilization, using early CPAP [5, 6], renewed interest among clinicians and questioned the need for routine surfactant administration. These observational data suggested significantly less bronchopulmonary dysplasia at one center that used much less mechanical ventilation. During the same period, Verder et al. [4] tested a novel approach, INSURE (intubation, surfactant administration, and extubation). This technique provides the benefits of surfactant administration but also eliminates continued mechanical ventilation. This approach, however, still requires skills for intubation and has the potential for trauma to the glottis and airway during intubation as well as the risks of surfactant administration enumerated above.

Over the last decade, randomized controlled trials have enrolled over 2500 infants to compare CPAP versus intubation and intermittent positive pressure ventilation (IPPV) at birth. Some trials (VON trial, IFDAS) also included INSURE as a third arm. Unfortunately, they reported no differences in the incidence of BPD or associated complications of prematurity [3].

With the uncertainty of initial management of these vulnerable premature infants, mechanical ventilation remains the "default" respiratory support. Some infants with mild surfactant deficiency may be managed without mechanical ventilation and surfactant administration for first few days, but the clinical problem is how to identify them. Borderline babies, who might do well initially, often develop signs of RDS over the next couple of days and may have a more difficult course because of delayed surfactant administration.

As mentioned above, intubation of the trachea can be hazardous and is usually undertaken after premedication, which may contribute to respiratory depression and a delay in extubation even after surfactant is administered. To incorporate the advantages of surfactant and to limit complications of endotracheal intubation, clinicians have been exploring other methods of surfactant administration, including delivery of surfactant via the upper airway or minimizing injury while administering intratracheal surfactant. Several techniques, collectively labeled "minimally invasive surfactant therapy" (MIST), have been described in which surfactant is delivered without tracheal intubation. These potential strategies include the following:

(1) intra-amniotic instillation,

(2) pharyngeal instillation,

(3) administration via laryngeal mask airway,

(4) administration via thin endotracheal catheter without IPPV,

(5) aerosolized/nebulized surfactant administration in spontaneously breathing infants.

2. Intra-Amniotic Instillation of Surfactant

There is only one feasibility report describing endoscopic delivery of surfactant directly to the fetus during active preterm labor. Using this technique, Petrikovsky et al. [7] introduced a gas-sterilized intraoperative fiberscope through the cervical canal into the amniotic cavity after spontaneous rupture of membranes during preterm labor. Using this approach the investigators injected surfactant into the mouths of 3 preterm fetuses through a catheter placed through the biopsy channel of the fiberscope. They reported no complications but suggested the need for further prospective studies to confirm the safety and efficacy of this method. Thus far, it has not been incorporated into clinical practice.

3. Pharyngeal Instillation of Surfactant

Babies born at term normally initiate respirations by first inspiring air and then closing the glottis while attempting to exhale. This creates a significant positive transpulmonary pressure and presumably forces fetal lung fluid into the interstitium of the lung [8]. It is likely that this process results in establishment of an air-fluid interface in the alveolus with deposition of surfactant from the fetal lung fluid. However, in the preterm lung, where surfactant is deficient, a similar positive pressure may result in histologic disruption of alveolar integrity [9], release of cytokines [10], leakage of serum proteins [11], and inactivation of both endogenous surfactant and any exogenously administered surfactant [12]. The pharyngeal instillation of surfactant before delivery has the potential to replicate the physiologic process. While the chest remains compressed in the birth canal, fetal lung fluid can be suctioned from the upper airway and replaced with a surfactant-containing solution. Then, as the chest expands, the baby is stimulated to aspirate the surfactant-containing solution providing surfactant at the advancing air-fluid interface. This process can be further facilitated by the application of mask CPAP.

Utilizing this approach, the initial report of pharyngeal instillation of surfactant was published in 2004 [13]. Twenty-three infants (560 to 1804 grams) born between 27 and 30 weeks' gestation had surfactant (Infasurf) administered within the nasopharynx before delivery of the shoulders after suctioning of the nasopharynx. Newborns received CPAP at 10 cm H_2O by mask as they initiated breathing, and this was continued at 6 cm H_2O for at least 48 hours. The investigators reported the technique to be relatively safe and simple to accomplish during vaginal deliveries. Unfortunately, this approach requires a cephalic delivery and a spontaneously breathing infant. Cesarean section, malpresentation (breech or transverse), or perinatal compromise limit the application of this approach. A Cochrane review did not find any articles

comparing this approach to no treatment or treatment with intubation and surfactant [14].

4. Administering via Laryngeal Mask Airway (LMA)

The LMA is a supraglottic device consisting of a curved plastic tube with an elliptical inflatable mask that is inserted blindly into the posterior pharynx of the baby. The mask may be inflated in the hypopharynx to create an airtight seal around the upper esophagus. It offers the possibility of rapidly establishing effective ventilation and access to the airway without the need for tracheal intubation, even when performed by relatively inexperienced personnel. There are different types of LMAs available (Classic; ProSeal; i-Gel; PAX press; CobraPLA).

A protocol for LMA surfactant administration suggested by Trevisanuto [15] involves positioning the LMA, followed by instilling the surfactant in two to four aliquots via the LMA. Each aliquot is usually followed by brief IPPV until the surfactant disappears from the LMA. Once the surfactant aliquots have been completely administered, the LMA is removed and the baby is placed on CPAP for subsequent management.

There are no reported studies of prophylactic or early LMA surfactant administration [16]. One small study reported a comparison of late rescue LMA administration of surfactant versus no surfactant. This study enrolled 26 preterm infants ≥1200 g with RDS who required CPAP. LMA surfactant administration resulted in a reduction in the mean FiO_2 required to maintain pulse oximetry between 88% and 92% for 12 hours after the intervention. No significant differences in subsequent mechanical ventilation, pneumothorax, days of intermittent positive airway pressure (IPPV), and days of IPPV or oxygen were reported [17].

Possible adverse effects of LMA surfactant administration include hypoxia and bradycardia during administration, laryngospasm, and malposition of the LMA, with potential effects on the newborn [18]. The limitations of surfactant administration using LMA are related to the nonavailability of smaller LMA sizes for use in extremely premature infants [18]. The technique is relatively simple and seems promising, but well-designed studies are needed to confer safety and efficacy.

5. Administration via Thin Endotracheal Catheter/Feeding Tube without IPPV

This method of surfactant administration delivers exogenous surfactant using a thin intravascular catheter or feeding tube inserted below the vocal cords. It is classified as a "MIST" technique. Using Magill forceps, a nasogastric tube is inserted into the trachea under direct laryngoscopic visualization of the vocal cords during nasal CPAP therapy. After placement of the catheter, surfactant is administered over a period of 1–3 minutes, while the infant continues to receive nasal CPAP. The procedure was first described in a feasibility study including premature infants ≤27 weeks of gestation. In this observational study, the intervention data were compared to historical controls. Reduced mortality (11.9% versus 35.3%, $P = 0.025$) and a reduced rate of severe IVH (grade 2 or 3) in survivors (5.1% versus 31.8%, $P = 0.01$) were observed [19]. After the publication of these results, some German centers adopted this method and they conducted a retrospective analysis of data from 15 centers. A total of 1541 infants <31 weeks of gestation was analyzed [20]. One thousand two hundred and twenty-two infants received standard care, and 319 were treated with the new method. Although smaller (945 versus 1018 g, $P < 0.001$) and less mature (27.3 versus 27.9 weeks, $P < 0.001$), infants treated with the new method showed less death or BPD (13.3% versus 19.9%, $P = 0.007$) and less need for any respiratory support. The technical difficulties associated with this method include the use of a highly flexible feeding tube and the need to use Magill forceps to advance the tube tip into the trachea. The necessary skills set may limit more widespread application.

To overcome the limitations imposed by the flexible nasogastric tube, Dargaville et al. tested the MIST technique utilizing placement of a 16-gauge vascular catheter below the vocal cords without using Magill forceps or premedication. This study enrolled 11 infants 25–28 weeks' gestation requiring any CPAP pressure or FiO_2, and 14 infants 29–34 weeks' gestation at CPAP pressure ≥7 cm H_2O and FiO_2 ≥0.35. In all cases, surfactant was successfully administered and CPAP was reestablished. Coughing (32%) and bradycardia (44%) were transiently noted and 44% received positive pressure inflations. There was a clear surfactant effect, with lower FiO_2 after MIST (pre-MIST: 0.39 ± 0.092; after 4 hour: 0.26 ± 0.093; $P < 0.01$), and a modest reduction in CPAP pressure. Few adverse outcomes were reported: intubation within 72 h ($n = 3$), pneumothorax ($n = 1$), BPD ($n = 3$), and death ($n = 1$), all in the 25–28-week group. Favorable outcomes were reported in both gestation groups, with a trend towards reduction in intubation in the first 72 h in the 25–28-week infants compared to historical controls [21].

The main limitations of the MIST methods are the need for laryngoscopy and the use of Magill forceps. There is still concern about potential trauma from both the laryngoscope and the catheters. In active preterm infants, in particular, placement of the catheter without sedation may be difficult and potentially traumatic, as well as uncomfortable. Additionally, this technique utilized a Benevista gas jet valve to provide CPAP while administering surfactant. This facilitates dispersion of surfactant without IPPV using a high flow CPAP system. It is unclear whether this method will be effective when used with Bubble CPAP or Infant flow driver CPAP.

6. Aerosolized/Nebulized Surfactant Administration in Spontaneously Breathing Infants

Many believe that the noninvasive administration of an aerosolized or nebulized surfactant might represent the best of all possible worlds by sparing manipulation of the airway but being able to administer surfactant early in the course

of RDS. Until recently, aerosolization has remained elusive. In order for the parent surfactant to be effective, four steps need to be accomplished. First, the surfactant needs to be aerosolized. The energy to do so may denature surfactant proteins. Second, the appropriate particle size needs to be achieved so that it does not "rain out" in the airway and is capable of penetrating deep into the lung. Third, the particles must be able to reaggregate at their site of action. Finally, the reaggregated surfactant has to regain and maintain its biological activity.

Use of nebulized surfactant seems to be the most sophisticated and minimally invasive technique. Several pilot trials have utilized this technique [22–26]. The majority of these trials used nasal CPAP delivery. One of the studies showed an improvement in (A-a) O_2-gradient, Silverman score, and $PaCO_2$ [22] and another study failed to demonstrate efficacy [23]. The studies are difficult to compare, as different surfactant preparations and different devices for nebulization and delivery were used, including jet nebulizers, ultrasonic nebulizers, and vibrating membrane nebulizers. The postnatal ages at application also varied between less than 30 minutes to less than 3 days of age.

Arzhavitina examined an *in vitro* model comparing six different nebulizers. They reported differences in the process of aerosol droplet generation between drugs with and without properties of surface activity and according to the type of nebulizer. They hypothesized that a vibrating membrane nebulizer is the best device for substances with surface activity, such as surfactant, as the residual volume in the device is minimal and the substance output is maximal [27].

The typical protocol for nebulized surfactant administration involves the use of an aerosol generator with surfactant administered by a nasal CPAP system, using either a tight face mask or nasopharyngeal tube [24]. Multiple factors are reported to influence aerosol surfactant dose delivery, including patient weight or size, minute ventilation [28], aerosol flow and patient peak inspiratory flow, aerosol particle size (as large as possible to avoid potential exhalation, yet small enough to bypass the oropharynx) [26], type of aerosol generator used, and type of surfactant [29] (Table 1). Nebulized surfactant may reduce the need for endotracheal intubation and is well tolerated [22, 24], apart from transient oxygen desaturation during dosing. There are no trials comparing the efficacy of nebulized surfactant delivery in premature infants compared to the standard approach or other delivery methods. Further refinements may, however, make it an attractive technique for future consideration.

Table 1: Factors influencing the success of aerosolized/nebulized surfactant.

(1) Patient weight
(2) Minute ventilation
(3) Aerosol flow
(4) Patient peak inspiratory flow
(5) Aerosol particle size
(6) Type of aerosol generator
(7) Type of surfactant

7. Summary

The current evidence regarding noninvasive surfactant delivery techniques in premature infants is limited to pilot data and feasibility studies. This is further complicated by varying delivery methods and nonavailability of smaller devices for use in very preterm infants. With the growing interest in noninvasive respiratory support techniques, until conclusive data on superiority of approach is documented, the gold standard of respiratory support is endotracheal intubation, administration of surfactant, and optimal mechanical ventilation. Data from clinical trials of the novel techniques will need to evaluate long-term respiratory and neurodevelopmental outcomes to prevent any untoward harm in vulnerable preterm infants and to assess the true cost effectiveness.

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Clinical Study

Flow-Synchronized Nasal Intermittent Positive Pressure Ventilation for Infants <32 Weeks' Gestation with Respiratory Distress Syndrome

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Aim. To evaluate whether synchronized-NIPPV (SNIPPV) used after the INSURE procedure can reduce mechanical ventilation (MV) need in preterm infants with RDS more effectively than NCPAP and to compare the clinical course and the incidence of short-term outcomes of infants managed with SNIPPV or NCPAP. *Methods*. Chart data of inborn infants <32 weeks undergoing INSURE approach in the period January 2009–December 2010 were reviewed. After INSURE, newborns born January –December 2009 received NCPAP, whereas those born January–December 2010 received SNIPPV. INSURE failure was defined as FiO_2 need >0.4, respiratory acidosis, or intractable apnoea that occurred within 72 hours of surfactant administration. *Results*. Eleven out of 31 (35.5%) infants in the NCPAP group and 2 out of 33 (6.1%) infants in the SNIPPV group failed the INSURE approach and underwent MV ($P < 0.004$). Fewer infants in the INSURE/SNIPPV group needed a second dose of surfactant, a high caffeine maintenance dose, and pharmacological treatment for PDA. Differences in O_2 dependency at 28 days and 36 weeks of postmenstrual age were at the limit of significance in favor of SNIPPV treated infants. *Conclusions*. SNIPPV use after INSURE technique in our NICU reduced MV need and favorably affected short-term morbidities of our premature infants.

1. Introduction

Respiratory distress syndrome (RDS) is the single most important cause of morbidity and mortality in preterm infants. In past years, the standard treatment for this condition was endotracheal intubation and mechanical ventilation (MV), as well as exogenous surfactant therapy. Although life saving, MV is invasive, resulting in airway and lung injury and contributing to the development of bronchopulmonary dysplasia (BPD). Nasal continuous positive airway pressure (NCPAP) has been advocated to be a gentler form of respiratory support that makes it possible to reduce the need for MV in preterm infants [1–3]. NCPAP combined with early surfactant replacement therapy, administered by intubation and rapid extubation (intubation-surfactant-extubation, INSURE), has been introduced as a primary mode of respiratory support in premature infants with RDS with a varying degree of success, depending on patients' gestational age (GA) and the severity of the radiological stage of RDS and FiO_2 at surfactant administration [4–10]. An evidence-based review showed that surfactant given at an early stage of RDS with extubation to NCPAP, compared with

surfactant given later and continued MV, is associated with a reduced need for MV, a lower incidence of BPD and fewer air-leak syndromes [11].

Nasal intermittent positive pressure ventilation (NIPPV) is a noninvasive mode of ventilation that offers more ventilatory support than NCPAP. NIPPV may be synchronized (SNIPPV) or nonsynchronized to the infant's breathing efforts. Several observations favor SNIPPV. Kiciman et al. [12] demonstrated that thoracoabdominal motion asynchrony and flow resistance through the nasal prongs decreased in neonates on SNIPPV, with improved stability of the chest wall and pulmonary mechanics. Moreover, delivering the peak inspiratory pressure immediately after the start of a respiratory effort, when the glottis is open, allows pressure to be effectively transmitted to the lungs, with little or no deviation through the esophagus into the stomach, obtaining the double advantage of increasing tidal volume (Vt) and reducing the risk of gastrointestinal side effects. In doing so, it is also possible that SNIPPV recruits collapsed alveoli and increases functional residual capacity (FRC). Recently, Owen et al. [13] described that during nonsynchronized NIPPV, Vt increases only when pressure peaks occur during spontaneous inspiration, suggesting that synchronization may be beneficial. In a previous study our group reported that application of SNIPPV was associated with increased tidal and minute volumes and decreased respiratory effort when compared with NCPAP in the same infant [14]. Finally, Aghai et al. [15] and Chang et al. [16] demonstrated that infants receiving SNIPPV have decreased work of breathing (WOB). Asynchronous breaths, on the contrary, may increase the risk of pneumothorax (PNX), blood pressure and cerebral blood flow velocity fluctuations and WOB [16, 17].

The purpose of our study was to evaluate whether in premature infants (GA < 32 wks) with RDS, SNIPPV used as ventilatory support immediately after surfactant administration using the INSURE technique is effective in further reducing the incidence of MV within the following 72 hours when compared to the conventional INSURE/NCPAP treatment. Our aim was also to compare the NICU clinical course and the incidence of short-term outcomes of pretem infants managed with SNIPPV or NCPAP after INSURE.

2. Patients and Methods

Chart data from inborn preterm infants with GA < 32 weeks admitted to our NICU from January 2009 to December 2010 were reviewed retrospectively with the aim of identifying newborns treated with nasal ventilation and the INSURE approach. Management of RDS before INSURE and the INSURE procedure were similar for all infants. Spontaneously breathing preterm newborns, not requiring intubation at birth for cardiopulmonary resuscitation, received early rescue NCPAP if chest retractions and/or grunting and/or tachypnea and/or oxygen need were present. Infants were routinely treated with caffeine (loading dose of 20 mg per kg of caffeine citrate followed by a daily maintenance dose of 5 mg per kg; daily maintenance dose could be doubled to 10 mg per kg in case of persistent apnoeic spells). Intubation for surfactant administration (INSURE technique) was performed if the FiO_2 requirement on NCPAP (CPAP level 5-6 cm H_2O) was >0.4 for more than 30 min, to maintain transcutaneous oxygen saturation between 85 and 93% in the presence of radiological signs of RDS. Poractant alfa (Curosurf-Chiesi Farmaceutici, Parma, Italy) 200 mg/kg was given endotracheally, followed by manual ventilation by bag for 2–5 min. A preterm size self-inflating ventilation bag was used for the procedure. During manual ventilation, titration of O_2 delivery was achieved by connecting the inlet of the self-inflating bag to an air/oxygen blender. Pressure was controlled by an attached disposable manometer. Pain control for elective endotracheal intubation was obtained by administering fentanyl 0.5–2 mcg/kg 5–10 min before intubation. After surfactant and manual ventilation, in the presence of a good respiratory drive and a satisfactory transcutaneous oxygen saturation value, infants were extubated. To reverse the potential respiratory depression because of opioids, infants without a good respiratory drive could receive a single dose of Naloxone (0.04 mg/kg). After extubation, infants referred from January to December 2009 (INSURE/NCPAP historical control group) were treated with ventilator-derived NCPAP (V.I.P. Bird Gold ventilator-Viasys Healthcare, Yorba Linda, CA, USA) as per standard protocol, while infants referred from January to December 2010 (INSURE/SNIPPV study group) were treated with flow-SNIPPV ("Giulia" Neonatal Nasal Ventilator-Ginevri Medical Technologies, Rome, Italy) according to a new institutional protocol for RDS. The device synchronizes NIPPV by means of a pneumothacograph interposed between the nasal prongs and the Y piece [18]. Before that time, in our unit, SNIPPV was mostly used to help infants weaning from MV after extubation and to treat apnoea of prematurity.

Nasal prongs of the same type (Ginevri Medical Technologies, Rome, Italy) were used for both ventilation modes. The size of the prongs was determined by the infant's weight. The largest possible prongs were used, with a snug fit to avoid leakage. No precautions were taken to avoid leakage from the mouth.

Mechanical ventilation was started in case of INSURE failure defined as: (1) $FiO_2 > 0.4$ to maintain SpO_2 85–93%; (2) significant apnoea defined as more than 4 episodes of apnoea/hour or more than 2 episodes of apnoea/hour if bag and mask ventilation were required; (3) respiratory acidosis defined as $pCO_2 > 65$ mmHg (8.5 kPa) and pH < 7.20 on arterial or capillary blood gas.

A second dose and additional doses of surfactant of 100 mg/kg could be administered to infants who were on MV, while a second INSURE was never tried. All infants were started on parenteral nutrition (PN) within the first 24 h of life, with dextrose, amino acids, and lipids. Total fluid volumes were increased daily until a goal of 140 to150 mL/kg/day was achieved during the first week of life. Infants were started on trophic feeds when clinically stable. Enteral nutrition was increased by 10–20 mL/kg every day as tolerated until a goal of 150 mL/kg/day. PN was stopped when full feeds (120 mL/kg/day) were tolerated.

Echocardiography was performed in all infants at 24–72 h of life and intravenous treatment with ibuprofen or indomethacin for a patent ductus arteriosus (PDA) was based on echocardiography and clinical signs. Cerebral echography was performed within the first 48 h of life, repeated at 7 days, and then every week until discharge. Intracranial hemorrhages (IVH) were classified as described by Volpe [19] and periventricular leucomalacia (PVL) as described by de Vries et al. [20]. Late-onset sepsis was diagnosed when a positive blood culture occurred in a sick infant after the first 72 hours of life. Necrotizing enterocolitis (NEC) was classified based on Bell's criteria [21]. Retinopathy of prematurity (ROP) grades I-V were defined as per international classification [22]. The INSURE/SNIPPV group was compared with the INSURE/NCPAP group to evaluate whether SNIPPV reduced the need for MV in the 72 hours after INSURE. The two groups were also compared in terms of incidence of air leaks, need for a second dose of surfactant, need for a high maintenance dose of caffeine, need for postnatal steroids, O_2 dependency at 28 days and 36 weeks of postmenstrual age (PMA), late-onset sepsis, nasal complications, feeding intolerance, NEC, PDA, IVH, PVL, ROP, and death. Duration of MV for infants who failed the INSURE approach, days on nasal ventilation, days on oxygen, days on parenteral nutrition, and length of hospital stay were also evaluated in the two study groups.

The maternal variables examined included type of delivery, antenatal steroid treatment, pregnancy induced hypertension, prolonged premature rupture of membranes (pPROM) > 18 h, placental abruption, intrauterine growth restriction (IUGR), and clinical chorionamnionitis (defined as the presence of fever with one or more of the following: maternal leukocytosis > 15,000/mm^3, uterine tenderness, fetal tachycardia, foul-smelling amniotic fluid).

Approval for this study was obtained from the Ethics Committee of the "S. Giovanni Calibita" Fatebenefratelli Hospital, Isola Tiberina, Rome.

3. Statistical Methods

All data were collected in an Excel database and analyzed using the statistical package STATA 12.0. Continuous normally distributed variables were compared using the *t* Student test for unpaired data and categorical variables were compared using the chi-square test. The Mann-Whitney test was performed to compare continuous variables that were not normally distributed. The Shapiro Wilk test was used to evaluate normally distributed assumptions.

A P value less than 0.05 was considered statistically significant.

4. Results

One hundred and sixty-seven infants with GA < 32 weeks were referred to our NICU in the 2 study periods. Surfactant was administered to 101 infants (60.5%); 64 of them underwent INSURE treatment (31 newborns in the INSURE/NCPAP historical control group and 33 in the INSURE/SNIPPV study group) and were included in this review. Characteristics of the newborns included in the 2 groups did not demonstrate significant differences (Table 1). In particular, Clinical Risk Index for Babies (CRIB) scores [23, 24], radiographic classification [25], FiO_2 values, and transcutaneous PCO_2 ($tcPCO_2$) values before surfactant treatment indicated that the RDS severity was similar for the 2 groups. After INSURE, infants in the historical group received NCPAP at a pressure level of 5-6 cm H_2O with a flow rate of 8.0 ± 0.5 L/min while infants in the study group received SNIPPV in the assist/control mode (i.e., ventilator assisting each spontaneous breath) with the following initial respiratory parameters: Ti 0.32 ± 0.02 sec, back-up rate 35 ± 5 bpm, PIP 15 ± 2 cm H_2O, PEEP 5.5 ± 0.5 cm H_2O, flow rate 8.0 ± 0.5 L/min.

Table 2 reports neonatal outcomes in the 2 study groups. Eleven (GA 27.9 ± 1.7 weeks, BW 1056 ± 222 g) out of 31 infants in the INSURE/NCPAP group versus 2 (GA 25 and 27 weeks, BW 670 and 1080 g, resp.) out of 33 infants in the INSURE/SNIPPV group met the INSURE failure criteria and underwent endotracheal MV (35.5% versus 6.1%; $P = 0.004$). Failure was due to pneumothorax in 1 infant, intractable apnoea in 4 infants, hypercapnia in 3 infants, and increased oxygen requirement in 3 infants in the INSURE/NCPAP group, while in the INSURE/SNIPPV group both infants failed because of increased oxygen requirement.

INSURE failure occurred at median (range) 48.1 (5–71) hours after surfactant administration in the INSURE/NCPAP group and at 9.5 (6–13) hours in the INSURE/SNIPPV group. Six hours after surfactant administration, the FiO_2 requirement for infants still on nasal ventilation was significantly higher in the INSURE/NCPAP group (median (range): 0.30 (0.21–0.45) versus 0.22 (0.21–0.40); $P < 0.001$). More infants in the INSURE/NCPAP group needed a second dose of surfactant (22.6% versus 3%; $P = 0.025$) and a high maintenance dose of caffeine (29% versus 9.9%; $P = 0.041$). Treated PDA was also more frequent in the INSURE/NCPAP group (25% versus 6.5%; $P = 0.041$). Among the 10 patients with treated PDA, only 1 in the INSURE/NCPAP group required surgical ligation. Four (2 per group) of those infants who were successfully treated with the INSURE approach subsequently needed MV due to late-onset sepsis at median age of 13 days (range 8–21 days). Although fewer infants in the INSURE/SNIPPV group were O_2 dependent at 28 days and 36 weeks PMA, this was at the limit of statistical significance. Other neonatal outcomes did not differ in the 2 groups, as reported in Table 2. Nasal complications, including columella nasi bleeding, flaring of the nostrils, and snubbing of the nose, were all transient, and the incidence was similar in the two groups. Some infants in both groups had moderate abdominal distention; however the incidence of feeding intolerance was similar in the two groups. One infant in the INSURE/NCPAP group developed NEC (Bell stage IIA) by day 20 of life. The observed ROP case was grade I. NICU course did not differ between groups (Table 3).

Table 1: Neonatal characteristic in the two study groups.

	INSURE/NCPAP (n.31)	INSURE/SNIPPV (n.33)	*P* value
Gestational age (wks)	29.1 ± 1.4	28.7 ± 1.3	0.768
Birth weight (g)	1305 ± 364	1283 ± 278	0.786
M/F	14/17	13/20	0.641
Multiple births	7 (22.6)	8 (24.2)	0.085
Antenatal steroids	27 (87.1)	26 (78.8)	0.689
Main maternal pregnancy diseases			
(i) hypertensive disorders	6 (19.3)	9 (27.2)	0.455
(ii) pPROM	6 (19.3)	5 (15.1)	0.729
(iii) placental abruption	4 (12.9)	3 (9.1)	0.704
(iv) corionamnionitis	3 (9.6)	4 (12.1)	1.000
(v) IUGR	5 (16.1)	5 (15.1)	1.000
Cesarean section	27 (87.0)	28 (84.8)	0.796
Apgar score at 5′	8 (5–9)	8 (6–9)	0.947
CRIB score	2 (0–11)	1 (0–8)	0.078
RDS moderate to severe*	20 (64.5)	23 (69.7)	0.625
Age at NCPAP (min)	30 (15–120)	30 (15–120)	0.994
Age at INSURE (hours)	4 (0.5–17)	4 (0.5–23)	0.736
FiO_2 at INSURE	0.44 ± 0.05	0.43 ± 0.03	0.332
$tcPCO_2$ at INSURE (mm Hg)	46.6 ± 6.6	48.6 ± 7.9	0.278

Values are given as mean ± SD, median (range), or number and (%).
*Radiographic classification.

Table 2: Neonatal outcomes in the two study groups.

	INSURE/NCPAP (n.31)	INSURE/SNIPPV (n.33)	*P* value
INSURE failure	11 (35.5)	2 (6.1)	0.004
Pneumothorax	1 (3.2)	0	0.484
Surfactant second dose	7 (22.6)	1 (3.0)	0.025
Caffeine high maintenance dose	9 (29.0)	3 (9.9)	0.041
PDA treated	8 (25.8)	2 (6.1)	0.041
Postnatal steroids	4 (12.9)	1 (3.0)	0.190
O_2 dep. at 28 days	6 (19.3)	1 (3.0)	0.050
O_2 dep. at 36 weeks PMA	4 (12.9)	0	0.050
Late onset sepsis	4 (12.9)	4 (12.1)	1.000
Feeding intolerance	3 (9.7)	4 (12.1)	1.000
NEC	1 (3.2)	0	0.484
IVH (1-2°)	2 (6.4)	2 (6.0)	1.000
IVH (3-4°)	1 (3.2)	1 (3.0)	1.000
PVL	0	0	1.000
ROP	0	1 (3.0)	1.000
Death	0	0	1.000

Values are given as number and (%).

Table 3: NICU course in the two study groups.

	INSURE/NCPAP (n.31)	INSURE/SNIPPV (n.33)	*P* value
Duration of MV* (h)	29 ± 21	39 ± 22.8	0.073
Days on nasal ventilation	4.8 (1–62)	4.9 (1–25)	1.000
Days on oxygen	7.4 (1–62)	6 (1–35)	0.704
Days on parenteral nutrition	13.2 ± 8.2	15.6 ± 9.8	0.294
Length of hospital stay (days)	49 ± 19	48 ± 25	1.000

Values are given as mean ± SD or median (range).
*For infants who failed INSURE approach.

5. Discussion

Early surfactant therapy administered by INSURE technique and combined with NCPAP has been applied in preterm infants with RDS to prevent ventilator-associated lung injury. This strategy is effective in improving respiratory outcome and reducing the need for MV, although several studies reported an INSURE/NCPAP approach failure ranging between 26% and 50% [10]. In our pre-SNIPPV period, INSURE failure occurred in about 35% of infants <32 weeks' gestation, similar to literature reports. The introduction of flow-SNIPPV in our Unit and its use after INSURE significantly reduced the need for MV.

We previously observed that flow-SNIPPV in the post extubation period supports respiratory effort more effectively than NCPAP [14, 18]. According to present data, flow-SNIPPV also seems to be promising in treating infants in the acute phase of RDS, as a primary mode of ventilation after INSURE.

Compared with NCPAP, SNIPPV improves ventilation by increasing Vt [13, 14] and decreasing respiratory effort [12, 14–16], thus representing an ideal mode of noninvasive support. These mechanisms of action probably account for the higher success of the INSURE/SNIPPV strategy over the classical INSURE/NCPAP reported in our study. Indeed, in our series of preterm infants the prominent effects of flow-SNIPPV were those of augmenting and stimulating spontaneous breathing as demonstrated by the absence of respiratory acidosis and apnoeic episodes as reasons for failure in infants who received this mode of ventilation.

It has been observed that SNIPPV significantly reduces PCO_2 values when compared with NCPAP in preterm infants [14, 26] as a consequence of improved alveolar ventilation. Moreover, apnoeic spells are common in premature infants and are recognized as a significant reason for MV use. Barrington et al. [27] found a trend towards a reduction of apnoeic episodes per day in infants treated with SNIPPV after extubation, while Lin et al. [28] suggested that synchronization may increase the success of NIPPV in reducing apnoeic spells. Conversely, Ryan et al. [29] and Pantalitschka et al. [30] found that NIPPV offers no advantages over NCPAP in treating apnoea of prematurity. According to these observations, fewer infants in the INSURE/SNIPPV group needed a high maintenance dose of caffeine for persistent apnoeic spells and no infant underwent MV due to apnoea in this group. Synchronized NIPPV may effectively help preterm infants suffering apnoeic episodes to counteract the mechanisms that contribute to this pathology better than NCPAP and NIPPV.

FiO_2 requirement 6 hours after INSURE was lower in SNIPPV treated infants. One possible explanation for this association is that SNIPPV, favoring alveolar recruitment and keeping the lung open by applying a higher mean airway pressure, may prevent RDS worsening more effectively than NCPAP. For the same reasons, SNIPPV probably helps exogenous surfactant distribution in the lungs and its more effective action, as suggested by a reduced need for a second surfactant dose in this group.

Recently, 3 randomized controlled trials studied the effects of NIPPV applied in the acute phase of RDS as a primary mode of respiratory support before surfactant replacement. Kugelman et al. [31] observed that NIPPV, compared with NCPAP, decreased the requirement for endotracheal ventilation in premature infants <35 weeks with RDS, and this was associated with a reduced incidence of BPD. In this study however the INSURE approach was not used. Sai Sunil Kishore et al. [32] used NIPPV at the first signs of RDS, and coupled this technique with the INSURE approach in premature infants with GA ≥ 28 weeks. Similar to our reports, they found that the need for intubation and MV was lower with NIPPV. Finally, Meneses et al. [33] could obtain similar results using NIPPV only in infants weighing > 1000 g. According to these reports, nonsynchronized early NIPPV seems to be beneficial for slightly older and heavier infants when compared with NCPAP. However, as SNIPPV presents potential advantages over NCPAP and NIPPV, its use soon after birth in preterm infants <1000 g deserves further investigation.

A significant reduction in BPD has been reported when NIPPV is used as respiratory support after extubation or as a primary mode for RDS [34–37]. In our study, the difference in O_2 dependency at 28 days and 36 weeks between the two groups was at the limit of statistical significance, probably due to the small number of patients included. As MV in the first days of a preterm infant is a major factor for BPD [38, 39], avoiding endotracheal tube ventilation remains of paramount importance in preventing ventilator-induced lung injury.

RDS and PDA are common comorbidities in premature infants. In our series, more infants in the INSURE/NCPAP group needed pharmacological treatment for PDA. Symptomatic PDA is an identified risk factor for INSURE failure [40] and may have contributed to the higher need for MV in this group. Moreover, as mechanical ventilation strategies may influence ductal closure [41], whether flow-SNIPPV may have a direct effect on PDA should be investigated further.

In our study a second INSURE after the first INSURE failure was not attempted. Recently, Dani et al. [42] found that multiple INSURE procedures were followed by a similar respiratory outcome to the single procedure in a cohort of extremely premature infants. Whether the multiple INSURE approach might be a useful alternative to surfactant given during MV requires specific studies.

Abdominal distension is commonly observed in infants undergoing nasal ventilation. Although mild abdominal distension usually causes no severe complications, it may play a role either in delaying the speed of oral feeding or in reducing the efficacy of ventilation. In our series, one infant per group had to discontinue oral feeding for 24 hours while on nasal ventilation, while 2 infants in the INSURE/NCPAP group and 3 infants in the INSURE/SNIPPV group delayed the daily increase of oral feeds. Overall, days on parenteral nutrition were similar in the two groups. Nasal complications were also observed, but not serious enough to cause ventilation to be suspended.

The incidence of cerebral damage was very low in the whole group, confirming the safety of this ventilatory approach in a population of relatively large preterm infants. Moreover, the two study groups did not differ in terms of short-term outcomes at discharge, suggesting that flow-SNIPPV could provide effective as well as safe ventilatory support for the treatment of infants with RDS after surfactant treatment.

The main limitations of this study are in the retrospective design and in the small number of patients included. Although this is a small retrospective study, it has been conducted over a relatively short clinical period, during which we are not aware of any significant shift in clinical practice other than the introduction of flow-SNIPPV as a respiratory support after INSURE, therefore we do not believe that other changes in clinical practice not recorded for this analysis might explain the differences between the two groups. Nevertheless, these data need to be confirmed in a randomized controlled trial, and the possible underlying protective mechanisms of SNIPPV on acidosis and/or on apnoea deserve specific investigation.

Another limitation of our study relates to the small number of infants included weighing less than 1000 g at birth, since most of the difficulties in keeping infants away from MV are encountered with these tiny newborns. In our series, 5 infants in each group had a birth weight <1000 g. Among these, 4 in the NCPAP group and 1 in the SNIPPV group failed the INSURE approach. Thus, further work is needed to establish the effectiveness of SNIPPV in extremely low birth weight infants.

6. Conclusions

Our data suggest that, for infants being treated with nasal ventilation for RDS, the use of flow-SNIPPV after surfactant administration using the INSURE technique is safe and beneficial, as evidenced by the decreased need for MV, with no worsening of prematurity-related outcomes, compared with infants who underwent the classical INSURE/NCPAP approach. Further studies should be conducted to confirm these findings, to evaluate the real improvement in long-term outcome in SNIPPV treated newborns, to determine the optimal ventilatory settings of the SNIPPV system and to investigate the possibility of using SNIPPV to treat apnoea of prematurity and as a primary support for idiopathic RDS before surfactant administration particularly in extremely low birth weight infants.

Conflict of Interests

C. Moretti has been a consultant to Ginevri Medical Technologies. Ginevri Medical Technologies has not contributed any financial support for this paper or had any part in the authorship.

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