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Bronchopulmonary dysplasia: Latest advances

Edited by
Jia-Yuh Chen, Xiaoguang Zhou, Chung-Ming Chen, Shahana Perveen and Hisanori Sobajima

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Bronchopulmonary dysplasia: Latest advances

Topic editors

Jia-Yuh Chen — Chung Shan Medical University, Taiwan
Xiaoguang Zhou — Department of Neonatology, Nanjing Children's Hospital, China
Chung-Ming Chen — Taipei Medical University, Taiwan
Shahana Perveen — Cohen Children's Medical Center, United States
Hisanori Sobajima — Saitama Medical University Hospital, Saitama Medical University, Japan

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Eugene Dempsey,
University College Cork, Ireland

*CORRESPONDENCE
Jia-Yuh Chen
✉ 182288@cch.org.tw

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Editorial: Bronchopulmonary dysplasia: latest advances

Shahana Perveen[1,2], Chung-Ming Chen[3], Hisanori Sobajima[4], Xiaoguang Zhou[5] and Jia-Yuh Chen[6]*

[1]Department of Pediatrics, Cohen Children Medical Center, Manhasset, NY, United States, [2]Department of Pediatrics, Zucker School of Medicine at Hofstra/Northwell, Manhasset, NY, United States, [3]Department of Pediatrics, Taipei Medical University, Taipei, Taiwan, [4]Division of Neonatology, Department of Pediatrics, Saitama Medical Center, Saitama Medical University, Saitama, Japan, [5]Department of Neonatology, The Eighth Affiliated Hospital, Sun Yat-Sen University, Kangdon, China, [6]Division of Neonatology, Changhua Christian Children's Hospital, Changhua, Taiwan

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Editorial on the Research Topic
Bronchopulmonary dysplasia: latest advances

Bronchopulmonary dysplasia (BPD) is a complex and devastating condition related to prematurity. BPD is the most common chronic condition of infancy and is associated with significant morbidity and mortality and high financial burden on healthcare systems, families, and communities (1). Infants are not born with BPD; the condition evolves with time with prolonged use of mechanical ventilation and oxygen therapy leading to lung damage. The pathogenesis of BPD is complex, poorly understood, and multifactorial. The incidence has not changed in last two decades despite many advances in neonatology because of the improved survival of extreme preterm infants, including babies less than 500 g (2). Advances in technologies and improving clinical care have led to a lower incidence of "Classic BPD" described as heterogeneity and severe airway epithelial lesions, airway smooth muscle hyperplasia, extensive alveolar septal fibrosis, and hypertensive remodeling of the pulmonary arteries (3). It has been replaced it with a "New BPD" described as less heterogeneity, with large simplified alveolar structures, reduced and dysmorphic vascular bed and mild airway smooth muscle thickening (4) which present with distinct clinical and pathological features in surviving infants (5). Regardless of changing definition BPD is a chronic condition which mainly affects premature infants with disparity in lung injury and repair processes in the presence of immature developing lung (6). The overall incidence of BPD in infants born prematurely <28-week gestation age is approximately 48%–68% and inversely related to gestational age (7). The pathogenesis of BPD remains unclear and involves several prenatal as well as post-natal factor in the presence of prematurity leading to lung damage (8). These multiple factors, including mechanical ventilation, oxygen therapy, infections, exposure to toxins and genetic factors play a key role in the development of BPD. The diagnosis of BPD in preterm infants remains associated with significant short term and long-term morbidity (2). The general approach is to prevent lung injury with optimal ventilator adjustment, prevent infection and promoting lung growth by providing adequate nutrition.

The research topic "Bronchopulmonary Dysplasia: Latest Advances" presents a total of 12 articles focusing on underlying etiology, pathogenesis, prevention bundles for improving the understanding and management strategies of the BPD. One of the articles "Association

of newer definitions of bronchopulmonary dysplasia with pulmonary hypertension and long-term outcomes" by Hwang et al. describes the importance of using new evolving definition of BPD and its impact on predicting long term outcome and development of pulmonary hypertension. This retrospective study enrolled preterm infants born at <32-week GA B/W 2014–2018 at Guri Hospital, Korea. This is the first study that compares the different criteria of BPD regarding pulmonary hypertension in preterm infants and showed an association between recently described criteria for defining BPD as NICHD 2018 & NICHD 2019 criteria with severity of BPD and later outcome including pulmonary hypertension in preterm infants. This is an important concept to recognize as BPD is now described as a form of pulmonary vascular disease (9) and development of pulmonary hypertension could be spectrum of vascular disease with increasing severity and poor prognosis (10).

"A prediction nomogram for moderate-to-severe bronchopulmonary dysplasia in preterm infants <32 weeks of gestation: A multicenter retrospective study" by Zhang et al. presents a multicenter retrospective study conducted with aim to develop a dynamic nomogram for early prediction of BPD using perinatal factors in preterm infants born at <32 weeks' gestation. This was conducted at three hospitals in China between January 2017 and December 2021. There are several prediction models in extreme premature babies predicting the BPD and poor outcome (11, 12), however metanalysis have showed the limited utility and validity in these prediction models (13, 14). Using machine learning the authors indentified GA, Apgar 5-min score, early onset sepsis score (EOS), small for gestational age (SGA), and duration of invasive ventilation as predictors for BPD. Predicting significant BPD as early as within 7 days of life is an important aspect in future management of the babies at risk for BPD.

"Association between the development of bronchopulmonary dysplasia and platelet transfusion: a protocol for a systematic review and meta-analysis" by Chioma et al. describes an potentially important link to development of bronchopulmonary dysplasia. Platelets play a role in the formation of pulmonary blood vessels and thrombocytopenia has been described with pulmonary disease including BPD (15). Chioma et al. describe a protocol and plan to evaluate the correlation of platelet transfusion in preterm infant with development of BPD based on a systemic review and metanalysis. As platelet transfusion associated with release of bioactive factors can enhances oxidative stress leading to altered angiogenesis and BPD.

"Development of a novel humanized mouse model to study bronchopulmonary dysplasia" by Birkett et al. addresses the role of fetal circulating monocytes from cord blood in the pathogenesis of bronchopulmonary dysplasia. It also explores the relationship of chorioamnionitis and pre-eclampsia on fetal circulating monocytes using humanized mouse model. The study describes that fetal monocyte exposed to preeclampsia shows accelerated alveolarization as compared to exposure to chorioamnionitis which causes arrest of alveolarization. This study provides a remarkably interesting and novel approach to study basic underlying molecular mechanism and lung disease. Fetal monocytes play a role in in early lung development in humanized mouse model of BPD. Fetal monocyte induced vascular development was inhibited by hyperoxia and provide a platform for further exploration and patient targeted therapeutic approach and interventions.

"Two-stage learning-based prediction of bronchopulmonary dysplasia in very low birth weight infants: a nationwide cohort study" by Hwang et al. This study describes the development of machine-based learning model for the prediction of bronchopulmonary dysplasia and its severity through two stage approach incorporating the duration of respiratory support and prenatal and early postnatal variables. This study provides potential useful tool for clinician for early prediction of BPD and its severity with high predictive accuracy. There is great need for a reliable prediction model for BPD in preterm infants and future studies evaluating this model may be very helpful in achieving and validating this prediction model in neonatal intensive care units.

Extracellular vesicles (EVs) are a diverse array of nano-sized membranous structures that are becoming recognized as intercellular and inter-organ communication mediators. Wu et al. describe the isolation methods, characterization techniques, and the functions of EVs. The phospholipid membrane of EVs protects their payload from the extracellular environment, allowing for safe transit and distribution of their cargo to local or distant target cells and consequently, alters the target cell's gene expression, signaling pathways, and function. The extremely selective, sophisticated network via which EVs promote cell communication and modify cellular processes makes EVs as pathogenic messengers, biomarkers, and potential treatments for newborn lung disorders.

Various biomarkers have been studied for early prediction of BPD (9, 16–18). Biomarkers may be valuable for early diagnosis of BPD, enabling initiation of therapies to reduce the incidence of BPD and long-term cardiorespiratory impairment. Wang et al. describe that a combining clinical data, molecular biomarkers, and echocardiogram measurements can be valuable in predicting BPD. The tricuspid regurgitation jet, N-terminal-pro-B-brain natriuretic peptide (NT-pro BNP), ventilator associated pneumonia, days of FiO_2 ≧40%, red blood cell volume, and proportion of infants who receive total enteral milk (120 Kcal/kg/day) ≧24 days after birth are the most practical factors for predicting the risk of BPD. Gaertner et al. use electrical impendence tomography (EIT) to measure regional ventilation distribution and overall lung aeration. They describe that EIT markers of aeration at 30 min after birth accurately predict the need of oxygen supplement at 28 days of age but not the need of intubation or BPD.

Conclusion

The Research Topic "Bronchopulmonary dysplasia: Latest Advances" goal was to incorporate the most recent advances in basic science research, translational research, clinical data, and preventive measures towards the better understanding and

management of patients with BPD. Even though there have been promising advancements in understanding and management of the BPD, this condition remains complex and without any definitive cure. Continued research and collaboration among healthcare professionals and researchers are crucial to further improve the understanding, prevention, and treatment of bronchopulmonary dysplasia.

Author contributions

SP: Conceptualization, Writing – original draft. CC: Writing – review & editing. HS: Writing – review & editing. XZ: Writing – review & editing. JC: Conceptualization, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Xiaoguang Zhou,
Nanjing Children's Hospital, China

REVIEWED BY
Chuanzhong Yang,
Shenzhen Maternity and Child Healthcare Hospital, China
Xiaoqing Chen,
Nanjing Medical University, China

*CORRESPONDENCE
Yuan Shi
shiyuan@hospital.cqmu.edu.cn

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The value of plasma insulin-like growth factor 1 and interleukin-18 in the diagnosis of bronchopulmonary dysplasia in premature infants

Lie Huang[1], Ning Guo[2], Meile Cheng[2], Jianhui Wang[1], Feifan Chen[1] and Yuan Shi[1]*

[1]Department of Neonatology, Children's Hospital of Chongqing Medical University, National Clinical Research Center for Child Health and Disorders, Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing Key Laboratory of Pediatrics, Chongqing, China, [2]Department of Neonatology, The First People's Hospital of Yinchuan, Ningxia Medical University, Yinchuan, China

Objective: To explore the diagnostic value of IGF-1 and IL-18 in premature infants with BPD.

Methods: Through a prospective observational study, the serum samples of infants in the BPD group and the non-BPD group were collected at different targeted time points, and the serum IGF-1 and IL-18 concentrations were dynamically monitored by ELISA. The Student *t*-test and one-way analysis of variance were adopted to analyze data, and the receiver operating characteristic (ROC) curve was used to test the diagnostic value.

Result: A total of 90 VLBW premature infants admitted to NICU between January 2020 and 2021 were finally included. Compared with the non-BPD group, infants diagnosed with BPD had a significantly lower serum concentration of IGF-1 ($P < 0.05$) but a higher level of IL-18 ($P < 0.05$) on days 1, 7, 14, and 28 after birth. With the ROC curve analysis, the serum concentration IGF-1 on day 14 and IL-18 on day 28 reported high sensitivity and specificity to predict the risk of BPD (IGF-1: sensitivity: 89.29%, specificity: 77.78%, AUC: 0.8710; IL-18: sensitivity: 53.57%, specificity: 83.33%, AUC: 0.7887). And more substantial predictive power was found in combined analysis of IGF-1 and serum IL-18 on day 14: the sensitivity was 91.07% and the specificity was 83.33%, with the AUC of 0.9142.

Conclusion: IGF-1 and IL-18 might be closely involved in the occurrence and development of BPD. The serum concentration of IGF-1 combined with IL-18 could be potentially sensitive markers for the early diagnosis and severity of BPD.

KEYWORDS

bronchopulmonary dysplasia, very low birth weight (VLBW), IGF-1 (insulin-like growth factor 1), IL-18, inflammation

Introduction

Bronchopulmonary dysplasia (BPD), a chronic neonatal lung disease, is one of the most common and severe sequelae of premature births (1–5). Up to 15%–25% of infants born at <32 weeks of gestational age and 60% of those born at <28 weeks will develop BPD, with potential adverse events that can persist into their adulthood and lead to lifelong respiratory and neurodevelopmental consequences (6, 7).

However, the pathogenesis of BPD remains unclear and specific treatments are still lacking, so preventive measures and early diagnosis are indeed crucial. The pathogenesis of BPD is multifactorial (8, 9), involving immature lung tissue, excessive inflammatory injury, and abnormal repair processes, especially the abnormal repair after injury may significantly impair the development of airways, lung parenchyma, pulmonary interstitial, pulmonary vessels, and lymphatic system (10). Many previous studies have reported that growth factors and cytokines involved in the early immune response can initiate the immune cascade signaling pathways of the inflammatory response, participate in the occurrence and progression of BPD, and ultimately may affect the pulmonary structure of infants (11).

Insulin-like growth factor 1 (IGF-1) is a key factor in embryonic and postnatal growth and development, including stimulating cell proliferation and differentiation, inhibiting apoptosis, and regulating gene transcription (12), which is also found to play an important role in all stages of lung differentiation and maturation. IGF-1 can help repair and remodel airways by promoting the proliferation and differentiation of airway epithelial cells and fibroblasts (13). IGF-1 knockout mice exhibit impaired lung development and maturation, and neonatal rats exposed to hyperoxia are detected with decreased IGF-1 expression levels in the lungs and inhibited alveolarization processes (14). Besides IGF-1, Interleukin-18 (IL-18), a pro-inflammatory mediator and a member of the IL-1 cytokine family, maybe another potential predictor of BPD. It has been proved to be critical in many pulmonary diseases such as acute respiratory distress syndrome (ARDS) and chronic obstructive pulmonary disease (COPD) in adults (15). IL-18 can participate in the progression of lung inflammation by promoting TH1-type helper T-cell responses and promote fibroblast proliferation and collagen deposition in the pulmonary fibrosis process (16).

All the above findings suggest that IGF-1 and IL-18 may participate in the occurrence and development of BPD. This study aimed to explore the relationship between IGF-1 and IL-18 and BPD by detecting the serum concentration, hoping to provide a new reference for possible biomarkers for BPD prediction in premature infants.

Materials and methods

Patient enrollment

The study population consisted of neonates admitted to the NICU of The First People's Hospital of Yinchuan between January 2020 and 2021, with a gestational age <32 weeks, and hospitalized for more than 28 days. Infants with major congenital malformations, the age more than 24 h upon admission to the NICU, severe infection, shock, inherited metabolic diseases and who withdrew during the study period were excluded. All the neonates were divided into two groups: the BPD group and the non-BPD group. Infants were diagnosed with BPD according to the criteria from the workshop of the National Institutes of Child Health and Human Development (NICHD) (17) and were classified as follows: mild: breathing room air, moderate: a fraction of inspired oxygen FiO_2<0.3, severe: FiO_2 ≥0.3 and/or positive pressure ventilation or mechanical ventilation.

This study was approved by the ethics committee of The First People's Hospital of Yinchuan (Number: 2020110). Informed consent was obtained from all the parents of the included infants.

Collection of clinical data

The following data were collected from hospital records: (1) general condition of the infants: gender, gestational age (according to the first day of the last menstrual period) at birth, birth weight, Apgar Score 1 min, Apgar Score 5 min, (2) maternal conditions: Chorioamnionitis, Premature rupture of membranes, Antenatal steroids, Gestational hypertension, Gestational diabetes, (3) birth injuries, conditions, and comorbidities present in the infants: patent ductus arteriosus, late-onset neonatal sepsis, necrotizing enterocolitis and so on.

Sample collection

Detection of serum IGF-1 and IL-18 levels: 1–1.5 ml of neonatal serum samples were collected each time from all the infants on days 1, 7, 14, and 28 after birth. The serum samples were transferred to Eppendorf tubes and stored at −20° C until assayed. Concentrations of IGF-1 and IL-18 were detected using commercial enzyme-linked immunoassay (ELISA) according to the manual instructions strictly.

Statistical analysis

Refer to the relevant literature (18), the sample size was calculated based on the mean serum value of IL-18 and IGF-1

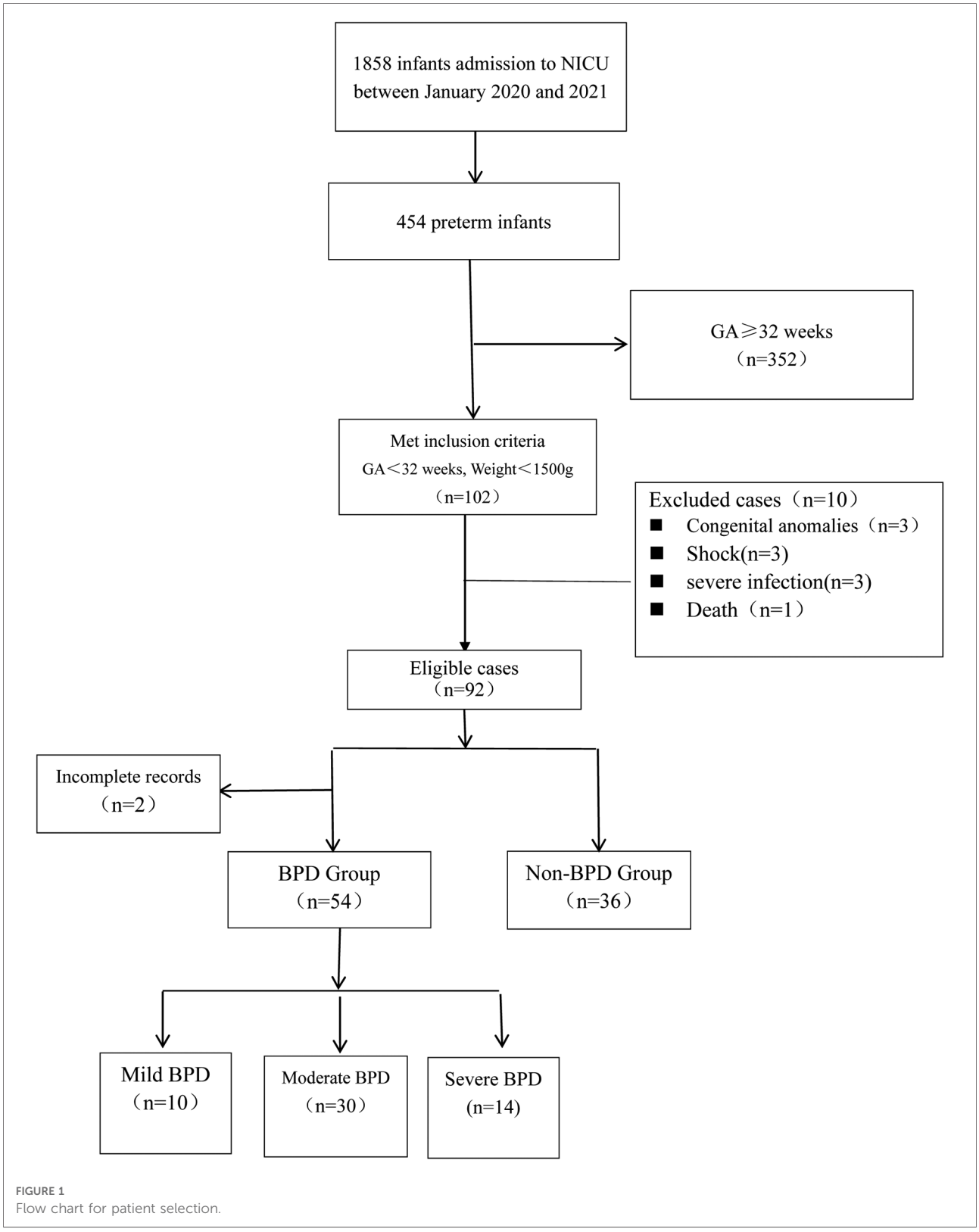

FIGURE 1
Flow chart for patient selection.

(213, 37), and the estimation was conduct with G*Power 3.1.7, with 75% power and a 2-sided α of 0.05 considering clinically significant. The finally required number of samples was about 88.

Statistical analysis was performed using SPSS24.0 statistical software. All quantitative variables were assessed for the normality test and described as the means ± standard deviations ($\bar{x} \pm s$) if conformed to a normal distribution. All qualitative variables were described as frequencies or percentages. For comparisons between two groups, the Student t-test was adopted for normally distributed variables, and the Mann-Whitney U test was used when the normality test failed. In the multiple-group comparisons, repeated measurement one-way analysis of variance (ANOVA) was adopted for normally distributed variables, and the non-parametric Kruskal-Wallis test was used when the normality test failed. Diagnostic efficacy for predicting the risk of BPD was evaluated by the receiver operating characteristic (ROC) analysis (AUROC of 0.5 indicates no capacity for differentiation and 1.0 indicates perfect differentiation). $P < 0.05$ was considered statistically significant in all tests.

Results

Patient characteristics

In the recruitment, the information of 102 patients was obtained from The First People's Hospital of Yinchuan and 90 premature infants were finally enrolled in the study (**Figure 1**). In the BPD group, 54 newborns were diagnosed with BPD, including 33 males (61.1%) and 21 females (38.9%), with a mean birth weight of 1332 ± 321.5 grams and a mean gestational age of 30.18 ± 1.36 weeks, of which 10 infants were categorized as mild BPD and 30 infants were categorized as moderate BPD and the other 14 infants were categorized as severe BPD. 36 non-BPD premature infants were included in the control group, with a mean birth weight of 1396 ± 237.8 grams and a mean gestational age of 30.28 ± 1.34 weeks. No significant difference in demographic data at birth was reported between the two groups ($P > 0.05$). The characteristics of the population are presented in **Table 1**.

TABLE 1 Baseline characteristics of included 90 infants.

Variable	BPD ($n = 54$)	Non-BPD ($n = 36$)	*P*
Infant characteristics			
Birth weight (gram), mean (SD)	1332 ± 321.5	1396 ± 237.8	0.31
Gestational age (week), mean (SD)	30.18 ± 1.36	30.28 ± 1.34	0.73
Gender (male), *n* (%)	33 (61.1)	18 (50)	0.3
Apgar 1min, median (IQR)	4 (0-8)	5 (0-9)	0.07
Apgar 5min, median (IQR)	6 (3-9)	7 (3-10)	0.065
Patent ductus arteriosus, *n* (%)	27 (50)	9 (25)	0.018
Late-onset neonatal sepsis, *n* (%)	14 (25.9)	8 (22.2)	0.69
Necrotizing enterocolitis, *n* (%)	3 (5.5)	1 (2.7)	0.65
Maternal characteristics			
Chorioamnionitis, *n* (%)	12 (22.2)	4 (11.1)	0.18
Premature rupture of membranes, *n* (%)	15 (27.7)	6 (16.7)	0.22
Antenatal steroids, *n* (%)	27 (50)	28 (77.8)	0.008
Gestational hypertension, *n* (%)	6 (11.1)	2 (5.5)	0.47
Gestational diabetes, *n* (%)	4 (7.4)	3 (8.3)	1

Serum IGF-1 concentrations in the BPD group and the non-BPD group

The concentrations of IGF-1 in serum samples in both the BPD and the non-BPD groups reached the peak value on day 1 and gradually decreased after day 1. The significantly lower level of IGF-1 in infants in the BPD group was always observed than that in the non-group on days 1, 7, 14, and 28 ($P < 0.05$). In the BPD group, the highest serum level of IGF-1 was on day 1 and the lowest was on day 14, and the value on days 7 and 14 were significantly lower than that on day 1 ($P < 0.05$). In the non-BPD group, the serum IGF-1 level reached the highest on day 1 and the lowest on day 7, and the value on days 7, 14, and 28 were significantly lower than that on day 1 ($P < 0.05$). All the details were shown in **Figures 2**.

Serum IGF-1 concentrations in infants with different severity of BPD

As shown in **Figures 3**, the concentrations of IGF-1 in serum samples from infants with different severity of BPD were always different on days 1, 7, 14, and 28, and infants diagnosed with severe BPD were significantly associated with a lower level of IGF-1 than those with mild BPD ($P < 0.05$).

Serum il-18 concentrations in the BPD group and the non-BPD group

The concentration of IL-18 in the serum samples from both the BPD group and the non-BPD group shared the same trend: the lowest value was on day 1, increased and reached the peak value on day 14, and then decreased gradually. Significantly higher concentration of serum IL-18 in the BPD group was always observed than that in the non-BPD group on days 1, 7, 14, and 28 ($P < 0.05$). In the BPD group, the serum IL-18

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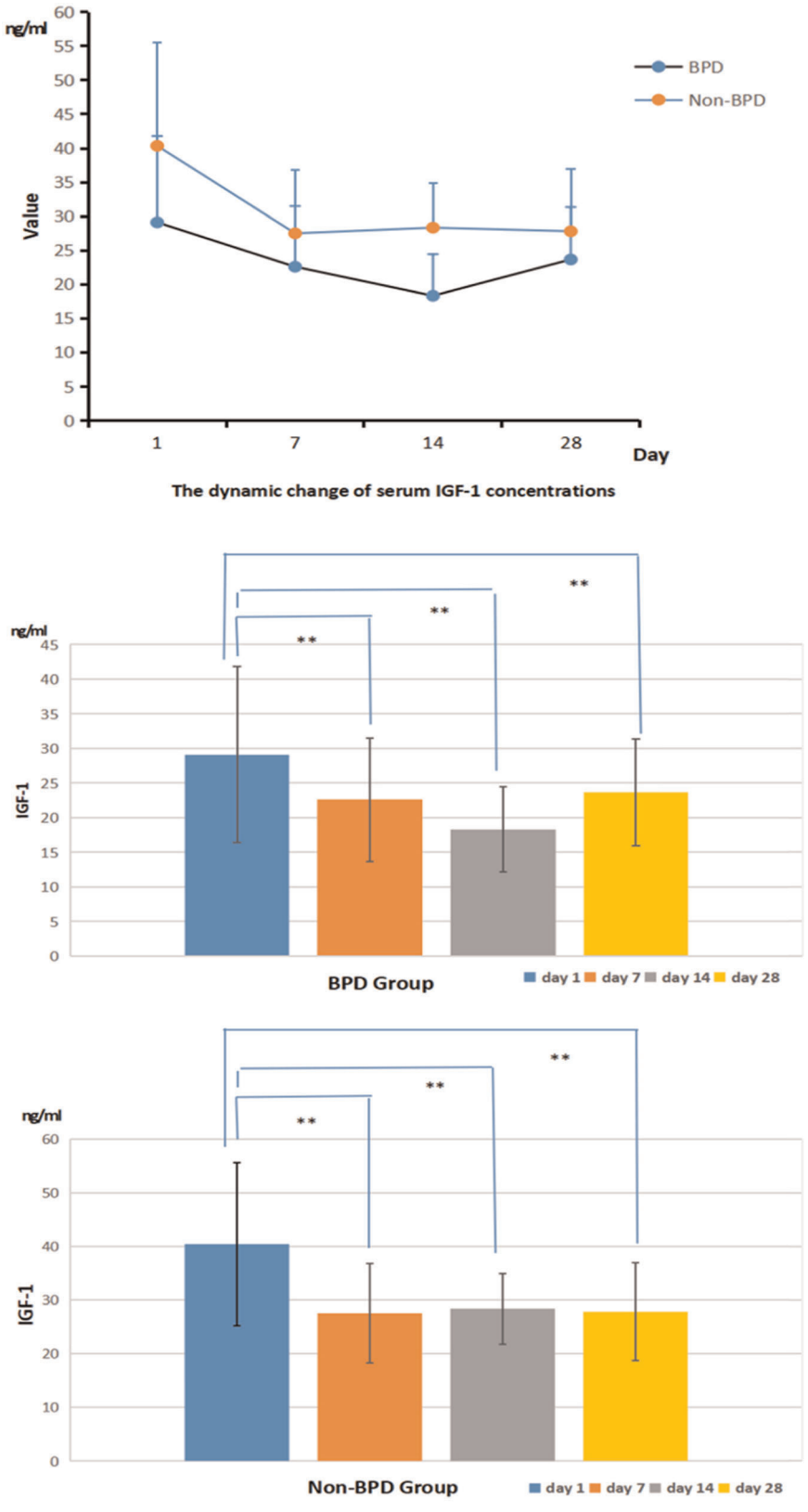

FIGURE 2
The dynamic serum IGF-1 concentrations in the BPD and the non-BPD group (ng/ml).

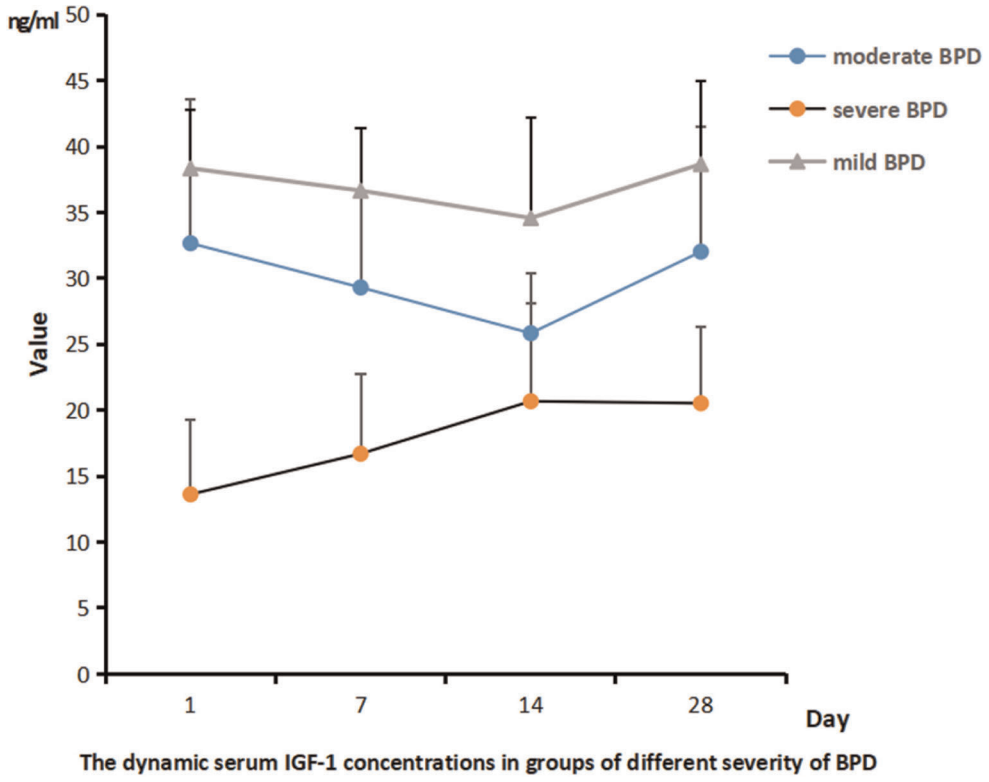

FIGURE 3
The dynamic serum IGF-1 concentrations in groups of different severity of BPD.

concentration on day 1 was significantly lower than that on days 7, 14, and 28 ($P < 0.05$). In the non-BPD group, the serum IL-18 concentration on day 14 was significantly higher than that on day 1 ($P < 0.05$). Details were shown in **Figures 4**.

Serum il-18 concentrations in infants with different severity of BPD

As shown in **Figures 5**, the serum level of IL-18 in infants with different severity of BPD were always different on days 1, 7, 14, and 28, and infants with severe BPD were significantly associated with a higher serum level of IL-18 than those with mild BPD ($P < 0.05$).

The diagnostic value of serum IGF-1 for BPD

With the ROC curve analysis, the diagnostic value of serum IGF-1 for BPD at different time points were shown in **Table 2** and **Figure 6A**. Serum IGF-1 concentrations on day 14 had a good predictive power with a cut-off value of 23.4 ng/ml (AUC: 0.871, sensitivity: 89.29%, specificity: 77.78%, $P < 0.0001$).

The diagnostic value of serum il-18 for BPD

Table 3 and **Figure 6B** demonstrated the results of serum IL-18 for BPD prediction at different time points. Serum IL-18 concentrations on day 28 had a good predictive power with a cut-off value of 248 ng/ml (AUC: 0.7837, sensitivity: 53.57%, specificity: 83.33%, $P < 0.05$).

The diagnostic value of IGF-1 combined with il-18 for predicting BPD

For a better prediction of BPD, a combined analysis of serum IGF-1 concentration and serum IL-18 concentration on day 14 was conducted in **Table 4** and **Figure 6C**: the sensitivity was 91.07% and the specificity was 83.33%, with the AUC of 0.9142, which demonstrated more substantial predictive power than that in a single variable included analysis.

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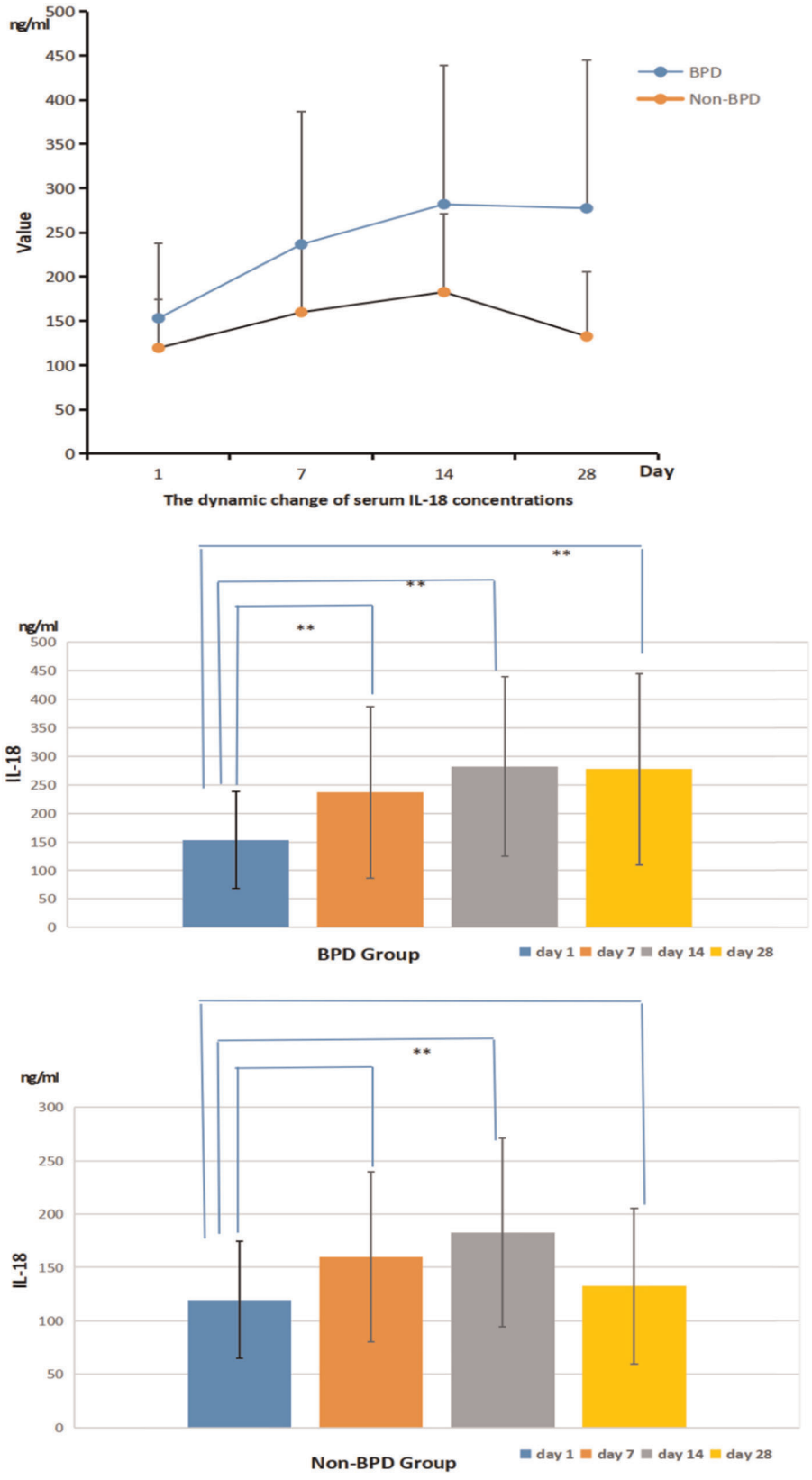

FIGURE 4
The dynamic serum IL-18 concentrations in the BPD and non-BPD group (ng/ml).

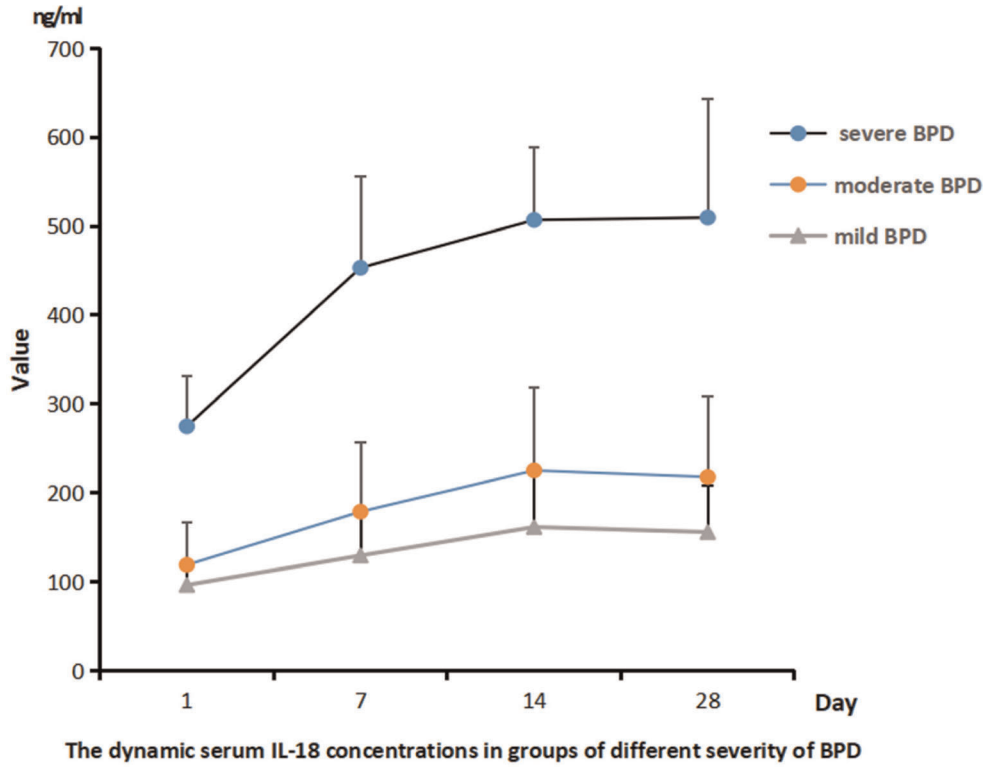

FIGURE 5
The dynamic serum IL-18 concentrations in groups with different severity of BPD (ng/ml).

TABLE 2 Diagnostic value of IGF-1 for predicting BPD at different time points.

Index	AUC	Sensitivity (%)	Specificity (%)	Cut-off value	*P*
Day 1	0.7475	87.5	74.75	36.43	<0.0001
Day 7	0.6915	73.21	63.89	24.12	0.002
Day 14	0.871	89.29	77.78	23.4	<0.0001
Day 28	0.626	57.14	62.60	24.33	<0.0001

Discussion

BPD is a chronic lung disease in premature infants, which has been increasingly recognized to result from pathological repair responses to prenatal and postnatal injuries during lung maturation. Various patterns of prenatal stress combined with different postnatal injuries may significantly impair the development of the airways, lung parenchyma, pulmonary interstitial, lymphatic system, and pulmonary vessels. In infants with severe BPD, cessation of alveolarization may persist into late childhood and lead to permanent injuries. Currently, lung inflammation has been considered central to many theories of etiology and pathogenesis of BPD. Chorioamnionitis, oxygen toxicity, mechanical ventilation, and postnatal infections can induce inflammatory responses in the immature airways and lung tissue (19). IGF-1 has been confirmed to involve in the growth and injury repair processes of many organs, including the lung (20). A previous study reported the positive expression of IGF-1 and its receptor in the processes of injury and repair of alveolar type II cells, and thus hypothesized that IGF-1 might play an important role in the regulation of proliferation and differentiation of alveolar epithelial cells (21). IGF-1 is a downstream factor of the vascular endothelial growth factor (VEGF) signaling pathway, which can participate in the occurrence and progression of BPD by affecting alveolar microvascular formation (22). Some evidence put forward that IGF-1 might have the potential to modulate neonatal immune responses, and the expression of IGF-1 and its receptors could promote the development and maturation of the immune system (23). IGF-1 may also influence immune system homeostasis by promoting lymphangiogenesis and cell proliferation and differentiation, and participate in inflammation by stimulating inflammatory cytokines and chemokines as a pro-inflammatory factor.

In this study, the serum IGF-1 concentration was reported highest on the first day after birth and gradually decreased as

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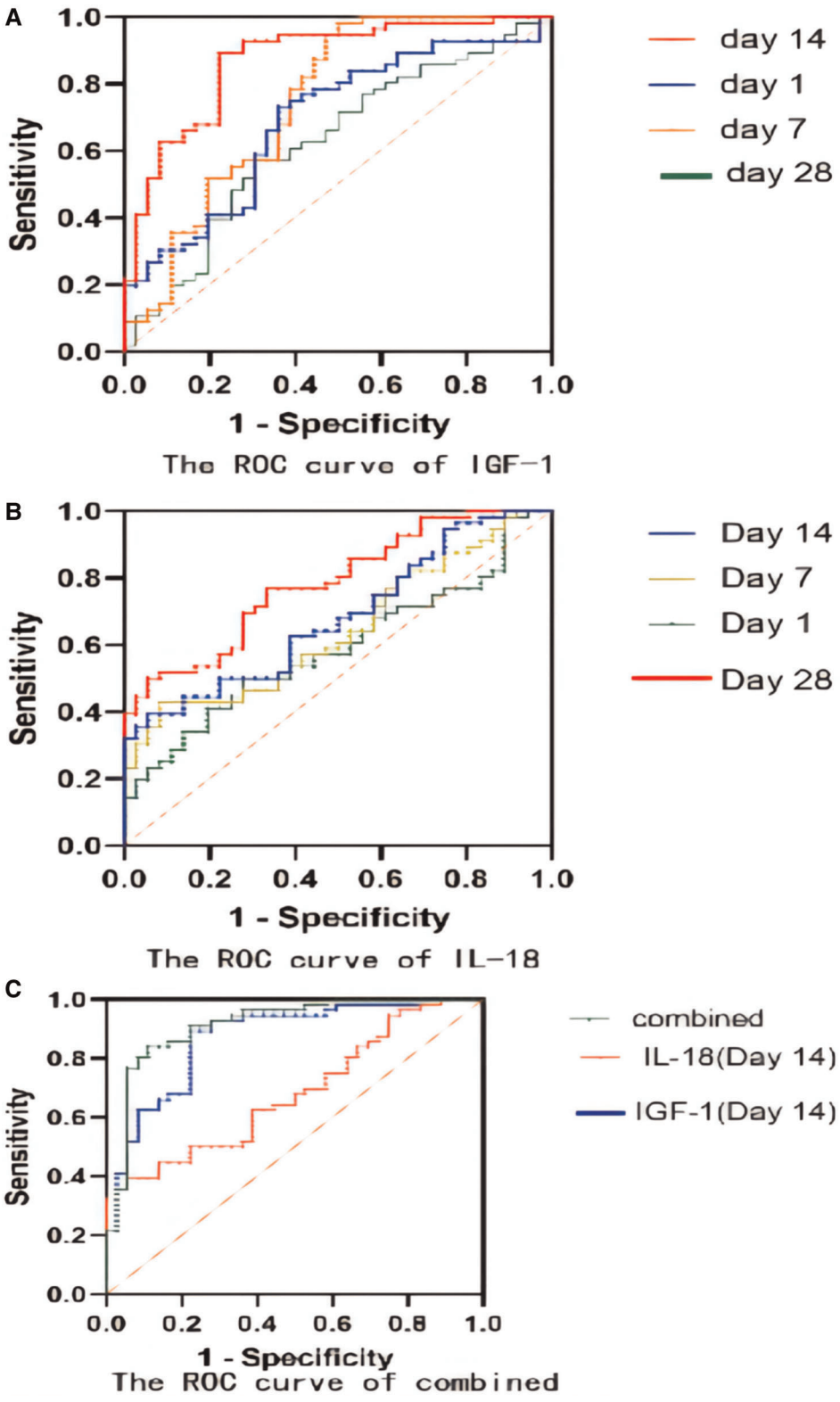

FIGURE 6
(**A**) The ROC curve of IGF-1 for BPD prediction; (**B**) The ROC curve of IL-18 for BPD prediction; (**C**) The ROC curve of IGF-1 combined with IL-18 for BPD prediction.

TABLE 3 Diagnostic value of IL-18 for predicting BPD at different time points.

Index	AUC	Sensitivity (%)	Specificity (%)	Cut-off value	*P*
Day 1	0.5903	50	72.22	135.1	0.1454
Day 7	0.6443	42.86	91.67	243.3	0.0199
Day 14	0.6815	51.79	77.78	314.9	0.0034
Day 28	0.7837	53.57	83.33	248	0.04

TABLE 4 The diagnostic value of IGF-1 combined with IL-18 for predicting BPD.

Index	AUC	Sensitivity (%)	Specificity (%)	*P*
IGF-1 (day 14)	0.871	89.29	77.78	<0.0001
IL-18 (day 14)	0.6815	51.79	77.78	0.0034
Combined	0.9142	91.07	83.33	<0.0001

time went by. Infants diagnosed with BPD were significantly associated with a lower serum IGF-1 level than those in the non-BPD group. The relationship between the severity of BPD and IGF-1 was also preliminarily analyzed and the result showed that infants with severe BPD usually had a lower expression IGF-1 level than those with mild BPD. The ROC analysis reported the highest sensitivity and specificity to predict the risk of BPD using the serum IGF-1 level on day 14. IL-18, a member of the IL-1 cytokine family, is a key pro-inflammatory mediator with particular importance in pulmonary infections and inflammation. IL-18 has been proposed as a novel biomarker for human ARDS, and its plasma level has been proved to correlate with ARDS severity and mortality. The organism can activate the NLRP3 inflammasome in response to infection, tissue damage, and oxidative stress, and then this activated complex transforms the cystatin-1 precursor into an active 20 kDa fragment that enables to promote of the IL-18 precursor into mature IL-18 and facilitate its release, which is considered significant in the initiation of inflammation (24). A growing number of studies have revealed that activation of the nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome is related to the pathogenesis of acute lung injury (25, 26). The activated NLRP3 inflammasome functions as a supramolecular platform for the caspase-1–dependent maturation and secretion of the proinflammatory cytokines IL-1β and IL-18 in macrophages (27, 28). Liao's research showed that NLRP3 activation is one of the primary causes of BPD (29). As our results shown, the concentration of IL-18 in the serum samples from both the BPD group and the non-BPD group shared the same trend: the lowest was on day 1, increased and reached the peak value on day 14, and then decreased gradually. The serum IL-18 concentration of the BPD group was significantly higher than that of the non-BPD group, and it also seemed to positively correlate with the clinical severity of BPD. Through the ROC analysis, the serum concentration of IGF-1 on day 14 and Il-18 on day 28 showed high sensitivity and specificity when predicting the risk of BPD respectively, which suggested their potential value in the diagnosis of BPD. With the combined analysis of IGF-1 on day 14 and IL-18 on day 14, the result reported the highest sensitivity (91.07%) and specificity (83.33%), also with the highest AUC (0.9142), which was superior to the prediction by IGF-1 and IL-18 alone. Compared with previous studies (30), it reported higher specificity and sensitivity. Consequently, the serum level of IGF-1 combined with the IL-18 may be more effective when establishing a predictive model of BPD. It is very convenient to collect sample from children clinically, and the detection of IL-18 and IGF-1 is also easy to conduct, which is feasible in clinical application.

However, this study has some limitations. The number of included patients was relatively small. Moreover, it was a single-center study, lacking multicenter clinical data support. Although it reported high sensitivity and specificity for predicting BPD, there is still a need for multicenter studies with larger sample sizes to improve the statistical power.

In conclusion, both IGF-1 and IL-18 might be closely involved in the occurrence and development of BPD. The decreased serum IGF-1 level and increased IL-18 level in preterm infants might be significantly associated with the severity of BPD. The serum concentration of IGF-1 combined with IL-18 could be potentially sensitive biomarkers for BPD prediction, but its diagnostic value still needs to be further verified in larger sample size research.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of The First People's Hospital of Yinchuan. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

YS had primary responsibility for protocol development, patient screening, enrollment, outcome assessment and preliminary data analysis. NG and MC collected the clinical data and measured the biological samples. LH and JW

designed the experiment, analyzed the data, and finished the manuscript. All the authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.1013537/full#supplementary-material.

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EDITED BY
Xiaoguang Zhou,
Nanjing Children's Hospital, China

REVIEWED BY
Christoph Bührer,
Charité Universitätsmedizin Berlin, Germany
Chung-Ming Chen,
Taipei Medical University, Taiwan

*CORRESPONDENCE
Lawrence Rhein
Lawrence.Rhein@umassmemorial.org

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Respiratory support strategies in the prevention of bronchopulmonary dysplasia: A single center quality improvement initiative

Heather White[1], Kamaris Merritt[1], Kirsti Martin[1], Emily Lauer[2,3] and Lawrence Rhein[1]*

[1]Division of Neonatology, Department of Pediatrics, University of Massachusetts Memorial Medical Center, Worcester, MA, United States, [2]Department of Family Medicine and Community Health, University of Massachusetts Chan Medical School, Worcester, MA, United States, [3]Eunice Kennedy Shriver Center, University of Massachusetts Chan Medical School, Worcester, MA, United States

Background and objectives: Bronchopulmonary dysplasia (BPD) continues to be a significant morbidity affecting very preterm infants, despite multiple advancements in therapies to treat respiratory distress syndrome and prevent BPD. Local quality improvement (QI) efforts have shown promise in reducing unit or system-wide rates of BPD. In preterm infants born between 23- and 32-weeks' gestation, our aim was to decrease the rate of BPD at 36 weeks corrected gestational age from 43% to 28% by January 2019.

Methods: Directed by a multidisciplinary respiratory QI team, we gradually implemented the following interventions to reach our aim: (1) early initiation of non-invasive ventilation in the delivery room, (2) initiation of caffeine prior to 24 h of life, (3) administration of early selective surfactant per a well-defined guideline, (4) continuation of non-invasive ventilation until 32 and 0/7 weeks corrected gestational age (CGA), and (5) a revision of the early selective surfactant guideline. Outcome measures included rates of BPD, and process measures included compliance with the above interventions.

Results: A total of 509 infants with an average gestational age of 29 1/7 weeks and birth weight of 1,254 (SD±401) grams were included. The rate of BPD in our unit decreased from a baseline of 43% to 19% from the start of the project in October 2016 until the first quarter of 2022 ($p < 0.00001$). The greatest reductions in BPD rates were seen after the initiation of the guideline to extend non-invasive ventilation until 32 0/7 weeks CGA. The rate of severe BPD decreased from 22% to 9%.

Conclusions: In preterm infants born between 23- and 32-weeks' gestation, our local QI interventions to reduce rates of BPD were associated with a reduction in rates by 56%. Increased use of antenatal steroids and higher birth weights post- vs. pre-intervention may have contributed to this successes.

KEYWORDS

bronchopulmonar dysplasia, surfactant, caffeine, non-invasive ventilation (NIV), quality improvement - outcomes

Introduction

Over the last two decades, survival without significant morbidity among very low birthweight (VLBW) infants has improved (1, 2). Steady decreases in the rate of morbidities such as severe intraventricular hemorrhage, retinopathy of prematurity, late onset sepsis, and necrotizing enterocolitis have been observed, yet rates of bronchopulmonary dysplasia (BPD) have remained consistent (1–3). Despite critical advances in treating respiratory distress syndrome (RDS) and BPD, almost half of surviving extremely preterm infants will develop BPD. The most widely accepted definition for BPD is an oxygen requirement at 36 weeks corrected gestational age (CGA) (4, 5).

While there has been extensive research regarding individual treatment strategies for preventing or reducing BPD, including use of caffeine (6, 7), early non-invasive respiratory support (8), and early selective surfactant administration (9), information regarding how individual neonatal intensive care units (NICUs) can implement these therapies to optimize local outcomes has only recently begun to emerge, in the form of published Quality Improvement (QI) efforts (10). Continued description of QI efforts may provide other NICUs with new ideas and strategies they can use in the quest to prevent and reduce BPD in their own unit.

In this article, we will describe the gradual implementation of the components of our unit's BPD Prevention Quality Improvement Bundle, focusing on optimizing our delivery room (DR) respiratory support strategies and early NICU course management. The initial intervention was a "DR Bundle" consisting of early initiation of non-invasive ventilation in the DR, initiation of caffeine prior to 24 h of life, and administration of early selective surfactant per a well-defined guideline. Subsequent interventions included continuation of non-invasive ventilation until 32 and 0/7 weeks corrected gestational age (CGA) and a revision of the early selective surfactant guideline. This initiative was conducted at The University of Massachusetts Chan Medical School and UMass Memorial Medical Center (UMMMC) NICU.

Aims

The goal of our initiative was to optimize our DR resuscitation guidelines and early neonatal course management for infants born at 32 0/7 weeks gestation or less, in order to decrease the incidence of BPD in our unit. During the UMMMC NICU's annual review of respiratory outcomes in late 2016, our BPD committee and QI team evaluated the respiratory management and BPD incidence rates of infants born ≤32 0/7 weeks previously admitted to our unit. At 36 0/7 weeks corrected gestational age (CGA), the BPD rate for infants born ≤32 0/7 was 43%, with rates in some quarters (3-month intervals) over 50%. Our team defined BPD as an oxygen requirement at 36 0/7 weeks CGA using the National Institute of Child Health and Human Development/National Heart, Lung and Blood Institute (NICHD/NHLBI) 2001 definition (4). The global aim of our ongoing respiratory QI initiative is to improve respiratory outcomes for preterm infants by decreasing our unit's BPD rate by 35%. Key drivers for this global aim are illustrated in the key driver diagram shown in **Supplementary Figure S1**.

As the first step in attempting to reach our global aim, our QI team targeted a SMART Aim to increase our compliance with our BPD Bundle guideline elements at birth to minimize lung injury from 43% to 90% by January 1, 2018. We set outcome measure goals for each of our interventions, keeping track of compliance with each intervention. Balancing measures of pneumothoraxes and ventilator utilization were also monitored during the project.

Methods

UMMMC is a 49-bed Level III NICU in Central Massachusetts with approximately 4500 deliveries and 650 admissions annually. The NICU serves as the only tertiary care center in Central Massachusetts and runs an active neonatal transport program serving this region. The NICU admits approximately 100 infants born under 32 0/7 weeks and 120 very low birth weight (VLBW) infants annually. The UMMMC NICU is in an academic center with a multidisciplinary team consisting of 7 neonatologists, 12 neonatal nurse practitioners, 1 physician assistant, 3 neonatal fellows, 2–4 rotating resident physicians per month, 7 respiratory therapists, 1 neonatal pharmacist, 1 full-time neonatal dietician, and 150 staff nurses that participate in carrying out the respiratory management of infants in the NICU. To change practice and sustain change over time, we therefore estimate that the number needed to influence is approximately 200 staff members. This work was reviewed by the Institutional Review Board of the UMass Chan Medical School and was determined to be exempt from full IRB review.

In October 2016, a multidisciplinary respiratory QI team was formed to assess the state of respiratory practice in the UMMMC NICU. Access to specific respiratory guidelines for infants born ≤32 0/7 weeks gestation prior to this time were not available. The team specifically tailored data collection tools to determine our unit's baseline BPD rate and to identify areas of potential focus.

In January 2017, a "Minimizing Lung Injury" delivery room bundle was introduced, which included (a) guidelines for early initiation of non-invasive ventilation in the DR, in order to avoid intubation for mechanical ventilation, (b) early surfactant administration for qualifying infants, and (c) early

administration of caffeine to prevent apnea of prematurity (**Supplementary Figure S2**).

Per the DR Bundle flow diagram, NICU providers were instructed to initially place infants born between 23 0/7 and 26 6/7 weeks on noninvasive positive pressure ventilation (NIPPV) *via* NeoTech RAM cannula (NeoTech Products, Valencia, CA, United States) at a positive inspiratory pressure (PIP) of 25 centimeters of water (cm H_20), positive end expiratory pressure (PEEP) of 7 cm H_20, and rate of 30 breaths per minute within 2 min of life. Infants born between 27 0/7 and 32 0/7 weeks were to be placed on continuous positive airway pressure (CPAP) *via* RAM cannula at PEEP of 6 to 8 cm H_20 within 2 min of life. If infants showed signs of increased work of breathing, apnea, or bradycardia, a trial of NIPPV was attempted, to avoid intubation for mechanical ventilation. Non-invasive ventilation was delivered *via* a NeoPuff T-piece resuscitator (Fisher & Paykel Healthcare, Auckland, New Zealand). Infants who required intubation in the DR due to lack of respiratory effect per the Neonatal Resuscitation Protocol were to be given surfactant within two hours of life. Infants who were maintained on non-invasive ventilation who had an FiO_2 requirement of greater than 40%, significant increased work of breathing with moderate to severe retractions and/or respiratory rate >80 times per minute were to receive surfactant *via* the INSURE method by 90 min of life. All infants ≤32 0/7 weeks were ordered to start caffeine at a dose of 20 mg/kg and were then maintained on daily caffeine doses of 10 mg/kg. Doses were given intravenously until infants were advanced to full enteral feeds and no longer had IV access.

We initiated these bundle guidelines *via* several plan-do-study-act (PDSA) cycles, including placing a large poster of the DR Bundle in the resuscitation room outside of the delivery/operating room, as well as provider and respiratory therapist training on T-piece resuscitator use. In conjunction with the roll out of a new electronic medical record system in October 2017, order sets for caffeine were implemented to ensure compliance with the early caffeine guideline. Since the interventions were focused on developing protocols, all PDSAs involved our multidisciplinary BPD committee members and were tested by all key disciplines for feedback prior to initiation and during implementation. We set outcome measure goals to have 90% compliance with each element of the DR bundle by January 1, 2018.

After further data collection and QI work, our BPD committee reconvened in December 2017 to review our respiratory outcomes. Although we noted improvements in the BPD rate, our NICU still had not reached our SMART aim of reducing BPD rates by 35%. In January 2018, we implemented a new guideline advising that all infants born <32 0/7 weeks gestation remain on non-invasive ventilation until at least 32 0/7 weeks CGA. This guideline was based on animal models showing that CPAP and resultant functional residual capacity (FRC) maintained by mechanical lung distention promoted lung development by providing stretch to the pulmonary tissue, and important stimulus for lung growth (11). We hoped that this strategy would not only promote further lung growth in our population, but also maintain FRC to prevent alveolar atelectasis that otherwise may have necessitated an escalation of respiratory support once CPAP was removed. This guideline was subsequently supported by the results of a study by Lam et al. which showed a greater increase in FRC after two weeks of continued CPAP and at hospital discharge for infants who were randomized to extended CPAP when compared to those randomized to usual CPAP discontinuation (12). We set outcome measure goals to have 90% compliance with continued non-invasive ventilation by January 1, 2019.

In January 2020, an internal audit on our unit's surfactant utilization and timing of administration identified that improvements could be made in identifying infants who would likely benefit from early selective surfactant and administering surfactant prior to two hours of life. At this time, we took on another PDSA cycle, developing a guideline aimed at early identification of these infants and the timely administration of surfactant. Criteria for early identification and administration of surfactant included assessment of each infant's gestational age, respiratory support in the DR and at 90 min of age (including modality and pressure used), FiO2 requirement, and physical exam/lab findings concerning for significant respiratory distress (**Supplementary Figure S3**). Infants who required intubation in the DR for resuscitation were to be given surfactant while they were intubated unless the need for intubation was deemed to be due to respiratory depression attributable to other factors such as maternal magnesium infusion. Surfactant was to be delivered *via* the INSURE method for infants who remained on non-invasive ventilation but met criteria for early selective surfactant administration (13). This guideline was implemented starting in August 2020. We set outcome measure goals to have 90% compliance with our new early selective surfactant guideline by August 1, 2021.

Measures

Clinical data was abstracted from the infants' electronic medical records, including respiratory support required in the delivery room, timing of first caffeine administration, timing of surfactant administration, days of invasive mechanical ventilation, last day of non-invasive ventilation, post-natal steroid administration, BPD status, presence of pneumothorax, length of stay, corrected gestational age at discharge, and discharge home on oxygen therapy. Demographic data was also collected, including gender, birth weight, gestational age, race/ethnicity, maternal age, and antenatal steroid status. Data

for outborn infants was collected but excluded for purposes of this analysis due to inability of these infants to consistently receive our DR Bundle at delivery. Infants who did not survive to 36 weeks CGA were also excluded from the analysis. Data was collected monthly for each infant and analyzed by quarter (3-month interval).

The primary outcome measure was the percentage of inborn infants born ≤32 0/7 who developed moderate or severe BPD per the NICHD/NHLBI 2001 consensus definition (infants requiring oxygen or positive pressure at 36 weeks CGA) (4, 5). Infants with mild BPD were not included as having BPD in this measure, as these infants did not require supplemental oxygen at 36 weeks CGA. A process control chart was utilized to track changes in the BPD rate in relation to the interventions throughout the project period.

Process measures included compliance with DR bundle interventions (measured separately as receipt of early non-invasive respiratory support in the delivery room, administration of caffeine prior to 24 h of life, and administration of early selective surfactant at less than two hours of life), compliance with continued CPAP until 32 0/7 weeks PMA, and compliance with the August 2020 revised early selective surfactant guideline. For the purposes of this analysis, infants who continued CPAP until 31 6/7 weeks CGA were considered compliant as infants often were removed from CPAP during evening rounds, within 4 h of turning 32 0/7 CGA. Overall compliance with the BPD Prevention QI bundle was also measured; compliance with protocol definitions changed with each additional guideline and PDSA cycle. Compliance rates for each guideline were analyzed using *P* charts for data involving classification of compliant vs. non-compliant. The balancing measure of days of invasive mechanical ventilation by patient were analyzed using a C chart. The balancing measure of pneumothorax rate was not placed into a *P* chart due to its low occurrence. Charts were prepared using Macros (KnowWare International, Inc, Denver, CO, United States) add-on in Microsoft Excel (Microsoft Corporation, Redmond, WA, United States) to display and analyze data over time. The center line (CL) was defined as the running average for 8-consecutive points on the same side of the CL, or successive increasing or decreasing points. The upper control limit (UCL) and lower control limit (LCL) define two of three points beyond 2 standard deviations from the mean on the same side of the CL (14).

Data was analyzed in four columns representing the four epochs before and after implementation of QI measures. Differences between groups on key demographic factors of mothers and infants were assessed with Friedman ANOVAs as shown in **Table 1**. Any demographics showing significant differences ($p < 0.05$) between groups were then analyzed with either Chi-squared or Fisher's exact tests for categorical variables, or post-hoc Mann Whitney *U* tests for continuous variables. Similarly, the association between groups and outcomes of BPD, death, death or BPD, invasive mechanical ventilation, postnatal steroid use and pneumothorax were first analyzed with Friedman ANOVAs as shown in **Table 2**. Specific comparisons between each set of groups were then assessed with the same tests as the demographic variables. In addition, logistic regressions were used selectively to look at the relationships between key findings based on the outcome of the group comparisons to test whether the group association with BPD as an outcome changed when controlling for particular factors as described in results. Bivariate and multivariable statistics were calculated with SAS 9.4 (Cary, NC, United States).

Results

A total of 611 infants were born between 23 0/7 and 32 0/7 weeks gestational age (GA) and admitted to the UMMMC NICU during the baseline and PDSA cycle periods. Cohort demographics are presented in **Table 1**. Significant differences included lower instances of maternal hypertension and higher utilization of full course antenatal steroids in later cohorts compared to baseline. Despite significance in group differences in an overall ANOVA test, Chi-squared tests for specific group differences did not indicate any significant differences in the number of Hispanic infants between specific groups. There was only a significant difference in median birth weights between the second intervention group (CPAP Until 32 CGA) and baseline ($p = 0.0055$). All other group comparisons on birthweights did not show differences in Mann Whitney *U* tests.

Following the roll out of the DR Bundle in January 2017, the rate of infants receiving early non-invasive ventilation in the delivery room quickly increased from a baseline of 51% to 87% (**Figure 1**). Further increases in compliance with this guideline were seen after four straight quarters of 100% compliance from Q4 2019 to Q3 2020, allowing the percent compliance to increase to 93%, past our goal of 90%. Compliance with initiation of caffeine within 24 h of life as part of this DR bundle saw similar improvements, increasing from a pre-intervention baseline of 32% to 86%. Compliance with this guideline increased past the goal of 90% to 94% in the quarters following the initiation of continued non-invasive ventilation until 32 0/7 weeks PMA (**Figure 2**).

Following PDSA cycle evaluations for BPD rates after our initial DR bundle intervention, we implemented the continued non-invasive ventilation until 32 0/7 weeks CGA guideline. Compliance rates for this guideline increased from 55% to 83% after implementation. After adjusting for infants who were taken off of non-invasive ventilation on night rounds at 31 6/7 weeks, just hours from turning 32 0/7, compliance increased to 97%, surpassing the goal of 90% (**Figure 3**).

TABLE 1 Cohort demographics Pre- and post-quality improvement initiative implementation.

		Pre-Delivery Room Bundle Baseline (Q1 2016-Q4 2016) *N* = 86	Post-Delivery Room Bundle and Other QI Interventions			
			DR Bundle Implementation (Q1 2017-Q4 2017) *N* = 109	CPAP Until 32 0/7 CGA (Q1 2018- Q2 2020) *N* = 249	Surfactant Guideline Revision (Q3 2020-Q1 2021) *N* = 167	
		N (%)				*p*-Value
Maternal Characteristics						
Chorioamnionitis		3 (4)	2 (2)	8 (3)	10 (6)	0.1239
Hypertension		40 (47)	41 (38)	70 (28)	56 (34)	*0.0497
Diabetes Mellitus		10 (12)	9 (8)	34 (14)	28 (17)	0.0664
Delivery Mode						
Vaginal Delivery		32 (37)	37 (34)	83 (33)	54 (32)	0.4895
C-section		54 (63)	72 (66)	166 (67)	113 (68)	
Antenatal Steroids						
Full course		49 (57)	59 (54)	152 (61)	110 (66)	*0.0012
Partial course		16 (19)	26 (24)	77 (31)	39 (23)	
No steroids		21 (24)	24 (22)	20 (8)	18 (11)	
Infant Characteristics						
Gender, Male		50 (58)	57 (52)	129 (52)	91 (55)	0.8008
Ethnicity						
Hispanic Latino		19 (22)	22 (20)	53 (21)	46 (28)	0.0074
Not specified		2 (2)	4 (4)	0 (0)	0 (0)	
Race						
Asian		1 (1)	4 (4)	14 (6)	9 (6)	0.1426
Black or African American		12 (14)	14 (13)	34 (14)	24 (14)	0.8097
White		62 (72)	72 (66)	158 (63)	107 (64)	0.2675
Inborn		76 (88)	100 (92)	228 (92)	159 (95)	0.0675
Outborn		10 (12)	9 (8)	21 (8)	8 (5)	
Death		8 (9)	10 (9)	25 (10)	18 (11)	0.6317
Maternal Age (yrs)	Median	30	30	31	31	
	Mean	29.8	30.6	30.3	30.9	
	Std. Dev	5.5	6.1	5.5	6.1	
Gestational Age (wks)	Median	29.3	29.3	29.7	29.4	
	Mean	28.5	28.9	28.9	29.0	
	Std. Dev	2.7	2.3	2.6	2.5	
Infant Birthweight (g)	Median	1120	1225	1263.5	1340	*0.0055
	Mean	1168	1221.8	1257.7	1320.1	
	Std. Dev	442.2	357.3	401.9	401.2	

*p-value of ≤0.05 was considered significantly significant.
No significant differences between groups in Mann–Whitney U test (two-sided p-value).
Chi squared test used for Ethnicity, hypertension and antenatal steroids given variable structure.

TABLE 2 Respiratory outcomes Pre- and post-quality improvement initiative implementation.

		Pre-Delivery Room Bundle Baseline (Q1 2016-Q4 2016) *N* = 86 *N (%)*	Post-Delivery Room Bundle and Other QI Interventions			*p*-Value
			DR Bundle Implementation (Q1 2017-Q4 2017) *N* = 109	CPAP Until 32 0/7 CGA (Q1 2018-Q2 2020) *N* = 249	Surfactant Guideline Revision (Q3 2020-Q1 2021) *N* = 167	
Moderate to Severe BPD		33 (38)	29 (27)	58 (23)	25 (15)	<0.0001*
Death		8 (9)	10 (9)	25 (10)	18 (11)	0.6317
Composite Moderate to Severe BPD or Death		41 (48)	39 (36)	83 (33)	43 (26)	0.0009*
Required Invasive Mechanical Ventilation		45 (52)	26 (24)	43 (17)	45 (27)	0.0025*
Postnatal Steroids		16 (19)	21 (19)	36 (14)	17 (10)	0.0212*
Pneumothorax		1 (1)	7 (6)	10 (4)	5 (3)	0.8320
Corrected Gestational Age Positive Pressure Removed	Median	32	32	32	32.1	
	Mean	32.4	32.5	32.8	32.9	
	Std. Dev	2.1	1.5	1.8	1.8	

*p-value of ≤0.05 was considered significantly significant.
BPD Status was defined using the NICHD criteria for moderate and severe BPD.

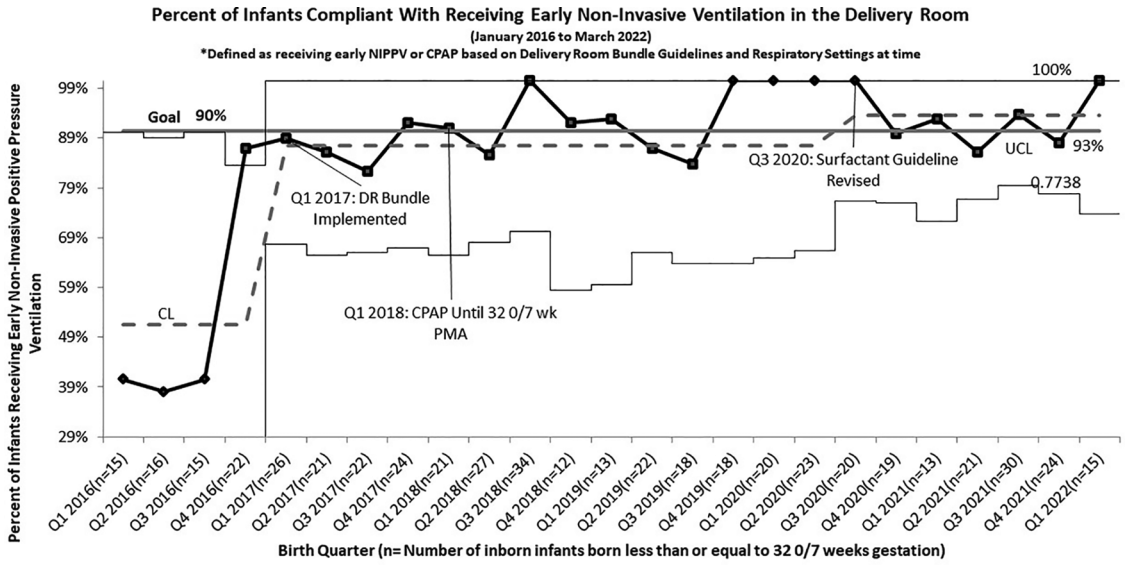

FIGURE 1
P chart of compliance with delivery room bundle for infants born less than or equal to 32 0/7 weeks gestation who received early continuous Non-invasive positive pressure by guidelines.

Compliance with the guideline for early selective surfactant that was rolled out as part of the DR bundle in January 2017 was tracked as receipt of surfactant prior to two hours of life; just 25% of infants were receiving surfactant at less than 2 h of life from Q1 2016 to Q2 2020. The new early selective surfactant guideline was created and implemented in Q3 2020. A baseline rate of compliance with this new guideline was collected retrospectively from Q4 of 2019 to Q2 of 2020 and was determined to be 36%. Implementation of this new guideline resulted in an increase in compliance with the guideline beyond 85%. It also resulted in an increase in administration of surfactant less than two hours to 51% of infants who received surfactant since August 2020 (38 of 74 infants; 137 total infants born during this time), compared to 36% of infants receiving surfactant in 2019.

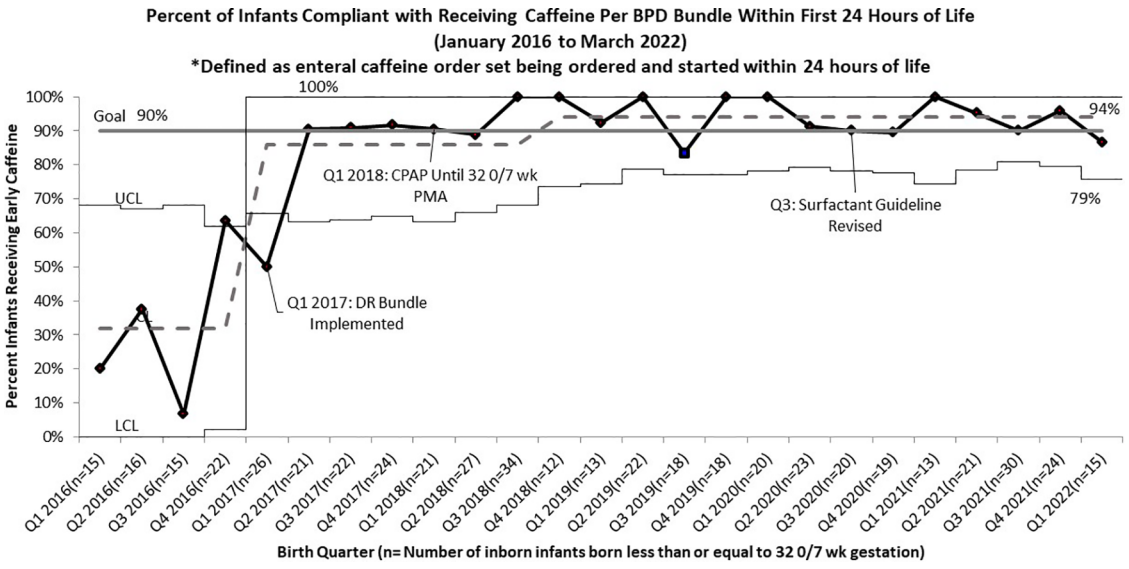

FIGURE 2
P chart of compliance with BPD bundle guidelines for infants born less than or equal to 32 0/7 Weeks Gestation to Receive Early Caffeine Within 24 h of life.

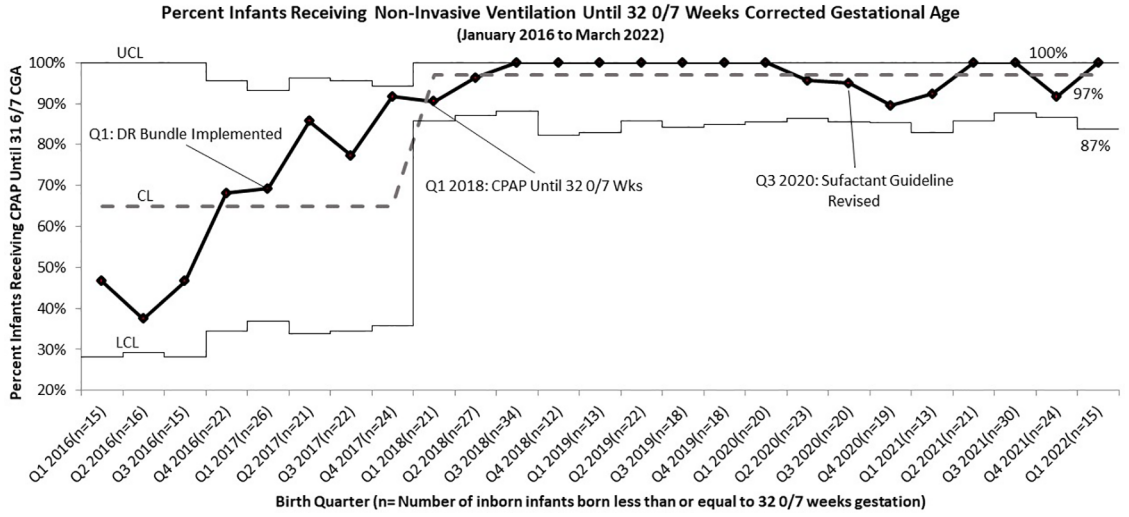

FIGURE 3
p Chart of Compliance with BPD Bundle Guidelines for Infants Born Less than or Equal to 32 0/7 Weeks Gestation to Receive Continuous Positive Airway Pressure Until 32 0/7 Weeks Corrected Gestational Age (Including those that were removed the evening of 31 6/7 Weeks).

Overall compliance with all aspects of our sequentially integrated guidelines improved from 9% at baseline to 81% at closeout (Figure 4). The pre-intervention cohort rate of BPD was 43%, which was utilized as the baseline unit rate of BPD. After implementation of the DR Bundle in January 2017 and continued non-invasive ventilation until 32 0/7 weeks CGA, unit-wide BPD rates decreased to a midpoint of 28%. Combined with these interventions, implementation of the revised early selective surfactant guideline in Q3 of 2020 saw a further decrease in unit-wide rate of BPD to 19% (Figure 5). Balancing measures of rate of pneumothorax remained low at 3.2% throughout the project period for all infants admitted to the UMMMC NICU. Additionally, there was a decrease in the average number of invasive mechanical ventilation days per infant from 6.7 days per infant at baseline to 2.6 days per infant (Figure 6).

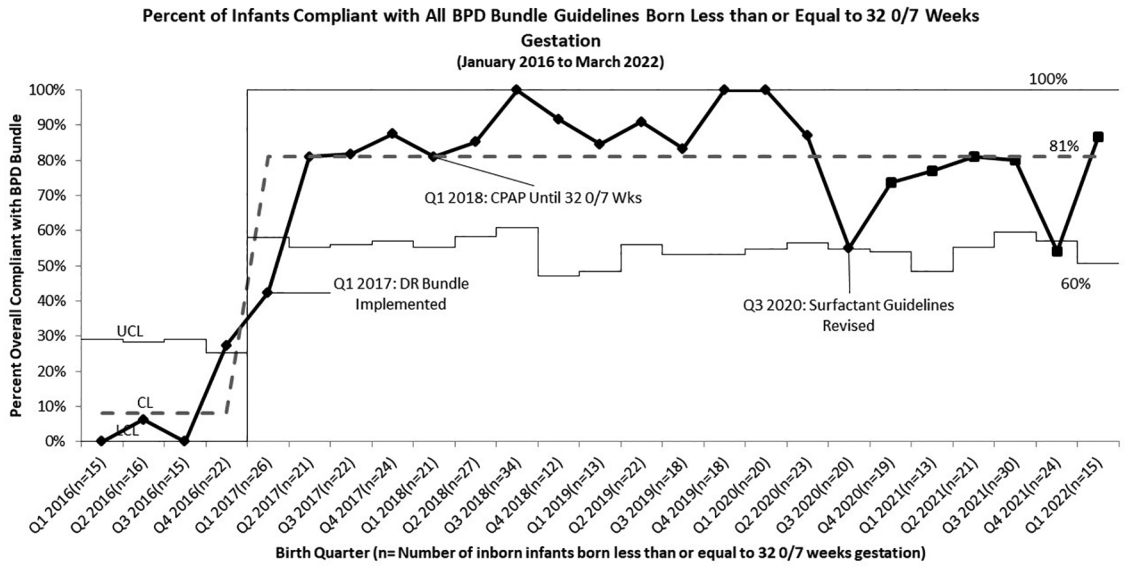

FIGURE 4
p chart of compliance with all BPD bundle guidelines for infants born less than or equal to 32 0/7 weeks gestation.

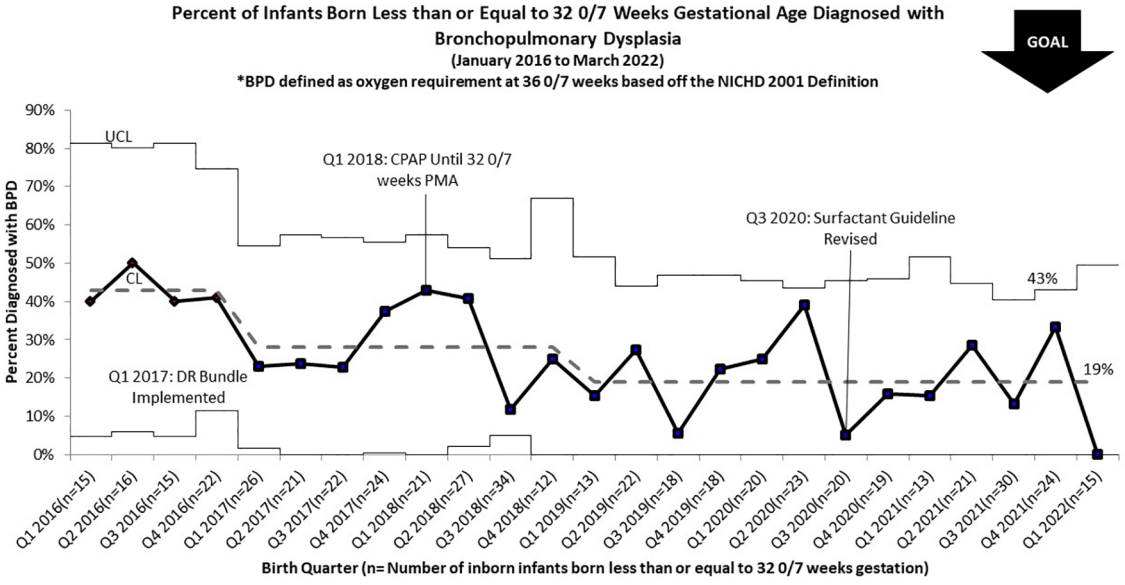

FIGURE 5
p chart for percent of infants born less than or equal to 32 0/7 weeks gestation diagnosed with BPD by NICHD criteria.

Respiratory outcomes are presented in Table 2. Significant decreases in BPD were seen after initiation of extending non-invasive ventilation until 32 0/7 weeks CGA and successful in incrementally reducing moderate and severe BPD from 38.4% to 15% ($p < 0.0001$). Following the initiation of a DR bundle guideline, the BPD rate decreased to 26.6% ($p = 0.089$). Subsequently, with implementation of the guideline for CPAP until 32 weeks CGA BPD rates further decreased to 23.3% representing a statistically significant difference from the baseline group ($p = 0.011$) and with revision of the surfactant guideline BPD rates were reduced to 15% ($p < 0.0001$) compared to baseline. After initiating our DR Bundle

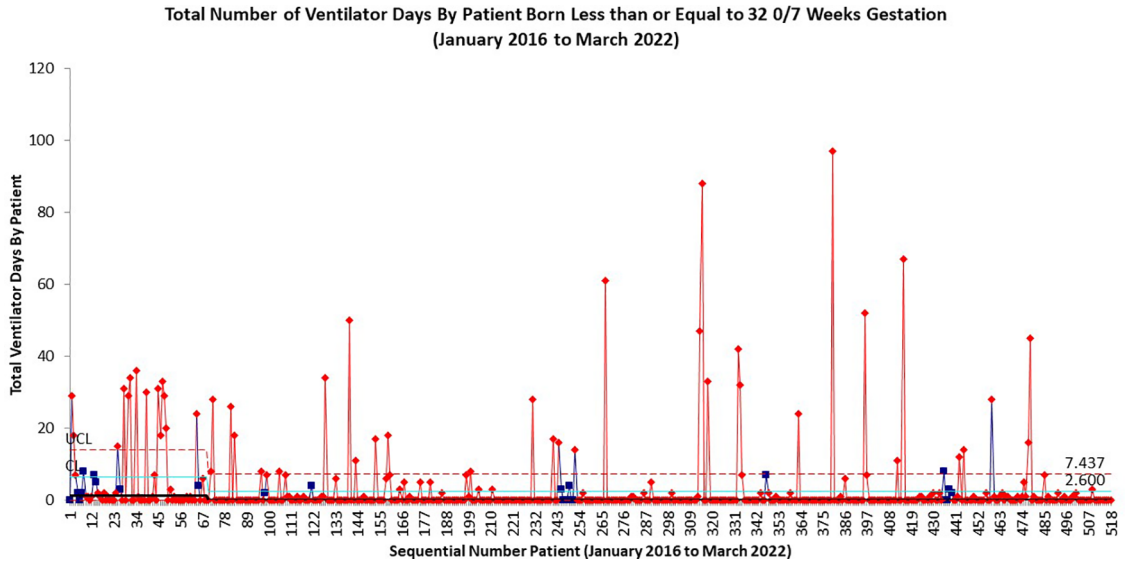

FIGURE 6
Ventilator days for infants born less than or equal to 32 0/7 weeks gestation.

mechanical ventilation utilization was significantly reduced ($p < 0.0001$) and continued to further decline with subsequent PDSA cycles ($p = 0.0025$).

In logistic regression modeling of moderate and severe BPD to rule out potential association due to the differences in birth weights amongst cohorts, SGA status was not significantly associated. The differences in antenatal steroid utilization were also explored. Use of antenatal steroids was not significantly associated with moderate or severe BPD status and did not modify the significant differences in this outcome across intervention groups. Babies treated with inpatient steroids had 41.1 the odds (95% C.I.: 21.4–79.1) of developing moderate or severe BPD compared to babies who did not receive inpatient steroids.

Discussion

In this work we describe our experiences with a multidisciplinary effort to decrease the incidence rate of BPD in our unit, whereas our unit's rates of BPD and among U.S. NICU's remains the most common morbidity of preterm infants. The interventions were derived from previously published literature on best practice for preventing and treating BPD and focused on use of non-invasive ventilation in the delivery room, early selective surfactant administration, early caffeine therapy, and continued non-invasive ventilation until 32 0/7 weeks CGA. Our unit was successful in implementing these guidelines, and the initiative achieved an impactful and sustained reduction in the incidence of BPD over the 5-year project period, representing an overall reduction of 56% without an increase in adverse outcomes.

Our results are reflective of real-world barriers and facilitators of implementing new guidelines. Our multidisciplinary BPD team worked together to research, inform, and create the guidelines. Additionally, our team educated and addressed the uncertainty staff members had around change to achieve adequate compliance with new guidelines.

A primary goal of our DR bundle was to optimize the delivery of non-invasive ventilation to avoid unnecessary need for mechanical ventilation. In clinical trials, mechanical ventilation has been linked in the development of BPD, so avoidance of invasive ventilation should correlate with lower rates of BPD in preterm infants (15, 16). Our protocol prioritizing noninvasive ventilation resulted in fewer infants in our cohort receiving invasive ventilation compared to baseline. Compliance rates reached or exceeded 90% for use of noninvasive ventilation in the DR. Despite rates of non-invasive ventilation in the delivery room of less than 100%, these rates may in fact have been the highest achievable rates, as a proportion of infants who received invasive mechanical ventilation in the delivery room and upon admission may have required mechanical ventilation per NRP guidelines and degree of illness and thus would not have qualified for non-invasive respiratory support. Our unit's utilization of mechanical ventilation has significantly decreased over the course of the project period, from a baseline of 6.7 days per infant to 2.6 days.

A recent systemic review by Healy et al. found that the most commonly utilized intervention in QI initiatives to decrease

BPD included reducing mechanical ventilation by promoting early non-invasive ventilation (10). Like other single center QI initiatives, our group introduced a DR Bundle that promoted and initiated set guidelines that utilized non-invasive ventilation and specified respiratory modality and pressures based on birth gestational age (17, 18). Other centers have described use of early selective surfactant and early enteral caffeine delivery within the first 24 h of birth, but few others have combined all these initiatives in their described bundles (18). The DR Bundle was significant in reducing the use of mechanical ventilation but it was not sufficient in reducing rates of moderate and severe BPD, further interventions were the primary driver of reducing BPD in our cohort.

Beyond a DR Bundle that promoted early prevention of lung injury, our team also initiated guidelines that promoted maintenance on non-invasive ventilation until 32 0/7 weeks CGA (12). No other published QI guidelines have described standardized protocols to discontinue CPAP at a certain CGA in addition to weaning criteria. Our data suggests that this intervention led to further reduction in BPD incidence in our unit, specifically for those at highest risk for developing severe BPD.

Previous published QI initiatives were also mostly single center work that optimized non-invasive ventilation, early surfactant delivery, and caffeine utilization. Prior work failed to show significant improvement in infants at the highest risk, infants under 28 weeks' gestation or with birth weights less than 1000 grams (10). Our guidelines applied to all infants below 32 weeks gestational age at birth, and were successful in reducing the severe BPD incidence rate by 60% from a baseline of 22%. In exploratory analysis of our cohort, SGA status at birth was not significantly associated with the development of BPD. Infants with severe BPD are at high risk for neurodevelopment impairment, and higher health care utilization rates post NICU discharge, including need for home oxygen and ventilator support, so reducing rates of severe BPD is particularly significant (10, 19, 20). Future QI initiatives may primarily focus on this cohort of infants to further reduce the rate of severe BPD (10, 20).

Despite high overall compliance rates, it is difficult to tease out which intervention contributed greatest or if the different interventions worked synergistically to improve our incidence rate of BPD. Our outcomes show the greatest reductions in BPD rates after the initiation of the guideline to extend non-invasive ventilation until 32 0/7 weeks CGA and was further reduced after revising our early selective surfactant guideline.

The limitations to our quality improvement work are that it reflects practice change guidelines in a single Level III NICU, some changes of which may not be broadly generalizable to other units. **Table 1** highlights one significant difference in birth weights between two cohort groups, but not overall. The rate of infants that are small for gestational age in our unit may differ from other units and may be an independent risk factor for BPD development. However, in logistic regression, being small for gestational age was not significantly associated with moderate or severe BPD status and did not modify interventional group associations with this outcome. Further, there were other non-respiratory management changes such as standardizing use of antenatal steroids for mothers at risk of preterm labor. Improvements in more mothers receiving full course of antenatal steroids came after an update in the opinion by the American College of Obstetricians and Gynecologists in late 2017 (21) and our OBGYN Departments response and QI work. This improvement is demonstrated by the increased utilization of full-course antenatal steroids after Q4 2017 and an observed decrease in our unit's utilization of post-natal steroids. However, the differences in antenatal steroid use were not significantly associated with moderate or severe BPD in infants. Further research would need to evaluate the significance and correlation of the observed trends.

Conclusion

Advances in neonatal care have led to higher amounts of infants developing BPD and reducing the incidence of BPD has become a primary focus of neonatal units. In this paper, we highlight our multidisciplinary team's QI work in reducing our units BPD incidence. Implementing a DR Bundle, continuing non-invasive ventilation until 32 0/7 weeks gestation, and revising early surfactant delivery guidelines helped reduce our overall BPD rate by 56% and our severe BPD rate by 60% over the 5-year project duration.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by UMass Chan Medical School Institutional Review Board. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

HW, KM, KM, and LR conceptualized and designed the study, coordinated and supervised data collection. HW and KM drafted the initial manuscript, and reviewed and revised the

manuscript. HW designed the data collection instruments, collected data, and carried out the initial analyses. EL conducted further analyses on the data and interpreted findings. KM, LR and ER revised and reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for the work presented. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.1012655/full#supplementary-material.

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EDITED BY
Shahana Perveen,
Cohen Children's Medical Center, United States

REVIEWED BY
Venkatakrishna Kakkilaya,
University of Texas Southwestern Medical Center, United States
Almudena Alonso-Ojembarrena,
Hospital Universitario Puerta del Mar, Spain

*CORRESPONDENCE
Seung Han Shin
revival421@snu.ac.kr

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Association of newer definitions of bronchopulmonary dysplasia with pulmonary hypertension and long-term outcomes

Jae Kyoon Hwang[1], Seung Han Shin[2]*, Ee-Kyung Kim[2], Seh Hyun Kim[2] and Han-Suk Kim[2]

[1]Department of Pediatrics, Hanyang University Guri Hospital, Gyeonggi-do, Republic of Korea, [2]Department of Pediatrics, Seoul National University College of Medicine, Seoul, Republic of Korea

Background: The definition of bronchopulmonary dysplasia (BPD) has been evolved recently from definition by the National Institute of Child Health and Human Development in 2001 (NICHD 2001) to the definition reported in 2018 (NICHD 2018) and that proposed by Jensen et al. in 2019 (NICHD 2019). The definition was developed based on the evolution of non-invasive respiratory support and to achieve better prediction of later outcomes. Our objective was to evaluate the association between different definitions of BPD and occurrence of pulmonary hypertension (PHN) and long term outcomes.

Methods: This retrospective study enrolled preterm infants born at < 32 weeks of gestation between 2014 and 2018. The association between re-hospitalization owing to a respiratory illness until a corrected age (CA) of 24 months, neurodevelopmental impairment (NDI) at a CA of 18–24 months, and PHN at a postmenstrual age (PMA) of 36 weeks was evaluated, with the severity of BPD defined based on these three definitions.

Results: Among 354 infants, the gestational age and birth weight were the lowest in severe BPD based on the NICHD 2019 definition. In total, 14.1% of the study population experienced NDI and 19.0% were re-hospitalized owing to a respiratory illness. At a PMA of 36 weeks, PHN was identified in 9.2% of infants with any BPD. Multiple logistic regression analysis showed that the adjusted odds ratio (OR) for re-hospitalization was the highest for Grade 3 BPD of the NICHD 2019 criteria (5.72, 95% confidence interval [CI]: 1.37–23.92), while the adjusted OR of Grade 3 BPD was 4.96 (95% CI: 1.73–14.23) in the NICHD 2018 definition. Moreover, no association of the severity of BPD was found in the NICHD 2001 definition. The adjusted ORs for NDI (12.09, 95% CI: 2.52–58.05) and PHN (40.37, 95% CI: 5.15–316.34) were also the highest for Grade 3 of the NICHD 2019 criteria.

Conclusion: Based on recently suggested criteria by the NICHD in 2019, BPD severity is associated with long-term outcomes and PHN at a PMA of 36 weeks in preterm infants.

KEYWORDS

bronchopulmonary dysplasia, developmental delay, prognosis, pulmonary hypertension, readmission

Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease that occurs in preterm infants and is one of the most important morbidities that determine later health outcomes in this population. Since it was first described in 1967 (1), the definition and characteristics of the disease have evolved over the past 50 years as more immature infants can now survive. To

date, the definition of BPD proposed in 2001 in a consensus conference of the National Institute of Child Health and Human Development (NICHD) is the most commonly used, and this categorized BPD severity based on the mode of respiratory support and the amount of supplemental oxygen required (2). Subsequently, a physiological definition of BPD has been suggested as the degree of respiratory support determined by each attending physician, rather than according to the basis of functional assessment (3). The diagnosis and severity of BPD have been associated with long-term adverse effects on respiratory, neurodevelopmental, and growth outcomes and contributes to early mortality as well (4, 5).

However, most commonly used definitions have some limitations (6). One of the most important issues is the fact that it does not incorporate recently introduced non-invasive respiratory support, such as high-flow nasal cannula, as those have been important strategies in the care of preterm infants (7, 8). In this context, revised refinements of the definition of BPD were suggested in the NICHD workshop in 2018, which considered non-invasive positive pressure ventilation (NIPPV) and heated humidified high flow nasal cannula as important modalities (9). More recently, Jensen et al. explored various criteria for diagnosing BPD (NICHD 2019) not only in view of current practices, but also in terms of the ability to accurately predict later health outcomes (10).

Moreover, etiology and pathophysiology of the disease were not considered in the BPD criteria in 2001 (6). Especially, pulmonary vascular disease has been recently recognized as an important pathophysiology of BPD in neonates born with immature lungs, and the association of BPD with this condition has been demonstrated as there is a relatively high prevalence of pulmonary hypertension (PHN) in patients with severe BPD (11, 12). Although it is difficult to include PHN in the current definition of BPD, a better-categorized definition of BPD may identify the association of PHN more clearly in severe forms of the disease.

In this study, the three criteria for diagnosing BPD in preterm infants, including the NICHD 2001, NICHD 2018, and NICHD 2019, were used to predict respiratory and neurodevelopmental outcomes at a corrected age (CA) of 18–24 months. Furthermore, the association between BPD severity and PHN at a postmenstrual age (PMA) of 36 weeks was explored using each criterion.

Methods

Population and data source

This retrospective study enrolled preterm infants who were born at < 32 weeks' gestation at our institution, between January 2014 and December 2018. Infants with congenital anomalies or congenital infections and infants who died before being discharged were excluded from the study population. Those who were lost to follow-up at a CA of 18–24 months were also excluded. Data on perinatal characteristics, clinical courses, PHN, and mode of respiratory support at a PMA of 36 weeks were collected. PHN was diagnosed at a PMA of 36 weeks using echocardiography based on the following findings: right-to-left or bidirectional shunt *via* patent ductus arteriosus or patent foramen ovale, velocity of tricuspid regurgitation ≥ 3 m/s, and left-deviated or flat configuration of the interventricular septum. Re-hospitalization due to a respiratory illness until a CA of 24 months and neurodevelopmental outcomes at a CA of 18–24 months were evaluated. Follow-up results were collected from the data achieved during regular outpatient follow-up schedule until 48 months of age, according to the institution's protocol. Hwang JK and Shin SH collected and reviewed the data from the electric medical records, and retrospectively categorized severity of BPD based on the three criteria. This study was approved by the Institutional Review Board of our institution (2109-008-1250). Obtaining informed consent was waived by the Institutional Review Board, and all methods were performed in accordance with the guidelines of the Human Research Protection Program.

Grading the severity of BPD based on the NICHD 2001, NICHD 2018, and NICHD 2019 criteria

First, BPD was diagnosed and graded as a mild, moderate, or severe disease according to the definition established by the NICHD in 2001 (NICHD 2001) (2). Second, the recently proposed skeletal definition of BPD by the NICHD in 2018 was used (NICHD 2018) (9). In 2019, Jensen et al. compared a number of diagnostic criteria for BPD, in terms of various types of non-invasive respiratory support (NICHD 2019) (10). Among the criteria, those that most accurately predicted later health outcomes were adopted in this study. These were used to categorize BPD that required respiratory support with low-flow (≤2 L/min) nasal cannula at PMA of 36 weeks (Grade 1); BPD that required high flow (>2 L/min) nasal cannula, nasal continuous positive airway pressure, or NIPPV at a PMA of 36 weeks (Grade 2); and BPD that required invasive positive pressure ventilation at a PMA of 36 weeks (Grade 3) (NICHD 2019). **Supplementary Table S1** compares the three definitions at a glance (**Supplementary Table S1**).

Outcomes

Pulmonary hypertension was diagnosed with echocardiography at a PMA of 36 weeks, based on the presence of at least one of the following criteria: (1) velocity of tricuspid valve regurgitation ≥ 3 m/s in the absence of pulmonary stenosis or (2) flat or left-deviated interventricular septal configuration and right ventricular hypertrophy with chamber dilation (13). Respiratory morbidity was defined as re-hospitalization due to a respiratory illness until a CA of 24 months in the study institution and other hospitals; this information was obtained as part of the routine follow-up protocol, based on information provided by the caregiver. The Bayley Scales of Infant and Toddler Development 3rd Edition (Bayley-III) results at a CA of 18–24 months were reviewed; scores < 85 (-1 SD) points in both cognitive and language domains or a motor score < 85 points were defined as developmental delay (14). The test was conducted by one nurse practitioner who has been appropriately trained and specialized for Bayley-III. Combined

neurodevelopmental impairment (NDI) was defined when there was any of the following: blindness, hearing impairment that required the use of hearing aids, cerebral palsy, and developmental delay in Bayley-III. Sepsis was defined if pathogen was demonstrated in blood culture and required systemic antibiotic treatment for more than 5 days. Periventricular leukomalacia was defined when cystic or non-cystic findings of white matter injury was found in imaging tests, such as ultrasound and magnetic resonance imaging (MRI) (13). Intraventricular hemorrhage (IVH) was defined according to Papile's classification (15).

Statistical analyses

Fisher's exact test was used for categorical variables, and one-way analysis of variance was conducted to compare continuous variables according to the severity of BPD with the post-hoc test of Bonferroni correction using the NICHD 2019 criteria. Gestational age, birth weight *z*-score, sex, sepsis, IVH (grade ≥ 3), and periventricular leukomalacia were adjusted in the multivariate logistic regression analysis for later respiratory and neurodevelopmental outcomes as well as PHN at a PMA of 36 weeks based on the three different definitions of BPD. Values are expressed as numbers (%) or medians (interquartile ranges), and a p-value < 0.05 indicated statistical significance. The STATA 12.0 software for Windows (Stata Corp., College Station, TX, United States) was used to analyze all data.

Results

During the study period, 479 infants who had <32 weeks' gestation were born at our facility. Infants with congenital anomalies, congenital infection, and died before discharged were excluded. Infant who died after being discharged and was lost to follow-up at a CA of 18–24 months were also excluded; the remaining 354 infants were included in the final analysis (**Supplementary Figure S1**).

Demographic findings of the study population are summarized according to the severity of BPD based on the NICHD 2019 (**Table 1**), NICHD 2001, and NICHD 2018 criteria (**Supplementary Tables S2,S3**). Based on the NICHD 2019 definition, gestational age (26.9 [25.3–29] weeks) and birth weight (730 [620–980] g) were the lowest among infants with severe BPD, but the incidence of small for gestational age was the highest among infants with severe BPD (20.8%). While the prevalence of multiple births was the highest among infants without BPD (74.3%), histologic chorioamnionitis and oligohydramnios were most common in the severe BPD group (62.3% and 35.8%, respectively). The prevalence of respiratory distress syndrome, patent ductus arteriosus requiring treatment, high-grade IVH, retinopathy of prematurity operation, and PHN at a PMA of 36 weeks were the highest in infants with severe BPD based on the NICHD 2019 (**Table 2**), NICHD 2001, and NICHD 2018 criteria (**Supplementary Tables S4,S5**).

In total, 50 (14.1%) infants experienced combined NDI at a CA of 18–24 months and 67 (19.0%) were re-hospitalized owing to a respiratory illness **Table 3**). Re-hospitalization due to a respiratory illness was higher in Grade 2 and Grade 3 BPD (38.5% and 55.6%, respectively) groups than in the no BPD group (14.1%). Scores of cognitive, language, and motor domains in Bayley-III were the lowest in Grade 3 BPD group compared with the other groups (85 [75–95], 83 [74–91], and 88 [70–94] points, respectively). Delays in both cognitive and language domains, as well as in the motor domain were most common in Grade 3 BPD group (42.9% and 42.9%, respectively). Cerebral palsy occurred most frequently in the severe BPD group (11.1%), but there were no cases of blindness or hearing impairment that required the use of hearing aids.

Multivariate logistic regression analysis was conducted to calculate the adjusted odds ratio (OR) for re-hospitalization and combined NDI based on the three different criteria for BPD

TABLE 1 Demographics of the study population according to the severity of bronchopulmonary dysplasia (NICHD 2019).

	No BPD ($n = 269$)	Grade 1 ($n = 37$)	Grade 2 ($n = 39$)	Grade 3 ($n = 9$)	p-value
GA (week)	30.3 (28.9–31.1)	28.1 (26.7–29.5)	26.4 (24.4–28.7)	27.6 (25.3–30.2)	<0.001
Birth weight (g)	1,290 (1075–1500)	980 (720–1220)	710 (600–990)	800 (645–1095)	<0.001
Birth weight *z*-score	−0.1 (−0.6–0.4)	−0.3 (−1.1–0.4)	−0.3 (−1.1–0.3)	0.0 (−1.6–0.5)	0.053
SGA	19 (7.1)	8 (21.6)	7 (17.9)	2 (22.2)	0.007
Male	136 (50.6)	22 (59.5)	18 (46.2)	3 (33.3)	0.470
C/S	155 (57.6)	21 (56.8)	26 (66.7)	4 (44.4)	0.593
Multiple birth	190 (70.6)	14 (37.8)	19 (48.7)	4 (44.4)	<0.001
hCAM	103 (38.6)	14 (38.9)	28 (71.8)	5 (55.6)	0.001
PROM	109 (40.8)	14 (37.8)	20 (52.6)	4 (44.4)	0.535
Oligohydramnios	48 (17.8)	8 (21.6)	14 (35.9)	5 (55.6)	0.004
Antenatal steroid	227 (84.4)	34 (91.9)	39 (100.0)	7 (77.8)	0.033

Values are expressed as numbers (%) or medians (interquartile ranges).
BPD, bronchopulmonary dysplasia; NICHD, National Institute of Child Health and Human Development; GA, gestational age; SGA, small for gestational age; C/S, Cesarean section; hCAM, histologic chorioamnionitis; PROM, premature rupture of membranes; NDI; neurodevelopmental impairment.

Figure 1). The adjusted OR for re-hospitalization was the highest for Grade 3 in the NICHD 2018 (4.96, 95% confidence interval [CI]: 1.73–14.23) and Grade 3 in the NICHD 2019 (5.72, 95% CI: 1.37–23.92), while no association was found in the NICHD 2001 definition (Figure 1A). Adjusted OR for combined NDI was also the highest for Grade 3 of the NICHD 2018 (6.47, 95% CI: 1.86–22.56) and Grade 3 of the NICHD 2019 (12.10, 95% CI: 2.52–58.05) (Figure 1B). Multivariate analysis for PHN at a PMA of 36 weeks based on each definition showed that PHN was associated with Grade 2 and Grade 3 BPD of the NICHD 2018 and NICHD 2019 criteria, with the highest adjusted OR observed for Grade 3 of the NICHD 2019 criteria (40.37, 95% CI: 5.15–316.35) (Figure 2).

TABLE 2 Clinical courses according to the severity of BPD (NICHD 2019).

	No BPD ($n = 269$)	Grade 1 ($n = 37$)	Grade 2 ($n = 39$)	Grade 3 ($n = 9$)	*p*-value
RDS	118 (43.9)	27 (73)	33 (84.6)	7 (77.8)	<0.001
PDA treated	56 (27.9)	21 (58.3)	24 (64.9)	7 (77.8)	<0.001
IVH (grade ≥3)	4 (1.5)	1 (2.7)	6 (15.4)	0 (0)	<0.001
NEC	7 (2.6)	2 (5.4)	4 (10.3)	1 (11.1)	0.079
ROP operation	5 (1.9)	3 (8.1)	19 (48.7)	5 (55.6)	<0.001
PHN at a PMA 36 weeks	2 (1.4)	3 (8.1)	6 (15.4)	4 (44.4)	<0.001

Values are expressed as numbers (%) or medians (interquartile ranges).
BPD, bronchopulmonary dysplasia; NICHD, National Institute of Child Health and Human Development; RDS, respiratory distress syndrome; PDA, patent ductus arteriosus; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity; PHN, pulmonary hypertension; PMA, postmenstrual age.

Discussion

In this study, the diagnostic criteria for BPD established in 2001, and newly proposed criteria established in 2018 and 2019 were used to define the severity of the disease considering the association of PHN at 36 weeks of PMA and later health outcomes of preterm infants born at < 32 weeks' gestation. A notable finding of the current study is that the association of PHN at a PMA of 36 weeks was well demonstrated in the newer criteria, especially in the NICHD 2019 criteria. To the best of our knowledge, this is the first study that compared different criteria of BPD regarding PHN in preterm infants.

This finding is important in terms of the pathophysiology of the disease as BPD is now recognized as a form of pulmonary vascular disease in premature infants. Abnormalities in the development of pulmonary blood vessels, along with lung damage, could lead to impairment in the structure and function of pulmonary blood vessels (16). As a result, pulmonary hypertension could develop in preterm infants as a spectrum of vascular disease, which is frequently accompanied by a severe form of BPD and is associated with poor outcomes (12). Therefore, the NICHD 2019 criteria might discriminate more severe diseases and reflect the pathophysiology of chronic lung disease in prematurity when compared with previous criteria.

Many previous studies have compared the prognosis of BPD based on various diagnostic criteria. Vyas-Read S. et al. showed findings from the Children's Hospitals Neonatal Consortium that the NICHD 2019 criteria had the highest odds of mortality or tracheostomy as short-term outcomes, followed by the NICHD 2018 criteria (17). A single-center retrospective study of infants born at a GA <32 weeks from China reported that the NICHD 2001 criteria had a lower specificity and worse positive predictive value than the NICHD 2018 criteria regarding severe respiratory morbidities or death at 18–24 months of CA (18).

TABLE 3 Neurodevelopmental and respiratory outcomes according to the severity of BPD (NICHD 2019).

	No BPD ($n = 269$)	Grade 1 ($n = 37$)	Grade 2 ($n = 39$)	Grade 3 ($n = 9$)	*p*-value
Re-hospitalization	35 (13)	12 (32.4)	15 (38.5)§	5 (55.6)§	<0.001
Bayley-III					
Cognitive	100 (90–110)	95 (90–100)	85 (80–95)§	85 (75–95)	<0.001
Language	97 (89–109)	91 (83–100)	83 (71–94)§	83 (74–91)	<0.001
Motor	100 (91–107)	97 (88–98.5)	91 (79–94)§	88 (70–94)§	<0.001
Cognitive and Language <85	13 (6.7)	4 (14.3)	8 (22.9)	3 (42.9)§	0.001
Motor <85	14 (7.3)	5 (17.9)	12 (34.3)§	3 (42.9)	<0.001
CP	10 (3.7)	0 (0)	4 (10.3)	1 (11.1)	0.039
Blindness	–	–	–	–	
Hearing aids	–	–	–	–	
Combined NDI	24 (8.9)	5 (13.5)	16 (41)§*	5 (55.6)§*	<0.001

Values are expressed as numbers (%) or medians (interquartile ranges). BPD, bronchopulmonary dysplasia; NICHD, National Institute of Child Health and Human Development; Bayley-III, Bayley Scales of Infant and Toddler Development 3rd Edition; CP, cerebral palsy; NDI, neurodevelopmental impairment.
§Indicates $p < 0.013$ compared with the no BPD group in the post-hoc analysis with Bonferroni correction.
*Indicates $p < 0.013$ compared with the mild BPD group in the post-hoc analysis with Bonferroni correction.

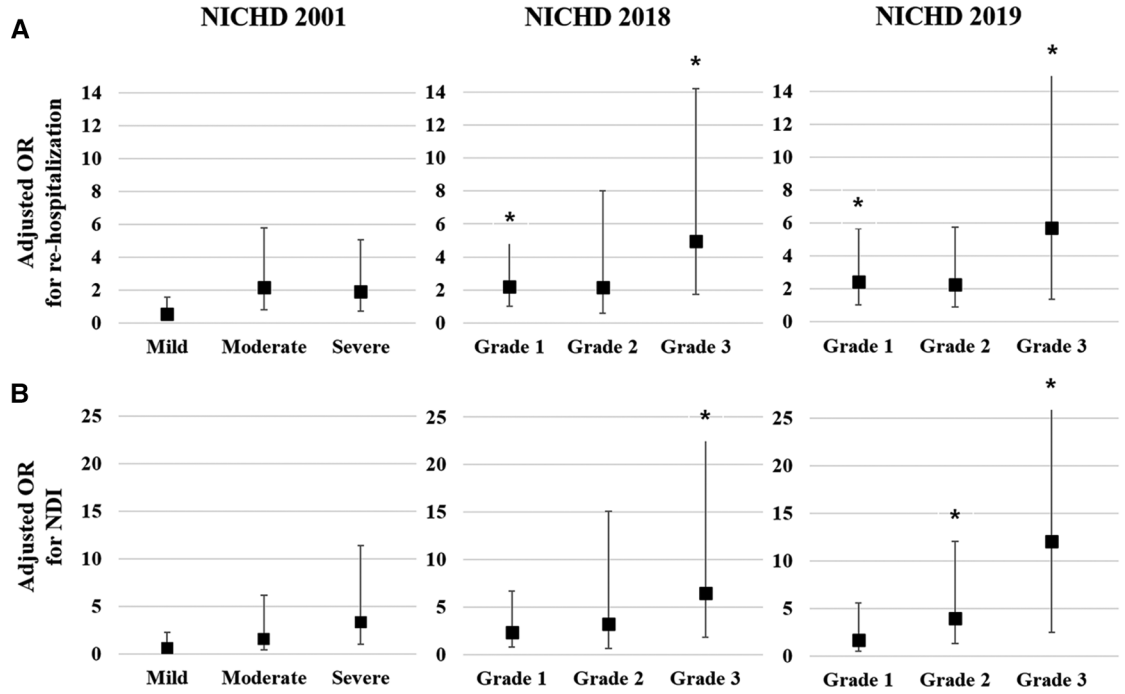

FIGURE 1
Multivariate analysis for respiratory and neurodevelopmental outcomes at a CA of 18–24 months based on each of the criteria for BPD. Gestational age, birthweight *z*-score, and sex were adjusted in the multivariate analysis. Adjusted OR of the severity of BPD based on the three criteria for re-hospitalization owing to a respiratory illness until a CA of 18–24 months (**A**). Adjusted OR of the severity of BPD based on the three criteria for NDI at a CA of 18–24 months (**B**). Asterisk (*) shows significantly adjusted OR ($p < 0.05$). CA, corrected age; BPD, bronchopulmonary dysplasia; OR, odds ratio; NDI, neurodevelopmental impairment; NICHD, National Institute of Child Health and Human Development.

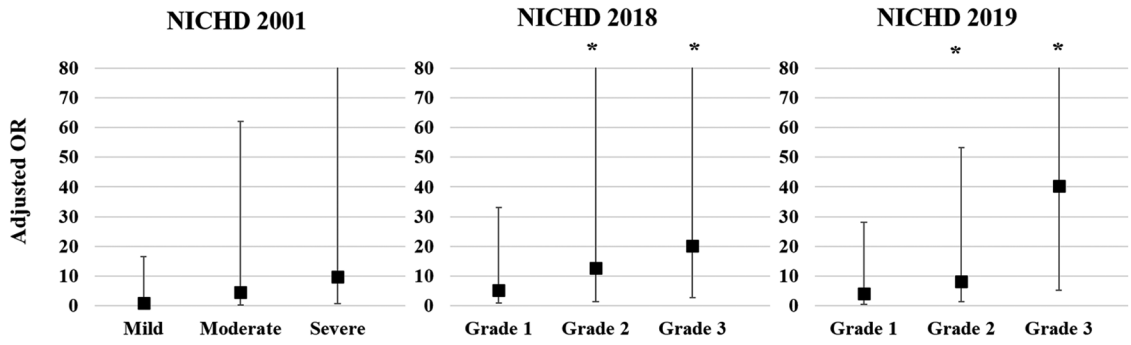

FIGURE 2
Adjusted OR for PHN at a PMA of 36 weeks based on each of the criteria for BPD. Gestational age, birthweight *z*-score, and sex were adjusted in the multivariate analysis. Asterisk (*) shows significant adjusted OR ($p < 0.05$). OR, odds ratio; PHN, pulmonary hypertension; PMA, postmenstrual age; BPD, bronchopulmonary dysplasia; NICHD, National Institute of Child Health and Human Development.

A study using nationwide registered data of extremely preterm infants in Korea to compare three definitions of BPD showed that the NICHD 2018 or NICHD 2019 criteria had better prediction in both respiratory morbidities and neurodevelopmental outcomes at 18–24 months of CA (19). Another study using nationwide data of infants with <32 weeks' gestation also compared the NICHD 2001 and NICHD 2019 criteria and showed that the latter was associated more with adverse respiratory and neurodevelopmental outcomes at 2 years of age (20). A previous study that analyzed the association of different definitions of BPD with economic impact showed that the NICHD 2019 criteria had the strongest correlation with hospital charges and the 1st year of life-associated hospital charges for preterm infants (21). The results of the present study are compatible with those of previous studies in that the association of BPD with long-term outcomes was not evident in the NICHD 2001 criteria, while both the NICHD 2018 and NICHD 2019 criteria had associations with those outcomes (22, 23).

Given the nature of the criteria, better prediction of later outcomes in the NICHD 2019 criteria seems obvious, as they were originally determined based on the best predictive ability among various pre-specified definitions of BPD (10). Further, in NICHD 2019 definition, the best predictability was found when BPD was

graded according to the mode of respiratory support regardless of oxygen use. These findings are intriguing, as a previous study reported that a higher capillary partial pressure of carbon dioxide, rather than the requirement for oxygen, was a good predictor of later respiratory outcomes among patients with BPD (24).

There are several limitations to this study. First, this was a single-center study; therefore, the number of participants was smaller than the corresponding of large-scale studies (10, 25). Second, only 74.3% of the study population was tested using Bayley-III at a CA of 18–24 months, although other neurodevelopmental outcomes, such as CP, hearing impairment, and blindness, have been well reported. Other aspects of respiratory outcomes, such as respiratory medication and respiratory symptoms, were not assessed. The incidence of PHN in patients with BPD was 9.2%, which was relatively lower than that in a previous study (11). This might be attributed to the study population, which was a relatively mature population of GA <32 weeks in this study. Furthermore, socioeconomic and environmental factors that influence respiratory and neurodevelopmental outcomes were not included in this study (11).

In conclusion, recently suggested BPD criteria, such as the NICHD 2018 and NICHD 2019 criteria, showed an association between the severity of BPD and later outcomes in preterm infants. Furthermore, the association of BPD severity based on these recent criteria and PHN was well demonstrated in this study. As PHN is an important aspect of the pathophysiology of BPD, it might be speculated that recent criteria of BPD could discriminate severity of disease not only more practically but also based on the entity of BPD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board, Seoul National University Children's Hospital, 101 Daehak-ro Jongno-gu, Seoul 03080, Republic of Korea IRB no. 2109-008-1250. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

JKH and SHS contributed to conception and design of the study. JKH, SHS, SHK curated and organized the data. JKH, SHS, EKK performed and validated the statistical analysis. JKH, SHS, SHK wrote the first draft of the manuscript. JHK, SHS, HSK wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1108925/full#supplementary-material.

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EDITED BY
Jia-Yuh Chen,
Chung Shan Medical University, Taiwan

REVIEWED BY
Chung-Ming Chen,
Taipei Medical University, Taiwan
Shahana Perveen,
Cohen Children's Medical Center, United States

*CORRESPONDENCE
Xueping Zhu
zhuxueping4637@hotmail.com
Xiaoli Zhu
zhuxiaoli90@hotmail.com

†These authors have contributed equally to this work

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Predictive values of clinical data, molecular biomarkers, and echocardiographic measurements in preterm infants with bronchopulmonary dysplasia

Huawei Wang[1†], Dongya Yan[1,2†], Zhixin Wu[1], Haifeng Geng[1], Xueping Zhu[1*] and Xiaoli Zhu[3*]

[1]Department of Neonatology, Children's Hospital of Soochow University, Suzhou, China, [2]Department of Neonatology, Children's Hospital of Anhui Province, Hefei, China, [3]Department of Intervention, The First Affiliated Hospital of Soochow University, Suzhou, China

Objective: We aimed to use molecular biomarkers and clinical data and echocardiograms that were collected during admission to predict bronchopulmonary dysplasia (BPD) in preterm infants with gestational age ≤32 weeks.
Methods: Eighty-two patients (40 with BPD, BPD group and 42 healthy as controls, non-BPD group) admitted to the Department of Neonatology of the Children's Hospital of Soochow University between October 1, 2018, and February 29, 2020, were enrolled in this study at the tertiary hospital. Basic clinical data on the perinatal period, echocardiographic measurements, and molecular biomarkers (N-terminal-pro-B-brain natriuretic peptide, NT-proBNP) were collected. We used multiple logistic regression analysis to establish an early predictive model for detecting BPD development in preterm infants of gestational age ≤32 weeks. We also used a receiver operating characteristic curve to assess the sensitivity and specificity of the model.
Results: No significant differences were found between the BPD and non-BPD groups in terms of sex, birth weight, gestational age, incidence of asphyxia, maternal age, gravidity, parity, mode of delivery, premature rupture of membranes >18 h, use of prenatal hormones, placental abruption, gestational diabetes mellitus, amniotic fluid contamination, prenatal infections, and maternal diseases. The use of caffeine, albumin, gamma globulin; ventilation; days of $FiO_2 \geq 40\%$; oxygen inhalation time; red blood cell suspension infusion volume (ml/kg); and proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 d after birth were higher in the BPD group than in the non-BPD group. The levels of hemoglobin, hematocrit, and albumin in the BPD group were significantly lower than those in the non-BPD group. The total calorie intake was significantly lower in the BPD group on the 3rd, 7th, and 14th day after birth than in the non-BPD group ($P < 0.05$). The incidence rates of patent ductus arteriosus (PDA), pulmonary hypertension, and tricuspid regurgitation were significantly higher in the BPD group than in the non-BPD group ($P < 0.05$). The serum level of NT-proBNP 24 h after birth was significantly higher in the BPD group than in the non-BPD group ($P < 0.05$). Serum NT-proBNP levels were significantly higher in infants with severe BPD than in those with mild or moderate BPD ($P < 0.05$).

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Conclusion: As there were various risk factors for BPD, a combining clinical data, molecular biomarkers, and echocardiogram measurements can be valuable in predicting the BPD. The tricuspid regurgitation flow rate (m/s), NT-proBNP (pg/ml), ventilator-associated pneumonia, days of $FiO_2 \geq 40\%$ (d), red blood cell suspension infusion volume (ml/kg), and proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 d after birth were the most practical factors considered for designing an appropriate model for predicting the risk of BPD.

KEYWORDS

predictive, NT-ProBNP, echocardiographic, preterm, bronchopulmonary dysplasia

Key messages

- Bronchopulmonary dysplasia seriously affects the treatment and long-term prognosis of preterm infants.
- Preventing BPD is important in clinical practice.
- Tricuspid regurgitation flow rate (m/s), NT-proBNP (pg/ml), ventilator-associated pneumonia, days of $FiO_2 \geq 40\%$, red blood cell suspension infusion volume (ml/kg), and proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 d after birth are BPD risk factors.

Introduction

Bronchopulmonary dysplasia (BPD) is a common chronic lung disease and one of the most severe sequelae of respiratory system in preterm infants (1). More than 40% of extremely premature and extremely low birth weight infants (gestational age <28 weeks/birth weight <1000 g) progress to BPD in developed countries, and this rate has not decreased substantially in the past 20 years (2, 3). Infants with BPD who survive may experience several neurodevelopmental impairments and respiratory problems, and some serious influences on neural development and cardiopulmonary function can extend into adolescence and adulthood (4, 5, 6).

BPD develops from the interaction of various types of damage influenced by inflammation related to chorioamnionitis, infections, ventilation, and high-concentration oxygen (7). However, there are currently no effective treatments for preventing the development of BPD. Furthermore, some existing therapeutic measures, such as systemic glucocorticoids, can cause adverse effects, including neurodevelopmental impairment (8).

Preterm and immature lung tissues are the key factors contributing to BPD development. Medical treatments, such as mechanical ventilation and the inhalation of high concentrations of oxygen, which are sometimes used to rescue preterm infants, may also cause, or aggravate BPD (9). Currently, there are no effective treatments for premature BPD. Therefore, the strategies to prevent BPD are crucial in clinical practice, and it is essential to explore relevant indicators for BPD. In a previous study, we collected perinatal clinical data and the neonatal critical illness score (NCIS) and certain identified molecular biomarkers to isolate risk factors for BPD. Another study showed that clinical data, echocardiographic measurements, and molecular biomarkers may assist in predicting the patients who will be subjected to the worst grades of BPD (10). Accordingly, we collected perinatal clinical and echocardiographic data and measured N-terminal-pro-B-brain natriuretic peptide (NT-pro BNP) levels to predict BPD in preterm infants (11). We explored the effectiveness of using clinical data, echocardiograms, and molecular biomarkers to predict BPD in preterm infants.

Patients and methods

This prospective study enrolled neonates admitted to the Department of Neonatology of the Children's Hospital of Soochow University from October 1, 2018 to February 29, 2020. The inclusion criteria for the participants were preterm infants with a gestational age ≤32 weeks and a hospital stay of ≥28 d. Clinical data and echocardiographic variables were recorded at various time points. The exclusion criteria were an admission age older than 24 h, infection at admission, major congenital abnormalities, surgical intervention requirements during the NICU stay, an unplanned discharge, and incomplete clinical data.

This study has already obtained an approvement from the Ethics Committee of the Children's Hospital of Soochow University. The parents of all infants provided written informed consent.

Data collection

Clinical variables

Clinical data were collected from medical records and the following maternal, infant, and prenatal factors were included: (1) maternal conditions, including maternal age, delivery mode, antenatal corticosteroid use, premature rupture of membranes (duration >18 h), placental abruption and placenta previa, gestational diabetes, gestational hypertension, gestational anemia, preeclampsia, amniotic fluid contamination, prenatal infection (fever, chorioamnionitis), and other maternal diseases. (2)

General conditions of the infants, including sex, gestational age, birth weight, age at admission, incidence of asphyxia, conception through *in vitro* fertilization, twins, or multiple births. (3) Primary diseases and complications that occurred during the hospitalization of preterm, including neonatal respiratory distress syndrome (NRDS), pneumonia, pneumothorax, ventilator-associated pneumonia (VAP), feeding intolerance, parenteral nutrition-associated cholestasis (PNAC), brain injury in premature infants (BIPI), periventricular/intraventricular hemorrhage (PVH/IVH), retinopathy of prematurity (ROP), hemodynamically significant patent ductus arteriosus (hs-PDA), frequent apnea, sepsis, bacterial meningitis, pulmonary hemorrhage, and necrotizing enterocolitis (NEC). (4) Treatment(s) during hospitalization: caffeine, albumin, gamma globulin, complete total enteral nutrition later than 24 d, more than three blood transfusions, invasive ventilation time, days of $FiO_2 \geq 40\%$, non-invasive ventilation time, oxygen inhalation time, and red blood cell suspension infusion volume (ml/kg). (5) Laboratory tests administered upon admission: white blood cell count (WBC, 10^9/L), red blood cell count (RBC, 10^{12}/L), platelets (PLT, 10^9/L), hemoglobin (Hb, g/L), neutrophil absolute value (NE, 10^9/L), lymphocyte absolute value (LY, 10^9/L), red blood cells deposited (Hct, L/L), average red blood cell volume (MCV, fL), albumin levels (propagated, g/L), and prealbumin levels (Pa, mg/L). (6) Oral fluid volume 3, 7, 14, 21, and 28 d postnatal, fluid intake, caloric intake (enteral, parenteral, and total), and the times for beginning feeding and reaching total enteral nutrition.

Diagnostic criteria

We firstly define the important clinical indicators for the sake of understanding.

(1) Definition of BPD and Clinical Grading (12)

The diagnostic criteria of BPD adopted in our study was based on the standard of the National Institute of Child Health and Human Development (NICHD) published in 2001, which defines BPD as follows: (i) preterm low birthweight infants treated with oxygen ($FiO_2 > 0.21$) for at least 28 days; (ii) persistent or progressive respiratory insufficiency; (iii) lungs with typical x-ray or CT scan findings (e.g., bilateral lungs with enhanced texture, reduced permeability, ground glass-like, localized emphysema, or cystic changes); (iv) exclusion of congenital cardiopathy, pneumothorax, pleural effusion, and sputum. The clinical grading was based on the supplemental O_2 of the infants at 36 weeks postmenstrual age or discharge (GA <32 weeks) and at 56 days postnatal age or discharge (GA ≥ 32 weeks).

The clinical grading was classified as follows:

Mild: breathing room air; moderate: a fraction of inspired oxygen (FiO_2) < 0.3; severe: $FiO_2 \geq 0.3$ and/or positive pressure ventilation or mechanical ventilation.

(2) Definition of UEGR (13)

Extrauterine growth restriction (EUGR) is a common condition in very low birth weight (VLBW) preterm infants (≤1,500 g). Most affected infants have a birth weight that is average for gestational age, but by the time of hospital discharge have a weight that is less than the tenth percentile for corrected gestational age.

(3) Definition of VAP (14)

VAP was defined as a nosocomial infection happening 48 h after mechanical ventilation.

(4) Diagnosis of NRDS (15)

NRDS was defined as the presensce of respiratory distress and increased oxygen requirement ($FiO_2 > 0.4$), which cannot be explained by other causes *via* chest x-ray and lab findings.

(4) Definition of BIPI (16)

BIPI refers to various pathologies due to prenatal, intrapartum, or/and postnatal conditions factors that lead to varying degrees of cerebral ischemia and/or hemorrhagic loss in preterm infants, it can lead to long-term nervous system sequelae and even death.

(5) Definition of PNAC (17)

PNAC was defined as cholestasis attributable to PN use, with other parameters excluded.

(6) Definition of has-PDA (18)

Echocardiographic evidence of a hs-PDA met one of the following criteria: ductal diameter ≥1.5 mm, unrestrictive pulsatile ductal flow (ductus arteriosus peak velocity <2.0 m/s), left heart volume loading (left atrium to aortic ratio >1.5), left heart pressure loading (early passive to a late atrial contractile phase of transmittal filling ratio >1.0 or isovolumic relaxation time ≥50).

Echocardiographic measurements

All included patients underwent echocardiography on the first day, and those with congenital heart disease were excluded. Patients were also examined for PDA, atrial septal defects, pulmonary vein stenosis, tricuspid regurgitation (TR), TR velocity, left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), and pulmonary hypertension (PH). The echocardiography protocol and examination results were discussed and agreed upon by pediatric cardiologists working for >10 years at our hospital. All parameters and indicators that could be quantified, including right atrial and right ventricular dilation, were recorded.

Analytical biomarker determination

Blood samples were collected and preserved on the first day. The levels of NT-proBNP in plasma were analyzed using a commercial enzyme-linked immunosorbent assay [ELISA, Roche Diagnostic Products (Shanghai) Co. Ltd, China].

Statistical analysis

SPSS 24.0 was used for analyzing the data in this study. Categorical variable data were analyzed using either the chi-square test or Fisher's exact test; normally distributed variable data are represented as the mean ± standard deviation and processed using independent t-test. Non-normally distributed variable data were represented as the median value ± interquartile range [M (P25, P75)] and analyzed using non-parametric tests. Significant factors were selected and recruited in the next step of logistic regression analysis to explore the independent risk factors for BPD occurrence in premature infants. The sensitivity and specificity of the predictive models were evaluated *via* the AUC. Statistical significance was set at $P < 0.05$.

Results

Clinical data of preterm infants

From October 1, 2018, to February 29, 2020, 232 preterm infants ≤32 weeks were admitted to our hospital. Of these infants, 12 patients were discharged automatically, 32 were hospitalized for less than 28 d, and 15 underwent surgery during hospitalization. Among these, 40 were diagnosed with BPD. A total of 42 preterm infants with no differences in general information from the BPD group were randomly matched to the non-BPD group. In total, 82 preterm infants were enrolled in this study. In the BPD group, 19, 15, and 6 cases were classified as mild, moderate, and severe, respectively. There were no significant differences in basic and clinical data between the two groups ($P > 0.05$, **Table 1**), including gestational age, sex, birth weight, incidence of asphyxia, maternal age, chorioamnionitis, use of prenatal steroids, oligohydramnios, gestational hypertension, gestational diabetes, and placental abruption.

Risk factors for BPD in preterm infants

The incidence of VAP, feeding intolerance, BIPI, PVH-IVH, apnea, sepsis, ROP, PNAC, and extrauterine growth restriction (EUGR) was significantly more frequent in the BPD group ($P < 0.05$, **Table 1**) than in the non-BPD group. The use of caffeine, albumin, and intravenous immunoglobulin and ventilation (including the invasive and non-invasive modes) was more frequent in the BPD group ($P < 0.05$) than in the non-BPD group. Days on oxygen inhalation ($FiO_2 > 40\%$), the proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 d after birth, and the duration of enteral nutrition was longer in the BPD group ($P < 0.05$) than in the non-BPD group. The number of red blood cell transfusions during the stay in the NICU was higher in the BPD group ($P < 0.05$, **Table 1**) than in the non-BPD group. The hemoglobin, hematocrit, and serum albumin levels were significantly different between the two groups ($P < 0.05$, **Table 2**). VAP (OR = 14.443, 95% CI: 1.045–199.522), days of $FiO_2 > 40\%$ (OR = 1.943, 95% CI: 1.047–3.608), the red blood cell transfusion volume (ml/kg) (OR = 1.108, 95% CI: 1.044–1.175), and the proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 d after birth (OR = 7.683, 95% CI: 1.320–44.714) were identified as possible risk factors for BPD development using multiple regression analysis (**Table 3**).

Echocardiographic evaluations in preterm infants

All preterm infants underwent complete echocardiographic examination upon inclusion (day 1), and congenital heart disease was ruled out. We interpreted echocardiograms for PDA, the TR velocity, LVEF, and LVFS and found that TR and Hs-PDA were more frequent in patients with BPD ($p < 0.05$) than in those without BPD. Furthermore, TR velocity was higher in the BPD group ($P < 0.05$, **Table 4**) than in the non-BPD group.

Serum NT-proBNP levels

Serum NT-proBNP levels were the lowest on the first day in the non-BPD group ($P < 0.05$, **Figure 1**) and were also significantly different from those in the BPD group ($P < 0.05$). Serum NT-proBNP levels gradually increased with BPD severity ($P < 0.05$, **Figure 2**).

Sensitivity and specificity of individual risk factors for BPD

ROC analysis using the TR velocity, NT-proBNP, VAP, days of $FiO_2 > 40\%$, transfusion volume of red blood cells, and the proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 d after birth indicated that these variables can be considered potential predictors or risk factors of BPD. The AUC, sensitivity, specificity, and Youden index values for these variables are shown in **Table 5**.

TABLE 1 Maternal and neonatal baseline characteristics

	BPD group (n=40)	Non-BPD group (n=42)	χ^2/U/t	*P*
Gender [male, n (%)]	25 (62.50)	25 (59.52)	0.076	0.782
Weight at birth (g, $\bar{x}$±s)	1353 ± 198.18	1410 ± 196.75	−1.300	0.197
Gestational age (week, $\bar{x}$±s)	29.96 ± 1.10	30.34 ± 0.60	−1.944	0.060
Neonatal asphyxia, n (%)	11 (27.50)	5 (11.90)	2.992	0.084
Mother's age < 20 or > 35 years, n (%)	10 (25.00)	8 (19.05)	0.424	0.598
Chorioamnionitis, n (%)	4 (10.00)	0	—	0.052
Prenatal steroids, n (%)	26 (65.00)	29 (69.05)	0.152	0.697
Oligohydramnios, n (%)	5 (12.50)	1 (2.38)	—	0.105
Gestational hypertension, n (%)	11 (27.50)	9 (21.43)	0.410	0.522
Gestational diabetes, n (%)	6 (15.00)	4 (9.52)	—	0.514
Placenta abruption, n (%)	1 (2.50)	3 (7.14)	—	0.616
Neonatal pneumonia, n (%)	39 (97.50)	40 (95.24)	—	1.000
NRDS, n (%)	16 (40.00)	9 (21.43)	3.334	0.068
Pneumothorax, n (%)	1 (2.50)	1 (2.38)	—	1.000
VAP, n (%)	12 (30.00)	1 (2.38)	—	0.001
Feeding intolerance, n (%)	22 (55.00)	10 (23.81)	8.376	0.004
BIPI, n (%)	14 (35.00)	5 (11.90)	6.139	0.013
PVH-IVH, n (%)	10 (25.00)	3 (7.14)	—	0.035
Apnea, n (%)	14 (35.00)	4 (9.52)	—	0.007
Sepsis, n (%)	7 (17.50)	1 (2.38)	—	0.027
CNS infection, n (%)	3 (7.50)	0	—	0.112
Pneumorrhagia, n (%)	3 (7.50)	0	—	0.112
NEC, n (%)	0	0	—	—
ROP, n (%)	12 (30.00)	3 (7.14)	—	0.010
PNAC, n (%)	14 (35.00)	2 (4.76)	—	0.000
EUGR, n (%)	21 (52.50)	10 (23.81)	7.172	0.007
Caffeine, n (%)	21 (52.5)	8 (19.0)	10.030	0.002
Albumin, n (%)	28 (70)	10 (23.8)	17.579	0.000
Intravenous immunoglobulin, n (%)	30 (75)	10 (23.8)	8.721	0.003
Invasive ventilation, n (%)	20 (50.00)	6 (14.29)	12.068	0.001
Duration of invasive ventilation [d, M (P_{25}, P_{75})]	7.5 (5, 16.25)	3 (1.50, 3)	481.5	0.000
Duration of non-invasive ventilation [d, M (P_{25}, P_{75})]	18.5 (12.25,26.75)	2.5 (0,7)	144	0.000
Days of FiO_2 >40% [d, M (P_{25}, P_{75})]	2 (2, 3)	3 (3, 3.75)	158	0.000
Days of oxygen inhalation [d, ($\bar{x}$±s)]	46.35 ± 14.83	19.39 ± 7.76	9.749	0.000
Red blood cells Transfusion, n (%)	29 (72.50%)	8 (19.05%)	23.64	0.000
Proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥ 24 days, n (%)	31 (77.5)	9 (21.4)	25.781	0.000
Time when enteral nutrition starts (d)	2 (2, 4)	2 (1,2)	498.5	0.001

BPD, bronchopulmonary dysplasia. NRDS, respiratory distress syndrome. VAP, ventilator-associated pneumonia. BIPI, brain injury in premature infants. PVH/IVH, periventricular or intraventricular hemorrhage. CNS, central nervous system. NEC, necrotizing enterocolitis. ROP, retinopathy of prematurity. PNAC, parenteral nutrition-associated cholestasis. EUGR, extrauterine growth retardation. ——: Not calculated.

Sensitivity and specificity of the BPD prediction model

The TR velocity, NT-proBNP, VAP, days of $FiO_2 > 40\%$, transfusion volume of red blood cells, and proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 d after birth were included to yield a predictive model for BPD. The X^2-value of the model was 89.203 ($P < 0.001$), suggesting these variables may predict the risk of BPD. The Hosmer-Lemeshow test was conducted using the classification interaction table (df = 8, $P > 0.05$), which demonstrated that the model was consistent with the indicators well. The test results indicated that the combination of these variables in the model yielded an AUC of 0.986. The sensitivity and specificity

TABLE 2 Laboratory findings of BPD and non-BPD patients.

	BPD ($n = 40$)	Non-BPD ($n = 42$)	χ^2/U/t	P
White blood cells [$\times 10^9$/L, M (P_{25}, P_{75})]	9.20 (6.53, 11.49)	8.78 (7.18, 11.24)	809	0.774
Red blood cells [$\times 10^{12}$/L, ($\bar{x} \pm s\pm$)]	4.21 ± 0.61	4.43 ± 0.48	−1.891	0.062
Blood platelets [$\times 10^9$/L, ($\bar{x} \pm s$)]	233.60 ± 64.22	247.24 ± 62.90	−0.971	0.334
Hemoglobin content [g/L, ($\bar{x} \pm s$)]	161.50 ± 20.36	173.26 ± 25.86	−2.281	0.025
Hematocrit [L/L, ($\bar{x} \pm s$)]	0.47 ± 0.06	0.50 ± 0.06	−2.220	0.029
Neutrophils [$\times 10^9$/L, M (P_{25}, P_{75})]	4.82 (3.04, 7.45)	4.48 (3.19, 6.28)	762	0.469
Lymphocyte [$\times 10^9$/L, M (P_{25}, P_{75})]	3.11 (1.89, 3.85)	2.78 (2.60, 3.70)	809	0.774
Serum albumin [g/L, ($\bar{x} \pm s$)]	31.08 ± 3.35	32.73 ± 3.10	−2.318	0.023
Serum prealbumin [mg/L, ($\bar{x} \pm s$)]	119.10 ± 23.63	116.71 ± 21.16	0.482	0.631

TABLE 3 Logistic regression results.

Variable	β	SE	Wald	P	OR	95%CI
VAP	2.670	1.340	3.973	0.046	14.443	1.045–199.522
Days of $FiO_2 > 40\%$	0.664	0.316	4.428	0.035	1.943	1.047–3.608
Transfusion volume of red blood cells (ml/kg)	0.102	0.030	11.631	0.001	1.108	1.044–1.175
Proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 days after birth (%)	2.039	0.899	5.148	0.023	7.683	1.320–44.714

VAP, ventilator-associated pneumonia.

were 97.60% and 92.50%, respectively. The predictive model yielded a higher AUC value, sensitivity, and specificity than any individual variables (Table 6).

Discussion

Prenatal and postnatal factors, among others, can influence the development of BPD in preterm infants. In this study, we systematically analyzed the clinical data, echocardiographic measurements, and molecular biomarkers of preterm infants of gestational age ≤32 weeks. We found that the TR flow rate (m/s), NT-proBNP (pg/ml), VAP, days of $FiO_2 > 0.4$, red blood cell suspension infusion volume (ml/kg), and proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 d after birth were practical risk factors contributing to the development of BPD.

TABLE 4 Echocardiographic evaluations of the non-BPD *vs.* BPD groups.

	BPD ($n = 40$)	Non-BPD ($n = 42$)	χ^2/U/t	P
Tricuspid regurgitation [n (%)]	28 (70.0)	19 (45.2)	5.135	0.023
Tricuspid regurgitation velocity [m/s, $\bar{x} \pm s$]	2.36 ± 0.77	1.33 ± 0.53	5.072	0.000
LVEF (%, $\bar{x} \pm s$)	70.16 ± 6.84	67.74 ± 4.90	5.579	0.071
LVFS (%, $\bar{x} \pm s$)	36.84 ± 5.26	34.95 ± 3.59	6.790	0.062
Hs-PDA	26 (65)	6 (14.3)	7.250	0.007

LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; hs-PDA, hemodynamically significant patent ductus arteriosus.

BPD is a serious pulmonary disease caused by multiple factors, including volume injury, infection, inflammation, and abnormal repair of the lung (19). Exploring the risk factors in the prenatal and postpartum periods is a possible area of research. Based on our findings, a neonatologist could anticipate and prevent BPD occurrences and develop therapeutic strategies for neonatal patients to decrease the damage to the pulmonary systems of infants in the future. Several predictive models currently exist, most of which depend on clinical variables, such as gestation, oxygen intake. For example, a study in Korea that included 4,600 very low birth weight preterm infants (VLBWIs, with a birthweight less than 1,500 grams) found that perinatal data, including 5 min Apgar scores, birth weights, necessary resuscitation procedures after birth, were significant indicators for VLBWIs (20). However, most of these existing models have limitations in terms of predicting the progression of high-risk preterm infants to BPD (21, 22). The incidence rate of BPD remains high as no single, specific indicator with a high predictive value has been widely accepted, making early intervention challenging (23).

Echocardiography is widely regarded as a useful and valuable screening tool for assessing the possibility of BPD in preterm infants, and echocardiographic measurements can be used to evaluate elevated pulmonary pressure (PAP). Echocardiography can be used to evaluate the elevated pulmonary vascular resistance index (PVRi) and classify severity based on pressure

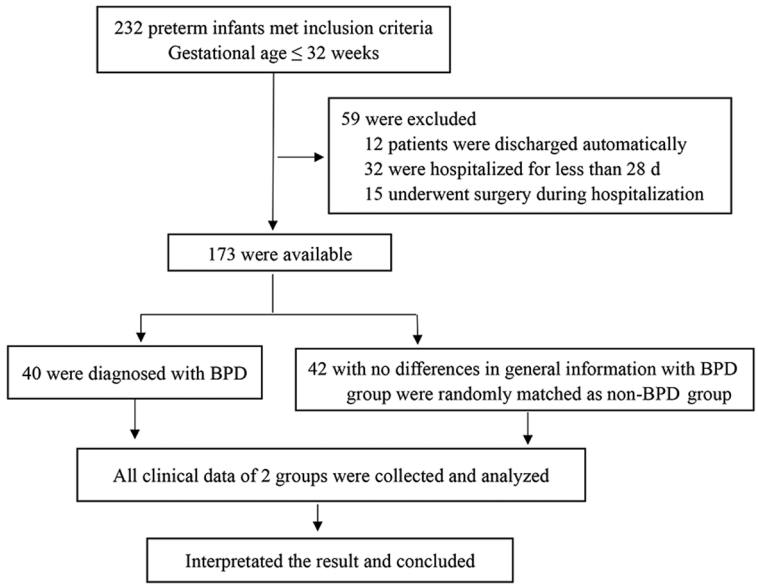

FIGURE 1
The flow chart of study design.

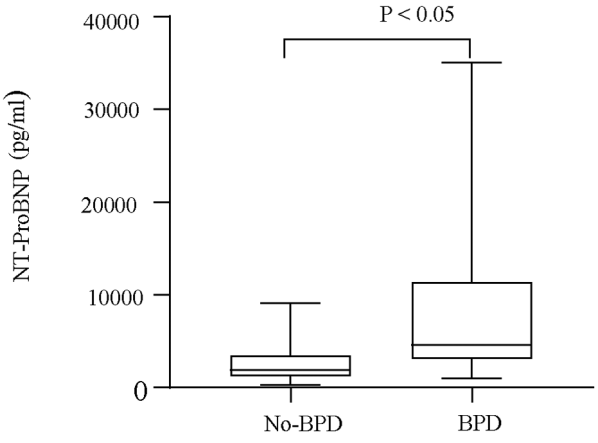

FIGURE 2
Serum NT-proBNP levels of BPD and no-BPD patients.

measurements. Mourani et al. (24) assessed the clinical value of using echocardiography to diagnose PH in infants with BPD and other lung diseases. We estimated PAP using echocardiography by monitoring the tricuspid valve regurgitation jet velocity in infants with all types of lung disease caused by various etiologies; echocardiographic abnormalities in the TR jet showed a high PH prediction accuracy. In infants with BPD, elevated pulmonary arterial pressure determined using echocardiography is usually associated with serious conditions and a substantial risk of mortality (25). In the United States, echocardiography is usually considered a less invasive tool for evaluating elevated PAP in preterm infants who have moderate or severe BPD (26, 27). Further, echocardiography is often used to measure PVR indirectly by calculating the blood flow velocity of the TR to estimate PAP (28).

In this study, the TR velocity was higher in patients with BPD than in those without BPD. Although TR velocity has not been reported to be related to the occurrence of BPD, elevated pulmonary arterial pressure leads to an increased and continuous deterioration of pulmonary circulation resistance and abnormal developments in pulmonary capillaries.

NT-proBNP has been widely used to diagnose heart failure and is often recognized by cardiomyocytes in response to excessive pressure and volume overload (29). NT-proBNP has a relatively stable chemical structure *in vitro* and can also remain in stable in blood samples after being drawn or preserved for over 72 h (30). NT-proBNP levels may also be valuable in predicting severe and moderate BPD, as indicated in a prospective study (31, 32). In preterm infants, excessive PAP as well as high-concentration oxygen absorption in immature lungs with an ongoing maturation process of the microstructure in the alveolar and microvascular regions may lead to textural anomalies of pulmonary vessels. Neonates with persistent PH show higher serum NT-proBNP levels (33), which can indicate the left ventricular load. After birth, the infant circulatory system transits from intrauterine fetal circulation to postnatal neonatal circulation, which is always accompanied by lung expansion; this may elevate systemic pulmonary vascular resistance and increase pulmonary blood flow volume. These changes may also increase ventricular

TABLE 5 Sensitivity, specificity, and youden Index of independent risk factors.

Variable	Sensitivity (%)	Specificity (%)	AUC	95%CI	*P*	Cut-off	Youden index
Tricuspid regurgitation velocity	88.10	62.50	0.735	0.623–0.848	0.000	1.45	0.506
NT-proBNP	69.00	80.00	0.802	0.709–0.896	0.000	2688.30	0.490
VAP	—	—	0.638	0.517–0.760	0.031	—	—
Days of $FiO_2 > 40\%$	85.7	87.5	0.846	0.751–0.941	0.000	1.50	0.732
Transfusion volume of red blood cells	88.10	85.00	0.903	0.832–0.974	0.000	0.73	16.616
Proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 days after birth (%)	—	—	0.780	0.676–0.885	0.000	—	—
Prediction model	97.60	92.50	0.986	0.968–0.999		0.60	0.901

NT-proBNP, *N*-terminal-pro-B-brain natriuretic peptide; VAP, ventilator-associated pneumonia; —, Not calculated.

TABLE 6 Predictive model for BPD.

Variable	β	SE	Wald	*P*	OR	95%CI
Tricuspid regurgitation velocity (m/s)	1.726	0.847	4.157	0.041	5.619	1.069–29.534
NT-proBNP	0.001	0.001	3.588	0.058	1.001	1.000–1.002
VAP	0.821	1.351	0.369	0.543	2.273	0.161–32.125
Days of $FiO_2 > 40\%$ (d)	1.409	0.576	5.974	0.015	4.092	1.322–12.665
Transfusion volume of red blood cells (ml/kg)	0.090	0.039	5.289	0.021	1.095	1.013–1.182
Proportion of infants who received total enteral nutrition (120 kcal/kg.d) ≥24 days after birth (%)	4.174	1.820	5.261	0.022	65.001	1.835–2301.904

NT-proBNP, *N*-terminal-pro-B-brain natriuretic peptide; VAP, ventilator-associated pneumonia.

volumes and pressure loads, which can stimulate BNP synthesis and secretion in the ventricle in the early days after birth (34). Serum BNP levels are higher after birth and decrease with the maturation of cardiac functions. Serum NT-proBNP levels often fluctuate with the mean pulmonary arterial pressure in the early days after birth in preterm neonates (35). Several studies have attempted to demonstrate the pathophysiological and clinical applications of serum NT-proBNP levels in patients with BPD. Sellmer et al. conducted a study involving 183 infants born at a gestational age ≤32 weeks and revealed that higher than normal levels of serum NT-proBNP three days after birth were closely related to an increased risk of BPD or higher mortality in preterm infants (36). Therefore, we speculate that increased NT-proBNP levels in premature infants within 24 h after birth is related to increased PAP, but these levels may also be related to an increase in early infection, inflammatory stimulation, and pro-inflammatory cytokines in premature infants with BPD.

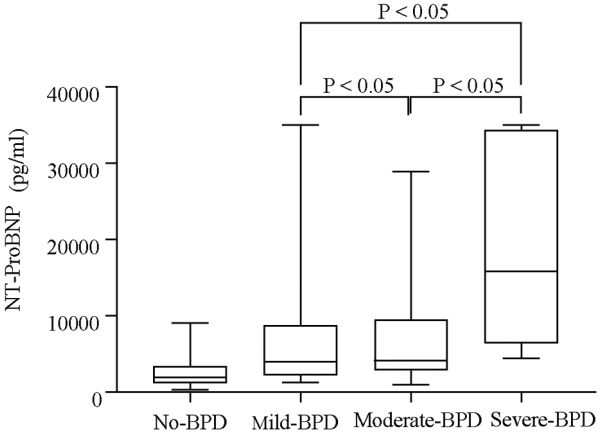

FIGURE 3
Serum NT-proBNP levels of different groups based on the severity of BPD (no-BPD, mild BPD, moderate BPD, and severe BPD).

Nutrition supplementation has an important function in the treatment and growth of infants; preterm infants with a higher volume of daily fluid and calorie intake and less body weight loss are at a higher risk for BPD in the early days of the first postnatal week (37). Greater quantities of fluid and nutrition intake to prevent weight loss may also cause pulmonary edema and worsen lung function in infants. The median age for reaching the desired calorie and energy intake (120 kcal/kg.d) through enteral feeding was 24 d in the 82 preterm infants included in this study. The proportion of infants who received total enteral nutrition (120 kcal/kg) d) ≥24 d after birth was higher in the BPD group; this also represented a potential risk factor for BPD depending on the logistic regression analysis, indicating that reaching the goal energy and calorie intake through enteral feeding for over 24 d was also a risk factor for developing BPD. Therefore, constant improvements and continuous optimization of the

administered nutritional formula may improve the treatment and prognoses of infants and reduce the morbidity of BPD.

Our study found that the number of preterm infants with oxygen inhaled days of $FiO_2 > 40\%$ and the proportion of preterm infants diagnosed with VAP was significantly higher in the BPD group; this result is consistent with previously reported results (38). High concentrations and long durations of oxygen intake may be harmful and toxic. Preterm infants requiring an oxygen supply concentration of over 30% during resuscitation, regardless of duration, were at a lower risk for developing BPD than those in the 90% or higher concentration oxygen group (39).

VAP is a severe mechanical ventilation complication that represents the second most common and difficult-to-cure infection in NICUs (40). Neonatal VAP seems to be significantly correlated with increased mortality, a longer duration of invasive mechanical ventilation, and longer hospital and NICU stays, especially in extremely preterm neonates (41, 42). Lung tissue inflammation and injury caused by VAP may substantially negatively influence lung alveolar and pulmonary alveolarization development in the early and critical stage in the postnatal period, and this pathophysiological process may partially explain the persistently high incidence rate of BPD (43, 44).

Anemia is commonly observed in preterm infants. Transfusions of RBC represent one of the most important methods of treating preterm infants; however, RBC transfusions are related to serious illnesses, especially BPD and cerebral hemorrhage, and they can also cause other diseases, such as NEC (45). Patel et al. suggested that serious diseases, such as BPD and NEC, in low gestational and birth weight infants are more likely to be associated with severe anemia rather than complications from the transfusion itself (46). In our study, the volume of RBC transfusions was one of the potential risk factors for BPD. In future, medical professionals should consider using pharmacological treatments to replace blood transfusions.

BPD is a multifactorial disease that is evolved by a complex combination of prenatal risk factors. Therefore, the present study aimed to establish a multifactorial prediction model for early detecting the BPD using a combination factors. The model that including molecular biomarkers and clinical data and echocardiograms can help to predict the development of BPD.

Limitations

This study has many limitations, including the small number of cases considered from a hospital and single clinical center. Although the incorporated multi-factor multifactorial model may help predict the occurrence and development of BPD in preterm infants with a relatively high sensitivity and specificity, it also displayed some disadvantages in that it could not predict the severity of BPD in preterm infants. Multicenter studies with variable grades of hospitals as well as a large sample size are needed to improve and enhance the BPD prediction models; this research could potentially increase prediction accuracy and improve effectiveness in preventing the progression of BPD.

Conclusions

In summary, inflammation, hyperoxia, blood transfusion, and malnutrition can lead to the development of BPD. TR flow rate and NT-proBNP levels were positively correlated with the occurrence of BPD. No single factor was effective in predicting BPD, but a combined regression model constructed using multiple indicators may predict the occurrence of BPD with an increased accuracy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by All parents or legal guardians of the participants provided written informed consent. This study was approved by the Ethics Committee of the Children's Hospital of Soochow University (ethics review number: 2020CS023). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

HW and DY designed the study and wrote the manuscript. ZW and HG conducted the clinical data collection and data analysis. XPZ and XLZ supervised the study design and execution, performed the final data analyses, and contributed to the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Xiaoguang Zhou,
Nanjing Children's Hospital, China

REVIEWED BY
Benjamim Ficial,
Integrated University Hospital Verona, Italy
Christoph Bührer,
Charité Universitätsmedizin Berlin, Germany

*CORRESPONDENCE
Wisam Muhsen
✉ wisam.muhsen@plymouth.ac.uk

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Echocardiography parameters used in identifying right ventricle dysfunction in preterm infants with early bronchopulmonary dysplasia: A scoping review

Wisam Muhsen[1,2]*, Eirik Nestaas[1,3,4], Joanne Hosking[5] and Jos M. Latour[1,6]

[1]Faculty of Health, University of Plymouth, Plymouth, United Kingdom, [2]Neonatal Intensive Care Unit, University Hospitals Plymouth NHS Trust, Plymouth, United Kingdom, [3]Faculty of Medicine, University of Oslo, Oslo, Norway, [4]Clinic of Pediatrics and Adolesence, Akershus University Hospital, Nordbyhagen, Norway, [5]Medical Statistics, Faculty of Health, University of Plymouth, Plymouth, United Kingdom, [6]Faculty of Health Sciences, Curtin University, Perth, WA, Australia

Background: Bronchopulmonary Dysplasia (BPD) is a chronic condition that affects preterm infants and is associated with long-term complications. Haemodynamic effects of BPD can lead to right ventricular (RV) dysfunction.
Objective: To synthesise and map the evidence of echo parameters used in identifying RV dysfunction in the first two weeks-after-birth (WAB) of preterm infants with early BPD.
Information Sources: This scoping review included the databases: Medline, CINAHL, PubMed, EMBASE, Scopus, ProQuest, Web of Science, Cochrane Library, JBI Evidence-Based Practise and Gray Literature.
Search Strategy: The search utilised Boolean operators and descriptors registered in Medical Subject Headings.
Inclusion and exclusion criteria: Included were studies utilising echo parameters to examine RV function in preterm infants with early BPD in the first two WAB.
Synthesis of results: The results are presented as a map of the extracted findings in a tabular format with a narrative summary.
Results: Eight studies were included. Differences were observed in the number and timing of echo scans performed in the first two WAB and the variations in the echo parameters used to compare preterm infants with and without early BPD. Only echo scans performed at the end of the first WAB, demonstrated significant differences in the echo parameters measurements between preterm infants with and without BPD. Studies using RV Myocardial Performance Index (MPI) to identify RV-dysfunction associated with early BPD demonstrated similar findings. The Pulsed-Wave Doppler technique identified differences in RV-MPI between preterm infants with and without BPD, while Tissue-Doppler-Imaging did not demonstrate similar results. Speckle tracking can measure strain (S) and strain rate (SR) and diagnose RV-dysfunction. However, the findings of studies that utilised speckle tracking varied. Finally, two of the included studies added blood tests to their diagnostic model of early BPD, which was able to demonstrate significant differences in blood test results between BPD-affected and control preterm infants.

Conclusion: BPD could adversely affect the myocardium function of the RV; these negative influences can be captured in the first two WAB. However, there are still knowledge gaps regarding the appropriate number, timing and the most suitable echo parameters to assess RV function.

KEYWORDS

preterm infants, right ventricular function, bronchopulmonar dysplasia, echocardiogaphy, haemodynamic effects, scoping review

1. Introduction

Bronchopulmonary Dysplasia (BPD) was first described in 1967 (1). It affects the immature lungs of preterm infants. Preterm infants born before 32 weeks of gestation are at a greater risk of developing BPD, especially those requiring respiratory support with higher oxygen requirements (2). The incidence rate of this disease remains high; a moderate and severe form of BPD affects almost a third of the preterm infants born before 32 weeks of gestation (3). The incidence rate of BPD is expected to continue to be elevated, especially when the viability threshold is reduced to 22 weeks of gestation, while more at-risk extreme preterm infants are expected to survive (4).

Bronchopulmonary Dysplasia negatively affects the normal growth and development of the immature lungs' alveoli and vascular bed through complex processes, which can reduce gas exchange surfaces and subsequently result in a decline in pulmonary function (5). The vascular pathogenesis theory hypothesises that as the premature heart and the premature lungs' vascular bed are closely interlinked, a negative effect on one of them will also be reflected on the other. Abnormally affected lungs by early BPD will also impair angiogenesis of the vascular bed of the lungs and the formation of pulmonary vascular disease (PVD), which can adversely affect the function of the right ventricle (RV) of the heart.

The pulmonary vascular remodelling results in a rise in the vascular tone, altered reactivity, vasoconstriction, and increased pulmonary vascular resistance (6). These histologic changes can increase the RV afterload pressure. Subsequently, the chronic increase in RV afterload pressure and hypoxic episodes can result in RV dysfunction, hypertrophy and, in severe cases, failure (7). Furthermore, severe forms of BPD are associated with a higher incidence of pulmonary arterial hypertension (8), which results from an increase in pulmonary arterial pressure and is associated with significant co-morbidities and high mortality rates (9).

The literature showed that the pathological effects of early BPD and respiratory insufficiency could manifest as early as day 7 of postnatal life (10). Data from 1,735 infants born between 23 and 30 weeks of gestational age showed that the proportion of these preterm infants needing oxygen decreases from birth to day 7 of postnatal life, followed by a steep rise in the number of infants requiring oxygen in the same cohort at the start of the second week postnatal (10). Additionally, studies, albeit limited, such as by Czernik et al., 2012 (11), demonstrated that the negative effect of PVD associated with early BPD on the function of the RV is present as early as the first two weeks after birth.

To address the gap in knowledge and provide state-of-the-art current knowledge, the aim of this scoping review is to identify, synthesise and map the evidence of echo parameters used in identifying dysfunction of the RV in the first two weeks of postnatal life of preterm infants with early BPD. This review will examine the echo parameters in preterm infants born before 32 weeks of gestation since these infants are at higher risk of developing BPD. In addition, the review will include studies which performed echo scans in the first two weeks of postnatal life as it is the period when early BPD starts to manifest clinically. Specifically, the scoping review questions were: (i) What techniques are used to capture echo images in preterm infants? (ii) When are the echo scans performed in the first two weeks after birth? (iii) What echo parameters are used to assess the haemodynamic effects of early BPD on the function of the right ventricle? and (iv) How are the echo parameters measured and analysed?

2. Methods

The preliminary literature search on 01 April 2022 in Medline (OVID), Cochrane Library, PROSPERO and the JBI Evidence Synthesis Database revealed no scoping or systematic review available or currently being developed about this subject.

The proposed scoping review is conducted according to Joanna Briggs Institute (JBI) methodology (Peters M. D. J., 2020). Also, this scoping review used the reporting guideline "Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension for scoping reviews (PRISMA-ScR): checklist and explanation" (12).

2.1. Participants, concept and context

Participants: The scoping review included studies that examined the first two weeks of postnatal life of preterm infants born before 32 weeks of gestation.

Concept: The review included studies that examined the haemodynamic effects of PVD associated with early BPD on the RV function through the analyses of the echo parameters. The included studies should have at least one echo scan performed in the first two weeks after the birth of the participating preterm infants. In addition, eligible studies should have compared the data collected from echo parameters analyses in preterm infants with and without BPD.

TABLE 1 Search strategies. (Search completed on the 02nd of April 2022).

Database	Search strategy		Records retrieved
Medline (OVID)	((preterm infants or premature infants or preterm baby or premature baby) and bronchopulmonary dysplasia and (Right ventricular function or right ventricular dysfunction) and (echocardiography or echo or echocardiogram))		11
CINAHL Plus with Full Text (EBSCO*host*)	((preterm infants) OR (premature infants) OR (preterm baby) OR (premature baby)) AND (bronchopulmonary dysplasia) AND ((right ventricular function) OR (right ventricular dysfunction)) AND (echocardiography OR echo OR echocardiogram)		8
EMBASE	((preterm infants or premature infants or preterm baby or premature baby) and bronchopulmonary dysplasia and (Right ventricular function or right ventricular dysfunction) and (echocardiography or echo or echocardiogram))		15
Pubmed	("preterm infants"[All Fields] OR "premature infants"[All Fields] OR "preterm baby"[All Fields] OR "premature baby"[All Fields]) AND "bronchopulmonary dysplasia"[All Fields] AND ("right ventricular function"[All Fields] OR "right ventricular dysfunction"[All Fields]) AND ("echocardiographies"[All Fields] OR "echocardiography"[MeSH Terms] OR "echocardiography"[All Fields] OR ("echo"[Journal] OR "echo"[All Fields]) OR ("echocardiography"[MeSH Terms] OR "echocardiography"[All Fields] OR "echocardiogram"[All Fields] OR "echocardiograms"[All Fields]))		14
Scopus	(Preterm AND infants OR premature AND infants OR preterm AND baby OR premature AND baby) AND ("bronchopulmonary dysplasia") AND (right AND ventricular AND function OR right AND ventricular AND dysfunction) AND (echocardiography OR echo OR echocardiogram)		72
ProQuest	(preterm OR premature) AND (infant OR baby) AND (bronchopulmonary dysplasia) AND ((right ventricular function) OR (right ventricular dysfunction)) AND (echocardiography OR echo OR echocardiogram)		143
Web of Science	(((ALL = ((preterm infants) OR (premature infants) OR (preterm baby) OR (premature baby))) AND ALL = ((bronchopulmonary dysplasia))) AND ALL = ((right ventricular function) OR (right ventricular dysfunction))) AND ALL = (echocardiography OR echo OR echocardiogram)		51
Cochrane library	6.3 (preterm infants) OR (premature infants) OR (preterm baby) OR (premature baby) in Title Abstract Keyword AND (bronchopulmonary dysplasia) in Title Abstract Keyword AND (right ventricular function) OR (right ventricular dysfunction) in Title Abstract Keyword AND echocardiography OR echo OR echocardiogram in Title Abstract Keyword Words variations have been searched.		2
JBI EBP Database	((preterm infants or premature infants or preterm baby or premature baby) and bronchopulmonary dysplasia and (Right ventricular function or right ventricular dysfunction) and (echocardiography or echo or echocardiogram))		Zero
British Library—explore further	The following terms were searched with "Anywhere" option [Preterm infants OR premature infants OR preterm baby OR premature baby] AND ["right ventricular function" OR "right ventricular dysfunction" AND "echocardiography" OR "echo" OR "echocardiogram"] AND ["bronchopulmonary dysplasia"] Material type: all items No limitations on the date Search scope: explore further. Limitations: only human		35
EThOS E-thesis online service	"\(preterm infants\) OR \(premature infants\) OR \(preterm baby\) OR \(premature baby\)" AND "\(bronchopulmonary dysplasia\)" AND "\(right ventricular function\) OR \(right ventricular dysfunction\)" AND "echocardiography OR echo OR echocardiogram"		Zero
Google Scholar	with **all** of the words	Right ventricular	150
	with the **exact phrase**	bronchopulmonary dysplasia	
	with **at least one** of the words	function dysfunction preterm premature infants baby echocardiography echo echocardiogram	
	without the words	catheterization OR MRI OR CT	
	Where my words occur	Anywhere in the article	
Total number of articles in all the searched databases			501

Context: The review included studies that recruited preterm infants admitted to a neonatal intensive care unit.

2.2. Types of evidence and sources

This scoping review considered all study designs, including experimental and quasi-experimental studies. Randomised controlled trials, non-randomised controlled trials, before and after studies and interrupted time-series studies were assessed for eligibility. Any systematic reviews that meet the inclusion criteria were considered, but none were retrieved. The review also considered analytical observational studies, such as prospective and retrospective cohort studies, case-control studies and analytical cross-sectional studies. There were no limitations regarding the date of the publication.

Papers were excluded if they did not fit in the conceptual framework of this review. In addition, studies were excluded if they used different imaging modalities, such as Magnetic Resonance Imaging or Computed Tomography or were not available in full text. Studies that did not assess the RV function or echo scans performed after the first two weeks after birth were also excluded. Studies were excluded if they were unavailable in English or were animal studies.

2.3. Search terms

The selected text words and index terms, such as preterm infants, right ventricular function, bronchopulmonary dysplasia, and echocardiography, were utilised to formulate a full search strategy (**Table 1**).

2.4. Search strategy

A comprehensive search strategy was followed and consisted of three steps.

First step: An initial limited search of two online databases, Medline (OVID) and CINHAL Plus Full text (EBSCOhost), was conducted to allocate related articles. The examination of the relevant articles resulted in the identification of the text words included in the titles and the abstracts and the index terms related to these articles to guide the subsequent detailed search as per the advice of a research librarian.

Second step: The full search strategy was applied to the included databases with the identified keywords and index terms. The descriptors registered in the Medical Subject Headings (MeSH) were used wherever possible. The synonyms were combined with the Boolean operator "OR" while the groups of words were combined with the operator "AND" (**Table 1**). The following databases were searched: Medline (OVID), CINAHL Plus with Full Text (EBSCOhost), PubMed, EMBASE, Scopus, ProQuest, Web of Science, Cochrane Library, JBI Evidence Based Practise (EBP) and Gray Literature (British Library, google scholar & EThOS).

Third step: A reference list containing all the identified studies was formed. There were no scoping nor systematic reviews identified.

A full search was performed by two reviewers (WM and JML). Any unresolved dispute was discussed with a third reviewer (EN).

2.5. Studies selection

The studies were selected from the included databases. All the identified citations were uploaded into EndNote 20 (Clarivate Analytics, PA, USA), followed by removing duplicates. Then, studies were selected by two reviewers (WM and JML) in two stages; titles and abstracts review; and full-text review, against the inclusion criteria. A detailed assessment of the full text of the selected articles against the inclusion criteria was performed by two independent reviewers (WM and JML). Any disagreements were discussed and resolved by both reviewers.

2.6. Data extraction

Two independent reviewers (WM and JL) performed the data extraction process using the tool developed by the reviewers for this purpose. The data extraction tool contained details of the population, concept, context, methodology and relevant findings. No modifications to the data extraction tool were performed.

3. Results

The final scoping review full report of the search results is presented in the PRISMA flow diagram (**Figure 1**). There were 501 records identified from 12 databases. Sixty-two duplicates were removed, leaving 439 records eligible for the first screening stage, i.e., titles and abstracts were screened. After the exclusion of 415 records, only 24 papers were eligible for the second stage of full-text screening. Sixteen articles were excluded (**Figure 1**), and eight studies were included in the final critical appraisal and analysis.

3.1. Data charting

Data were extracted from the full texts of the eight included studies and detailed into two tables (**Tables 2**, **3**). Table one described the studies' methodologies, while table two included details about the echo scans' timing, echo parameters analyses and the key findings. The results are presented in narrative descriptions and mapping of the data.

3.2. Narrative description of studies

The eight included studies were conducted in the USA and European countries. All studies were conducted in tertiary perinatal centres. Only four studies examined exclusively preterm infants born before 32 weeks of gestation (11, 13–15) (**Table 1**). The study by Helfer et al., 2014, included preterm infants born at 32 weeks gestation as the recruitment was per birth weight rather than gestational age (16). Another study included preterm infants with a higher gestation age, and the birth weight needed to be within a specific range (≤34 weeks gestation and birth weight between 500 and 1,250 grams) (17). The other two studies by Levy and colleagues focused on recruiting preterm infants with lower gestational age (23–28 weeks of gestation) (18, 19).

Different definitions to diagnose BPD were used in the selected eight studies. Czernik et al., 2012, diagnosed preterm infants with BPD based on their oxygen requirement to maintain pre-ductal arterial saturations of 92% at 36 weeks PMA (20, 21). Levy et al., 2017, utilized a modified Shennan definition (22). While the remaining six studies used the definition set in the NIH workshop (23). There is a lack of the definition of an early BPD, i.e., during the first two weeks after birth, in the selected studies.

3.2.1. What are the techniques used to capture the echo images

Several techniques were used to capture the echo images in the selected studies, such as 2D echo images, Pulsed wave (PW) Doppler, Tissue Doppler Imaging (TDI), 2D speckle tracking and M-mode.

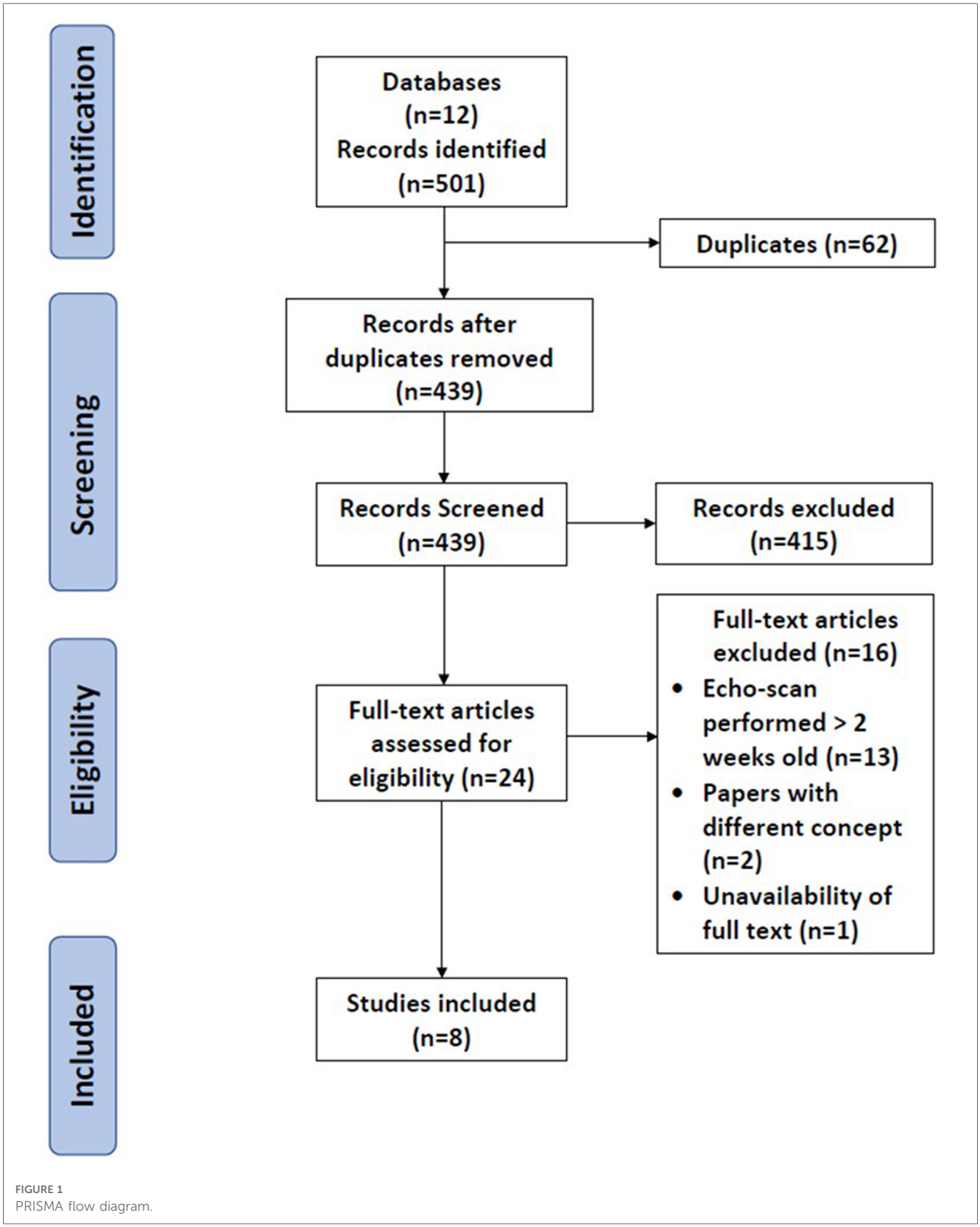

FIGURE 1
PRISMA flow diagram.

3.2.2. When were the echo scans performed in the first two weeks after birth?

The studies' timing and frequency of echo scans varied (Table 2). Three studies performed one echo scan within the first two weeks after birth (13, 15, 17); one study had the echo scans performed on the first day after birth (DAB) (17), and the other two studies performed the echo scans at the 7th DAB, respectively (13, 15). The remaining five studies had several echo

TABLE 2 Selected studies methodologies.

Authors & year	Country of origin	Aims	Population & sample size	Settings & methodology
Bokiniec et al., 2017	Poland	Evaluation of right ventricular function in preterm infants with & without BPD	A total of 89 preterm infants (<32 weeks gestation) were included: – No-BPD $n = 32$ – Mild-BPD $n = 35$ – Severe-BPD $n = 15$ – Excluded preterm infants $n = 7$ (Died in early days)	A prospective, single-centre study was conducted at a tertiary perinatal centre (2009–2014). Echo parameters were obtained *via* one of the following methods: – Pulsed wave (PW) Doppler. – Tissue Doppler Imaging (TDI). Appropriate statistical tests were used for analyses.
Czernik et al., 2012	Germany	Assessing the usefulness of the right ventricular index of myocardial performance (RIMP) to estimate pulmonary vascular resistance in very low birth weight infants.	A total of 143 preterm infants (<32 weeks gestation) were eligible for inclusion: Preterm infants included in the final analysis were $n = 121$: No-BPD $n = 85$ Preterm infants with BPD $n = 36$ Preterm infants were excluded from the final analysis were $n = 22$: Preterm infants with congenital heart disease $n = 4$. Preterm infants died within four weeks after birth $n = 18$.	A prospective, single-centre study was conducted at a tertiary perinatal centre from September 2008 till January 2010. The study was approved by the ethics committee at the institution. Echo parameters were obtained *via*: – Pulsed wave (PW) Doppler. Appropriate statistical tests were used for analyses.
Di Maria et al., 2015	USA	The aim was to prospectively study maturational changes in diastolic tissue velocities at two points in time; at seven days old and at 36 weeks post-menstrual age (PMA). Further analysis was performed to establish whether DTI measures were altered in infants with bronchopulmonary dysplasia (BPD) with or without pulmonary hypertension (PH).	Preterm infants were eligible for inclusion if they were ≤34 weeks gestation and had a birth weight between 500 and 1,250 grams. Enrolment is to take place within seven days after birth. The total number of enrolled preterm infants was 277 (274 received echo assessment at seven days after birth): Preterm infants included in the final analysis were: – Preterm infants without BPD $n = 111$. – Preterm infants with BPD $n = 166$.	A prospective, multi-centre trial was conducted from July 2006 till March 2012. The study team obtained ethical approvals from the participating institutions (University of Colorado Denver and Indiana University). Echo parameters were obtained *via* one of the following methods: – Pulsed wave (PW) Doppler. – Tissue Doppler Imaging (TDI). Appropriate statistical tests were used for analyses.
Helfer et al., 2014	Germany	The study aims to examine the development of cardiac function in preterm infants by measuring tissue Doppler-derived peak systolic strain (PSS) and strain rate (PSSR) in the first 28 days after birth. It is also to assess the impact of BPD on PSS & PSSR.	A total of 119 preterm infants (birth weight <1500 g) were included: Images from 110 preterm infants were suitable for analysis: – Preterm infants without BPD $n = 79$. – Preterm infants with BPD $n = 31$.	A prospective, single-centre trial was conducted at a tertiary perinatal centre from September 2008 to January 2010. The study was approved by the ethics committee at the research team institution. Echo parameters were obtained *via*: – Pulsed—Tissue Doppler Imaging (TDI). Appropriate statistical tests were used for analyses.
Levy et al., 2015	USA	The study aimed to determine the maturational (age- and weight-related) changes in the Right Ventricle fractional area of change (RV FAC) and RV areas and to establish reference values in healthy preterm and term neonates.	A total of 115 preterm infants (23–28 weeks of gestation) were enrolled: Preterm infants included in the final analysis were $n = 115$: – No or mild BPD $n = 60$ – Preterm infants with moderate-severe BPD $n = 55$.	A prospective, longitudinal and single centre was conducted at a tertiary perinatal centre from August 2011 till August 2013. The study was approved by the ethics committee at the institution. Echo parameters were obtained *via*: – 2D and RV-focused 4chambers (4ch) view. 2D speckle tracking. Appropriate statistical tests were used for analyses.
Levy et al, 2017	USA/Republic of Ireland	The study aims to determine the maturational changes in systolic strain mechanics of Ventricles by 2D speckle tracking echocardiography in extreme preterm neonates from birth to one year of age. Also, it assesses the impact of disorders, e.g., BPD, on the deformation measures.	A total of 239 preterm infants (23–28 weeks gestation) were enrolled; 17 participants died before discharge from the hospital, so only 222 preterm infants were included in the data analysis: Healthy preterm infants BPD $n = 103$. Preterm infants with the following diagnoses ($n = 119$): Preterm infants with BPD $n = 116$. Preterm infants with pulmonary hypertension $n = 17$. Preterm infants with PDA $n = 100$.	A prospective, longitudinal and multi-centre study was conducted at hospitals affiliated with two academic institutions (Washington University School of Medicine, Saint Louis Children's Hospital, and Royal College of Surgeons in Ireland, Rotunda Hospital) from August 2011 to January 2016. The study was approved by the institutional review board of Washington University, and the ethical committee on human research at the Royal College of Surgeons approved the protocol. Echo parameters were obtained *via*: – Two-Dimensional Speckle Tracking Imaging. Appropriate statistical tests were used for analyses.

(*continued*)

10.3389/fped.2023.1114587

TABLE 2 Continued

Authors & year	Country of origin	Aims	Population & sample size	Settings & methodology
Mendez-Abad et al., 2020	Spain	The study aims to assess the influence of BPD on the maturation of the myocardium in very low birth weight infants (VLBWIs).	Preterm infants born at ≤32 weeks gestation and/or ≤1500 g were eligible for inclusion. Preterm infants included in the final analysis were $n = 101$: – No-BPD $n = 86$ – Preterm infants with BPD $n = 15$	A prospective, observational, single-centre study was conducted at a tertiary perinatal centre from January 2015 to 2017. The study was approved by the ethics committee at the study institution. Echo parameters were obtained *via*: – Tissue Doppler Imaging (TDI). – M-Mode. Appropriate statistical tests were used for analyses.
Neumann et al., 2021	Germany/ Switzerland	The study aims to examine the prognostic capability of early echocardiographic assessment of right ventricular function and vasoactive peptides to predict BPD or death in very preterm infants.	Preterm infants born at <32 weeks gestation were eligible for inclusion. Preterm infants included in the final analysis were $n = 294$: – No-BPD $n = 229$ – Preterm infants with BPD/death $n = 65$	A prospective, observational, multi-centre study was conducted at two tertiary perinatal centres (University Children's Hospital Basel, Switzerland and Charité Universitätsmedizin Berlin, Germany) from January 2015 to November 2017. The study was approved by the ethics committees at the two study institutions. Echo parameters were obtained *via* one of the following methods: – Pulsed wave (PW) Doppler. – M-Mode. Appropriate statistical tests were used for analyses.

scans performed within the first two weeks after birth (11, 14, 16, 18, 19). There were variations between the studies regarding the timing when the echo scans were performed in the first 14 days after birth; Czernik et al., 2012 trial, the echo scans were performed on day 2, 7, 14 after birth (11); Helfer et al., 2014 study team performed the echo scans on day 1, 7, 14 after birth (16); Levy et al., 2015 study team performed the echo scans on day 1 and day 7 after birth (18); Levy et al., 2017 study teams performed the echo scans on day 1, 2, 5 and 7 after Birth (19); finally, Mendez-Abad et al., 2020 team per, the echo scans were performed on day 1, 3, 7, 14 after birth (14).

3.2.3. What echo parameters are used to assess the haemodynamic effects of early BPD on the function of the right ventricle?

The critically appraised studies utilised different echo parameters to assess the function of the RV in healthy preterm infants (controls) and the ones affected by BPD (cases); RV-myocardial performance index (MPI), RV strain (S) and strain rate (SR), RV fractional area of change (RV-FAC), TDI systolic- and diastolic-velocities and tricuspid annular plane systolic excursion (TAPSE).

3.2.4. How were the echo parameters measured and analysed?

Studies utilising RV-MPI to assess RV function, the calculation of RV-MPI was performed by using one of the following two equations:

– RV-MPI calculation in TDI echo images: RV-MPI (TDI) = IVCT + IVRT/ET.
(IVCT = Isovolumic Contraction Time; IVRT = Isovolumic Relaxation Time; ET = Ejection Time)
– RV-MPI calculation in PW echo images: RV-MPI (PW) = a−b/b.
(a = the measurement between cessation and onset of the tricuspid valve inflow; b = the ejection time of the RV outflow)

Four studies showed a delay in the normal maturation of the RV-MPI, i.e., persistently raised RV-MPI in BPD-affected preterm infants in comparison to the controls. The differences in the RV-MPI between the two groups (preterm infants with and without early BPD) were detected in the echo scans performed after the first three days after birth (11, 13–15). In addition, Neumann et al., 2021, demonstrated that TAPSE is lower in BPD-affected preterm infants (15).

Other findings were identified when examining all the included studies. Neumann et al., 2021, demonstrated that TAPSE is lower in BPD-affected preterm infants (15). Levy et al., 2015, showed differences in the RV-FAC measurements between controls and BPD-affected preterm infants when assessed at 32 weeks of PMA echo scans (18). Two studies utilising S and SR also demonstrated statistically significant differences in these measurements between their controls and the cases of preterm infants (16, 19). However, the study by Helfer et al., 2014, demonstrated that the differences in the S & SR measurements between controls and BPD-affected preterm infants would start to manifest in the echo scans at day 14 after birth while the

TABLE 3 Selected studies details related to authorship, the timing, analysis and the key findings of the echo scans. .

Authors & Year	Timing of Echo Scans	Echo Parameters measured	Echo Parameters Calculation	Key Findings
Bokiniec et al., 2017	Echo scans were performed at three-time points: – First day after birth. – 28 days after birth. – 36 weeks of PMA.	– B was calculated from pulsed Doppler waveforms of TV inflow. – A was calculated from pulsed Doppler waveforms of RV outflow. – TV diastolic velocities; A & E waves. – TDI velocities, e.g., (A', E', S')	RV-MPI (Tei Index) was calculated *via*: RV-MPI (TDI) =IVCT + IVRT/ET RV-MPI (PW) =a−b/b. E/E' RV ratio	Increased RV-MPI *via* PW-Doppler at 28 days after birth in preterm infants with severe-BPD group compared with both the preterm infants without BPD ($p = .014$) and the preterm infants with mild-BPD groups ($p = .031$). No difference in RV-MPI between preterm infants with no-BPD and preterm infants with mild-BPD groups was detected ($p = .919$). No difference in E/E' RV ratio between the groups at any time point.
Czernik et al., 2012	Echo scans were performed at four-time points: – At the second day after birth. – At the 7th day after birth. – At two weeks after birth. – At the 28th day after birth.	– B was calculated from pulsed Doppler waveforms of TV inflow. – A was calculated from pulsed Doppler waveforms of RV outflow.	RV-MPI (Tei Index) was calculated *via*: RV-MPI = a−b/b.	– RV-MPI was decreased in the last three echo scans (7th, 14th & 28th days old) in preterm infants without BPD, while remained elevated in those infants who are affected by BPD; 7 days old: 0.31 [0.22–0.39] vs. 0.35[0.29–0.48], $p = 0.014$; the 14 days old: 0.23[0.17–0.30] vs. 0.35[0.25–0.43], $p = 0.001$ and 28 days old: 0.21[0.15–0.28] vs. 0.31 [0.21–0.35], $p = 0.015$).
Di Maria et al., 2015	Echo scans were performed at two-time points: – At the 7th day after birth. ($n = 274$) – At 36 weeks PMA. ($n = 277$)	– RV-free Wall—E' (cm/sec) – RV-Free Wall-A' (cm/sec) TV diastolic velocities; A & E waves.	– RV Free Wall-E'/A' – TV E/E'	When grouping the study participants by BPD or PH status, there was no significant association between TDI measures and the presence or absence of PH or BPD.
Helfer et al., 2014	Echo scans were performed at four-time points: – At the first day after birth (0–3). – At the 7th day after birth (6–10). – At two weeks after birth (12–17). – At the 28th day after birth (22–31).	– Peak Systolic Strain (PSS) of RV-free walls. – Peak Systolic Strain Rate (PSSR) of RV-free walls.	N/A	In preterm infants who developed BPD, PSS was significantly lower on days 14 and 28.
Levy et al., 2015	All the study participants ($n = 115$) had echo scans performed at two-time points; 32 and 36 weeks of PMA. *Thirty (30)* of the participants had additional echo scans at two-time points, 24 and 72 h after birth, i.e., these infants got *four echo scans* in total.	Right Ventricle fractional area of change (RV FAC).	RV-FAC = 100 × [RV end-diastolic area (cm^2)—RV end-systolic area (cm^2)]/ RV end-diastolic area (cm^2).	From one to three days of age, there was no difference in right ventricle end-diastolic area, RVEDA and right ventricle end-systolic area, or RVESA between the reference cohort (infants with mild or no BPD) and the infants with moderate or severe BPD. By 32 weeks of PMA, there was a statistically significant increase in RVEDA and RVESA ($p = 0.034$) amongst infants with moderate or severe BPD.
Levy et al., 2017	All the study participants had echo scans performed at four-time points, in addition to one follow-up echo at one (1) year of corrected age: 1, 2, 5–7 days after birth and at 32 and 36 weeks of PMA. At the Washington University School of Medicine site, echo scans were performed at: Day 1, $n = 30$. Day 2, $n = 30$. 32 weeks PMA, $n = 117$. 36 weeks PMA, $n = 117$. At the Royal College of Surgeons in Ireland site, echo scans were performed at: Day 1, $n = 102$. Day 2, $n = 102$. Day 5–7, $n = 98$. 36 weeks PMA, $n = 47$.	RV FWLS (%) = RV—Free Wall Longitudinal Strain. RV FWLSRs (1/s) = RV—Free Wall Longitudinal Strain Rate. RV SLS (%) = RV—Segmental longitudinal strain. IVS GLS (%) = Intraventricular Septum (IVS) Global Longitudinal Strain. IVS GLSR (1/s) = IVS Global Longitudinal Strain Rate. IVS SLS (%) = IVS Segmental longitudinal strain.	N/A	IVS SLS with preterm infants with BPD and/or PH remains in an apex-to-base pattern (highest to lower), reflective of RV regional gradient. Preterm infants who developed BPD not only had decreased RV- GLS and IVS GLS from 32 weeks PMA to one year CA, but a persistent base-to-apex (reflective of an RV dominant pattern) IVS strain gradient that never reversed, even by one year of age.

(*continued*)

TABLE 3 Continued

Authors & Year	Timing of Echo Scans	Echo Parameters measured	Echo Parameters Calculation	Key Findings
Mendez-Abad et al., 2020	Echo scans were performed at numerous time points; 24 and 72 h old, then weekly until 36 weeks of PMA. N-terminal pro-B type natriuretic peptide (NTproBNP) plasma level on the 14th day after birth.	Early diastolic (E′) velocity. Late diastolic (A′) velocity. Tricuspid Annular Plane Systolic Excursion (TAPSE).	N/A	There is a negative correlation between the plasma NTproBNP levels and TAPSE (Spearman's Rho = −0.36, $p = 0.0001$), with similar results regarding diastolic velocities derived of TDI. Myocardial function maturation in VLWBIs that develop BPD seems to be delayed.
Neumann et al., 2021	Echo scan and blood tests were performed on the 7th day after birth. Blood tests to measure the Plasma concentrations of mid-regional pro-atrial natriuretic peptide (MR-proANP) and C-terminal pro-endothelin-1 (CT-proET1), were performed at the same time of the echo scans.	– TAPSE. – B was calculated from pulsed Doppler waveforms of TV inflow. – A was calculated from pulsed Doppler waveforms of RV outflow.	RV-MPI (Tei Index) was calculated *via*: RV-MPI = a−b/b.	RV-MPI values were higher, and TAPSE values were lower in infants with BPD/death than in infants without BPD. Both vasoactive peptides (MR-proANP and CT-proET1) were significantly higher in BPD/death infants as compared to controls.

study of Levy et al., 2017, the S & SR differences between the controls and the cases of preterm infants with BPD, did not manifest till the echo scan at 32 weeks of PMA (16, 19).

In addition to using echo parameters, two of the eight studies performed blood tests. Mendez-Abad et al., 2020, analysed N-terminal pro-B type natriuretic peptide (NTproBNP) plasma level on day 14 after birth (14). Neumann et al., 2021, analysed the plasma concentrations of mid-regional pro-atrial natriuretic peptide (MR-proANP) and C-terminal pro-endothelin-1 (CT-proET1) on day 7 after birth (15). Both studies demonstrated a statistically significant difference in the plasma levels of the selected tests between the controls and BPD-affected preterm infants.

4. Discussion

There were methodological variations between the selected studies. There are differences in the number and the timing of echo scans performed in the first two weeks of postnatal life, together with the variations in the echo parameters used to compare the preterm infants as controls and the preterm infants affected by BPD.

Echo scans performed in the first three days after birth did not demonstrate a difference in the echo parameters measurements between the control group and preterm infants with early BPD. In comparison, significant differences in the echo parameters measurements between the controls and preterm infants with BPD were seen in the echo scans performed at the end of the first week after birth and the subsequent weeks (11, 13, 15, 16). Similarly, the study by Levy et al., 2015, showed that RV-FAC measurements in the first three days after birth did not differ between preterm infants without BPD and the ones who have BPD (18).

Studies using RV-MPI to identify RV dysfunction associated with early BPD have similar findings. Bokiniec et al., 2017, showed that PW-Doppler could identify the differences in RV-MPI between controls and preterm infants affected by early BPD, while TDI could not demonstrate differences in RV-MPI between preterm infants with and without BPD (13). The trials conducted by Czernik et al., 2012, and Neumann et al., 2021 showed similar results when RV-MPI *via* PW-Doppler could identify RV dysfunction in preterm infants with early BPD (11, 15). While Mendez et al. 2020 findings demonstrated that RV-MPI and diastolic velocities *via* TDI can be still a useful tool since it could detect the cardiac maturational changes in preterm infants. The same study showed that diastolic velocities *via* TDI are capable of recognising RV dysfunction in infants with early BPD (14).

Despite the limitations of PW-Doppler in calculating RV-MPI, e.g., it utilises the measurements from different cardiac cycles, three of the eight selected studies used PW-Doppler and were able to diagnose RV dysfunction in preterm infants with early BPD. On the other hand, despite TDI being a valuable and practical tool in assessing RV's systolic and diastolic function, TDI was unable to identify the RV dysfunction (13, 17). This could be explained by the inherent limitations of TDI, which is angle-dependent and influenced by the global cardiac motion and the inability to differentiate between passive and active motion (24). In addition, velocities and displacements depend on the cardiac sizes; hence, larger hearts have higher velocities and displacements. Therefore, it is crucial to normalise the TDI measurements according to the cardiac size (25). None of the studies that utilised TDI technique normalised the cardiac TDI measurements, which might negatively affect the ability of these studies to demonstrate a significant difference in the TDI measurements between controls and cases.

Speckle tracking can be used to measure S and SR and diagnose RV dysfunction. However, the findings of the studies that utilised speckle tracking in this review varied. A study by Levy et al., 2017, could not diagnose the RV dysfunction in the echo scans performed at day 1, 2, and 5 to 7 after birth (19). In contrast, a study by Helfer et al., 2014, demonstrated a significant difference in the S and SR between preterm infants with and without BPD when echo scans were performed at day 14 after birth (16). The findings of these two studies demonstrate that results can vary even when a reliable technique such as speckle tracking was used. This might be well related to the timing of the echo scans, i.e., if the echo scan were performed too soon, it might not

capture the hemodynamic changes related to RV dysfunction that is associated with early BPD.

Furthermore, two of the selected eight studies added blood tests to their diagnostic model of early BPD (14, 15). Both studies demonstrated that adding specific blood tests would enhance the diagnostic ability of RV dysfunction associated with early BPD (14, 15).

Although, adding a specific blood test to a diagnostic model might enhance its diagnostic ability, it might also have a negative impact on its applicability in many perinatal centres worldwide, especially in developing countries, due to, for example, the cost and unavailability of these blood tests. Likewise, it will negate the idea of having a diagnostic model based on a practical, applicable and non-invasive test such as an echo scan.

The primary strength of this review was its broad literature search. The process of synthesising the scientific evidence was transparent and systematic in identifying and mapping the evidence related the use of echocardiography to diagnose RV dysfunction associated with early BPD. Nevertheless, as this review designed as a systematic scoping review, it did not assess the quality of the evidence, so it does not provide the scientific basis to inform clinical decision making. Finally, language restrictions may have led to the exclusion of a few studies.

5. Conclusion

This scoping review showed the need to develop a diagnostic model based on a non-invasive echo scan. The review demonstrated the emergence of unanswered questions regarding the practicalities of when and how many echo scans are needed and which cardiac parameter is required to diagnose early BPD in preterm infants.

BPD will adversely affect the myocardium function of the RV; these negative influences can be captured in the first two weeks of postnatal life (15, 17). However, there are still knowledge gaps regarding the appropriate number, timing of the echo scans and the most suitable cardiac parameters to assess RV function.

Author contribution

WM: contributed to the main design and detailed concept and discussion of this review article; he drafted the first manuscript. JML: as a senior author revised the manuscript and added details to it. EN and JH revised the manuscript and added details. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Shahana Perveen,
Cohen Children's Medical Center,
United States

REVIEWED BY
Bradley Yoder,
The University of Utah, United States
Laurence Magder,
University of Maryland, United States

*CORRESPONDENCE
Kai Mu
✉ mkbest@yeah.net

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A prediction nomogram for moderate-to-severe bronchopulmonary dysplasia in preterm infants < 32 weeks of gestation: A multicenter retrospective study

Jing Zhang[1], Kai Mu[1]*, Lihua Wei[2], Chunyan Fan[3], Rui Zhang[2] and Lingling Wang[3]

[1]Department of Pediatric, Department of Pediatrics, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Shandong Engineering and Technology Research Center for Pediatric Drug Development, Jinan, China, [2]Department of Neonatology, Affiliated Hospital of Jining Medical College, Jining, China, [3]Department of Pediatrics, Zibo First Hospital, Zibo, China

Background: Moderate-to-severe bronchopulmonary dysplasia (msBPD) is a serious complication in preterm infants. We aimed to develop a dynamic nomogram for early prediction of msBPD using perinatal factors in preterm infants born at <32 weeks' gestation.

Methods: This multicenter retrospective study conducted at three hospitals in China between January 2017 and December 2021 included data on preterm infants with gestational age (GA) < 32 weeks. All infants were randomly divided into training and validation cohorts (3:1 ratio). Variables were selected by Lasso regression. Multivariate logistic regression was used to build a dynamic nomogram to predict msBPD. The discrimination was verified by receiver operating characteristic curves. Hosmer-Lemeshow test and decision curve analysis (DCA) were used for evaluating calibration and clinical applicability.

Results: A total of 2,067 preterm infants. GA, Apgar 5-min score, small for gestational age (SGA), early onset sepsis, and duration of invasive ventilation were predictors for msBPD by Lasso regression. The area under the curve was 0.894 (95% CI 0.869–0.919) and 0.893 (95% CI 0.855–0.931) in training and validation cohorts. The Hosmer–Lemeshow test calculated *P* value of 0.059 showing a good fit of the nomogram. The DCA demonstrated significantly clinical benefit of the model in both cohorts. A dynamic nomogram predicting msBPD by perinatal days within postnatal day 7 is available at https://sdxxbxzz.shinyapps.io/BPDpredict/.

Conclusion: We assessed the perinatal predictors of msBPD in preterm infants with GA < 32 weeks and built a dynamic nomogram for early risk prediction, providing clinicians a visual tool for early identification of msBPD.

KEYWORDS

bronchopulmonar dysplasia, preterm, nomogram, prediction model, multi-center study, retrospective study

Introduction

Bronchopulmonary dysplasia (BPD) is one of the most common and serious complications in very preterm neonates. Infants with moderate-to-severe BPD (msBPD) are more likely to die, and those who survive have an increased risk of asthma, chronic lung disease in adulthood, neurodevelopmental impairment, and growth failure (1). However, there is no definite effective therapies for the prevention and treatment of msBPD.

Although management strategies for very premature infants have greatly progressed, studies have shown that BPD affects 10%–89% of very preterm infants (2, 3). Many studies have shown that perinatal factors were risk factors for developing msBPD (4–6). As the immature lung of very preterm infants is the most vulnerable period during the first week of life. Perinatal prediction may help clinicians perform interventions for msBPD as early as possible. Laughon et al. (7) firstly reported prediction model for BPD and death in preterm with 23–30 weeks' GA. Greenberg et al. (8) updated a web based estimator for BPD and death in preterm with GA ranging from 23 + 0/7 weeks to 28 + 6/7 weeks. Min Song et al. (5) developed a nomogram for msBPD in a single-center study, which included the N-terminal Pro-B-Natriuretic Peptide level, gestational age (GA) and ventilation-assisted ventilation. Amit Sharma et al. (9) reported early prediction of msBPD for extremely premature infants. A meta-analysis conducted by Onland et al. reported poor predictability in most established prediction models for BPD (10). Although many prediction models for have been developed using logistic regression, no machine learning model for predicting msBPD in Chinese multi-center populations.

A multi-center study was conducted to establish a dynamic nomogram using least absolute shrinkage and selection operator (Lasso) regression for predicting msBPD. The calibration of the nomogram was assessed using Hosmer–Lemeshow test. A dynamic diagnosis model nomogram was a simple statistical visual tool which has been widely used. The occurrence of msBPD is multifactorial and related to both intrauterine factors and postnatal clinical management; we selected perinatal risk factors for the analysis by Lasso regression. Hence, our study aimed to develop a dynamic nomogram for the early prediction of msBPD to improve predictive validity and provide the evidence for clinicians to prevent msBPD.

Methods

Study design and population

All data of this multi-center retrospective study were obtained from three hospitals including the First Affiliated Hospital of Shandong First Medical University, Affiliated Hospital of Jining Medical College, and the First Hospital of Zibo, between January 2017 and December 2021. All three hospitals underwent standardized training before the start of the project. This study was approved by the ethics review board of the First Affiliated Hospital of Shandong First Medical University [XMSBLL2021 (425)] and was recognized by all participating hospitals. A waiver of consent was granted at all sites, owing to the use of unidentified patient data.

The included infants were randomly assigned in a 3:1 ratio dividing into the training and validation cohorts. The inclusion criteria were as follows: (1) the preterm infants born at <32 weeks' gestation and >23 weeks' gestation (2) hospitalization for ≥28 days in the three hospitals. Infants with GA with 29 + 0/7 to 31 + 6/7 weeks were included, due to the incidence of BPD, which was 19.3% in Chinese preterm cohort study (3). The exclusion criteria were as follows: multiple malformations. The data on infants readmitted or transferred between hospitals in the collaborative network adopted a unique identification. All included infants were Han Chinese.

Definitions

The diagnosis and grading of BPD were based on the National Institutes of Child and Human Development (NICHD) 2001 (11). BPD is diagnosed when preterm infants require supplemental oxygen (fraction of inspired oxygen, $FiO_2 > 21\%$) for 28 days. Mild BPD id diagnosed when infants do not require FiO_2 at postmenstrual age (PMA) 36 weeks; moderate BPD as receiving $FiO_2 < 30\%$ and severe BPD as receiving $FiO_2 \geq 30\%$ or positive pressure at PMA 36 weeks. SGA was defined as birth weight ≤ the 10th percentile for the same GA. Early onset sepsis (EOS) was diagnosed in preterm infants with bacteremia or bacterial meningitis ≤ 72 h after birth (12). The duration of invasive ventilation was calculated as the number of days of invasive ventilation within 7 days after birth.

Data collection

Parental factors (maternal age, medication use during pregnancy, smoking and antenatal corticosteroid), risk factors before delivery (gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, hypertensive disorders of pregnancy, chorioamnionitis, premature rupture of membrane and infectious disease within 7 days before delivery), basic information of infants (GA, birth weight, Apgar score at 1 and 5 min and mode of delivery), disease diagnostic before 7 DOL (EOS and hemodynamically significant patent ductus arteriosus) and treatment during the first 7 DOL (pulmonary surfactant, caffeine, invasive mechanical ventilation, and parenteral nutrition) were collected.

Development and assessment of the nomogram

Multivariate logistic regression was used to build a nomogram model to predict the occurrence of msBPD and plot the receiver operating characteristic (ROC) curves. The areas under the ROC

curve (AUC) and concordance index (C-index) were calculated to measure the predictive ability and accuracy of the model. The goodness of fit was calibrated by using a calibration curve and Hosmer–Lemeshow test. The utility of the model for decision making was evaluated using decision curve analysis (DCA). DCA is critical method for assessing the clinical utility of clinical predictive models (13), and can address the limitations of ROC curve (14). Each variable corresponds to a point on the axis of the nomogram, and the corresponding score of the variable was obtained. The sum score of each variable was obtained, the total score corresponded to the point on the risk axis, and the risk value of msBPD was obtained.

Statistical analysis

The sample size calculation was performed using PASS version 11.0.7 (NCSS Statistical Software, Kaysville, UT, USA). The number of events per candidate variable was restricted to >20. The Lasso regression method performs variable selection as an alternative to the subset selection method to reduce model complexity. This method can prevent the model from overfitting and avoids the need for multiple test corrections (15). Normally distributed data are presented as mean ± standard deviation, and nonnormally distributed data are presented as medians and interquartile ranges. Categorical variables are expressed as frequencies and percentages. The demographic variables were summarized using descriptive statistics. Differences between the groups were compared using the chi-square or Fisher's tests. All statistical tests were two-sided. Statistical significance was set at $P < 0.05$. All analyses and a dynamic nomogram were used to visualize the model, which was achieved using R software (version4.0.4; R Foundation for Statistical Computing, Vienna, Austria).

Results

General characteristics

A total of 2,373 premature infants who met the inclusion criteria in the three hospitals between January 2017 and December 2021. 279 infants were excluded because of incomplete records. 2,067 patients were included in our study (**Figure 1**). The data was randomly divided into training (1,551 cases) and validation cohorts (516 cases) according to a 3:1 ratio. In the training cohort, the clinical information of 1,551 patients were obtained. The general characteristics including GA, birth weight, and sex, showed no statistical differences in the two cohort. Data from the training cohort were used to construct the dynamic nomogram. According to the NICHD 2001 grading of BPD (11), 331, 150, and 61 infants were diagnosed with mild, moderate, and severe BPD, respectively. The baseline characteristics of the training and validation sets are listed in **Table 1**.

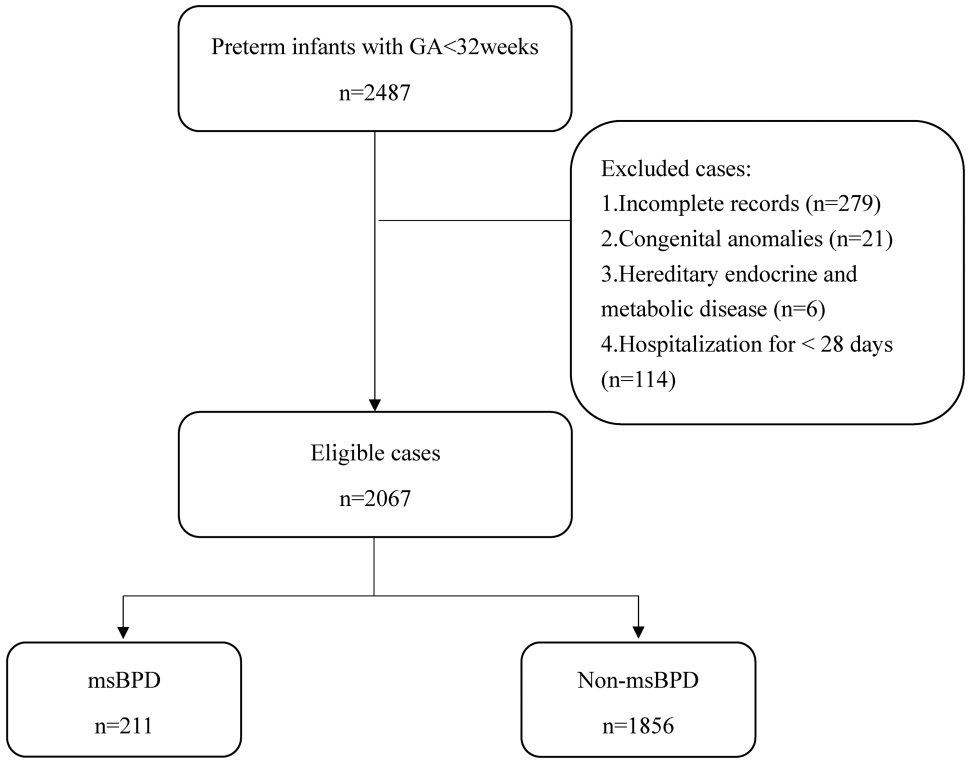

FIGURE 1
Flowchart for patient selection. GA, gestational age; msBPD, moderate-to-severe BPD.

TABLE 1 Baseline characteristics of all patients in the training cohort and validation cohort.

Variables		Training cohort ($n = 1551$)	Validation cohort ($n = 516$)	*P*-value
GA (weeks)		29.7 ± 1.6	29.6 ± 1.7	0.45
Birth weight (kilograms)		1.33 ± 0.34	1.33 ± 0.34	0.52
Gender	Male	850 (54.8%)	269 (52.1%)	0.29
	Female	701 (45.2%)	247 (47.9%)	
Apgar5-min score		8 (7,9)	8 (7,9)	0.43
Mode of delivery	Caesarean delivery	417 (26.9%)	130 (25.2%)	0.45
	Vaginal delivery	1,134 (73.1%)	386 (74.8%)	
PROM	+	493 (31.8%)	156 (30.2%)	0.76
	–	1,058 (68.2%)	360 (69.8%)	
ICP	+	124 (8.0%)	48 (9.3%)	0.12
	–	1,427 (92.0%)	468 (90.7%)	
GDM	+	244 (15.7%)	77 (14.9%)	0.66
	–	1,307 (84.3%)	439 (85.1%)	
HDP	+	367 (23.7%)	118 (22.9%)	0.71
	–	1,184 (76.3%)	398 (77.1%)	
Prenatal steroid	+	1,057 (68.1%)	329 (63.8%)	0.07
	–	494 (31.9%)	187 (36.2%)	
SGA	+	120 (7.7%)	53 (10.3%)	0.07
	–	1,431 (92.3%)	463 (89.7%)	
EOS	+	109 (7.0%)	51 (9.9%)	0.03
	–	1,442 (93.0%)	465 (90.1%)	
PDA	+	936 (60.3%)	342 (66.3%)	0.02
	–	615 (39.7%)	174 (33.7%)	
PS	+	427 (27.5%)	147 (28.5%)	0.67
Duration of invasive ventilation (day)		0 (0,5)	0 (0,5)	0.61
msBPD	+	171 (11.0%)	40(7.8%)	0.03
	–	1380(89.0%)	476(92.3%)	

+, yes; -, no; GA, gestational age; PROM, premature rupture of membrane; ICP, intrahepatic cholestasis of pregnancy; GDM, gestational diabetes mellitus; HDP, hypertensive disorders of pregnancy; Prenatal steroid, dexamethasone were given within 2 weeks before delivery; SGA, small for gestational age; EOS, early onset sepsis; PDA, patent ductus arteriosus; PS, pulmonary surfactant; msBPD, moderate-to-severe bronchopulmonary dysplasia.

Screening for predictive factors

Lasso regression was used to select the significant risk factors from the included data. We analyzed 21 risk factors for BPD according to literatures (16, 17). The Lasso regression analysis showed that five perinatal factors were best fit predicting factors for msBPD models: GA, SGA, Apgar 5-min score, EOS, and duration of invasive ventilation.

Risk prediction nomogram development

The five factors were integrated into the nomogram [Figure 2A and the available online website: https://sdxxbxzz.shinyapps.io/BPDpredict/ (Figure 2B)]. The dynamic nomogram predicts msBPD within postnatal day 7 based perinatal days. By selecting the corresponding tab, the scores for each variable can be obtained, and the prediction risk associated with the total score represents the risk of developing msBPD. For example, an infant born at 26 weeks, a diagnosis of SGA, Apgar 5-min score of 6, a diagnosis of EOS, and supporting by invasive ventilation for 7 days had the corresponding scores indicating an estimated msBPD of 86.9%. The predicted model had a good fit of the predicted model demonstrated by the Hosmer–Lemeshow test. ($P = 0.059$).

Predictive accuracy and net benefit of the nomogram

In the training cohort, the AUC and C-index were 0.894 (95% CI 0.869–0.919) (Figure 3A). The calibration curve approach to the ideal diagonal line (Figure 4A) showing high consistencies between the predicted and observed msBPD probability in the cohorts. The net benefit curves for the nomogram in training cohort was shown in Figure 5A. The X-axis indicates the threshold probability for msBPD, while the Y-axis indicates the net benefit. The DCA indicated a significantly better net benefit when the threshold probability was between 0.05 to 0.79, indicating the effective use of the nomogram in achieving net clinical benefit.

In the validation cohort, the AUC and C-index were 0.893 (95% CI 0.855–0.931) (Figure 3B). It demonstrated good predictive ability and accuracy of the nomogram. The calibration curve approached to the ideal diagonal line (Figure 4B), indicating that the prediction model had high goodness of fit. The DCA validation also showed a significant net benefit of the model (Figure 5B).

Discussion

Our study is the first to develop a dynamic nomogram for predicting the occurrence of msBPD using perinatal factors on a multicenter cohort and provide validation. We validated the high specificity, good sensitivity, and consistency of the prediction model. In their analysis of 26 studies, Onland et al. (10) identified that predictive models for BPD lacked calibration. Peng et al. (18) reported on 18 models for predicting BPD or death in preterm born at ≤32 weeks' gestation, most of which had many methodological shortcomings and lacked calibration assessment. To address these issues, our study included validation cohorts and calibration to enhance the predictive value of our model. Hosmer-Lemeshow test and DCA were used to evaluate calibration and clinical applicability. Thus, we aimed to enhance the predictive value of the model. The model developed by Greenberg et al. (8) included European-American multi-center data with GA of 23 + 0/7 weeks to 28 + 6/7 weeks. The model also lacked validation and calibration. To develop a broadly applicable prediction model for neonatologists, we also include preterm with GA < 32 weeks. As preterm with GA of 29 + 0/7 to 31 + 6/7 weeks accounted for 67.8% of preterm born at <32 weeks' gestation and had a BPD incidence with 19.3% in a Chinese national cohort study (3). We aimed to create a prediction model that can be widely used by neonatologists; our

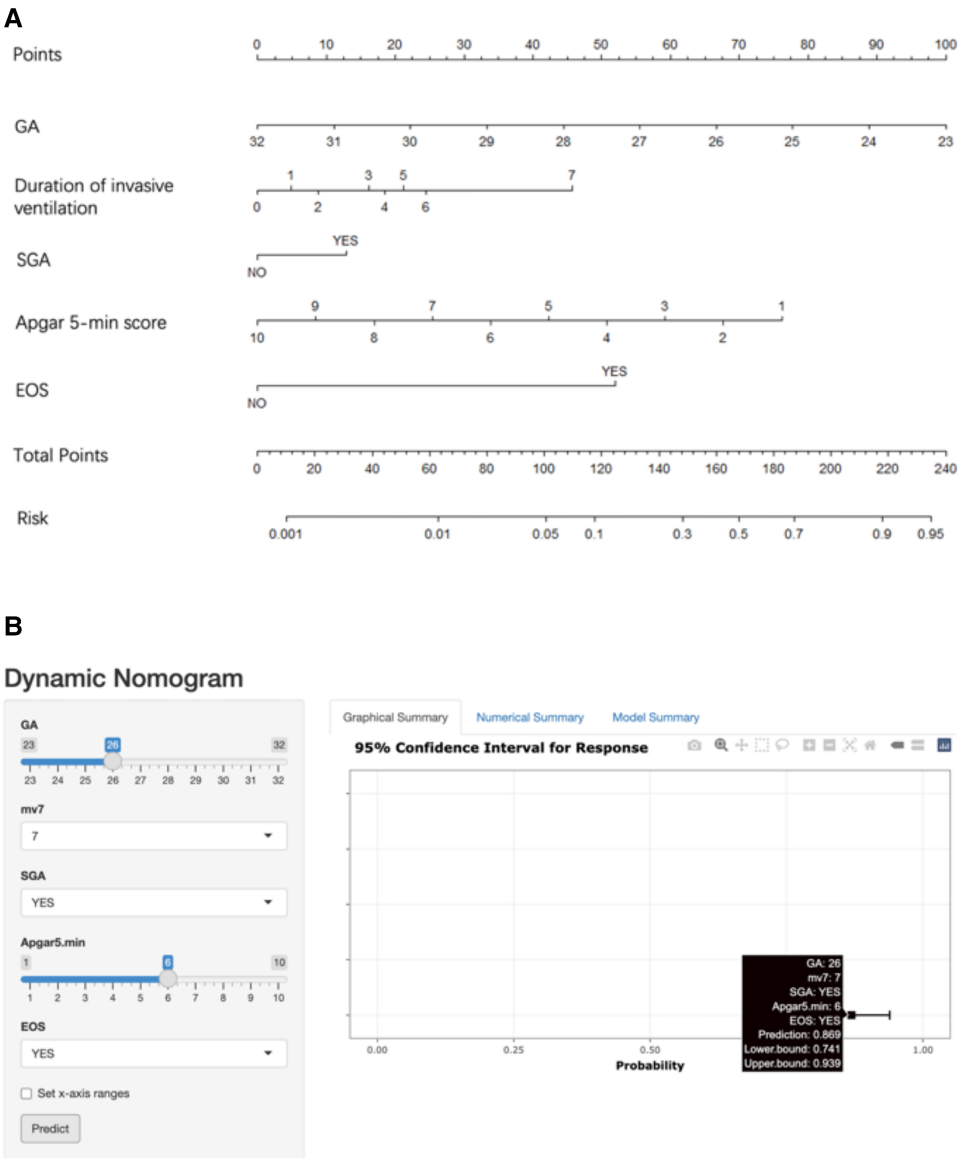

FIGURE 2
Nomogram. (A) Nomogram for the perinatal prediction of msBPD. (B) Dynamic nomogram for the perinatal prediction of msBPD. msBPD, moderate-to-severe BPD.

model utilized the Hosmer-Lemeshow test to showcase the calibration, making it more suitable for use in clinical practice.

Our study was a multi-center retrospective study aimed to develop a dynamic nomogram for predicting msBPD in preterm with GA < 32 weeks. Candidate predictors were screened by Lasso regression avoiding overfitting. The AUC of our model was higher than web-based calculator reported by R.G. Greenberg et al. (8) and Laughon MM et al. (7). C-index was 0.894 in training cohort showing favorable discrimination by the dynamic nomogram, which was validated in the validation cohort. DCA evaluates the consequences of the decisions made based on a model. DCA curves demonstrating significant net clinical benefit indicated better prediction of nomogram for predicting msBPD.

The dynamic nomogram can be utilized within postnatal day 7 to predict msBPD. Early prediction allows for early intervention/prevention in NICU. Early predicting msBPD will provide the preterm with msBPD for individualized therapy or precautions. For example, the use of postnatal steroids remains controversial. But the infants with highest risk for developing msBPD need postnatal dexamethasone after the first week of life (16, 19). Early predicting msBPD will also provide clinician for evidence of prevention strategies like diuretic, Vitamin A and nutritional strategies.

The dynamic nomogram computes the incidence of msBPD based on GA, SGA, Apgar 5-min score, EOS, and duration of invasive ventilation. GA is known to be strongly associated with the prognosis of preterm infants as the incidence of BPD is inversely related to GA. Our study also showed that GA was an independent predictor of msBPD and was therefore included in the predictive nomogram. The lower the GA, the higher the

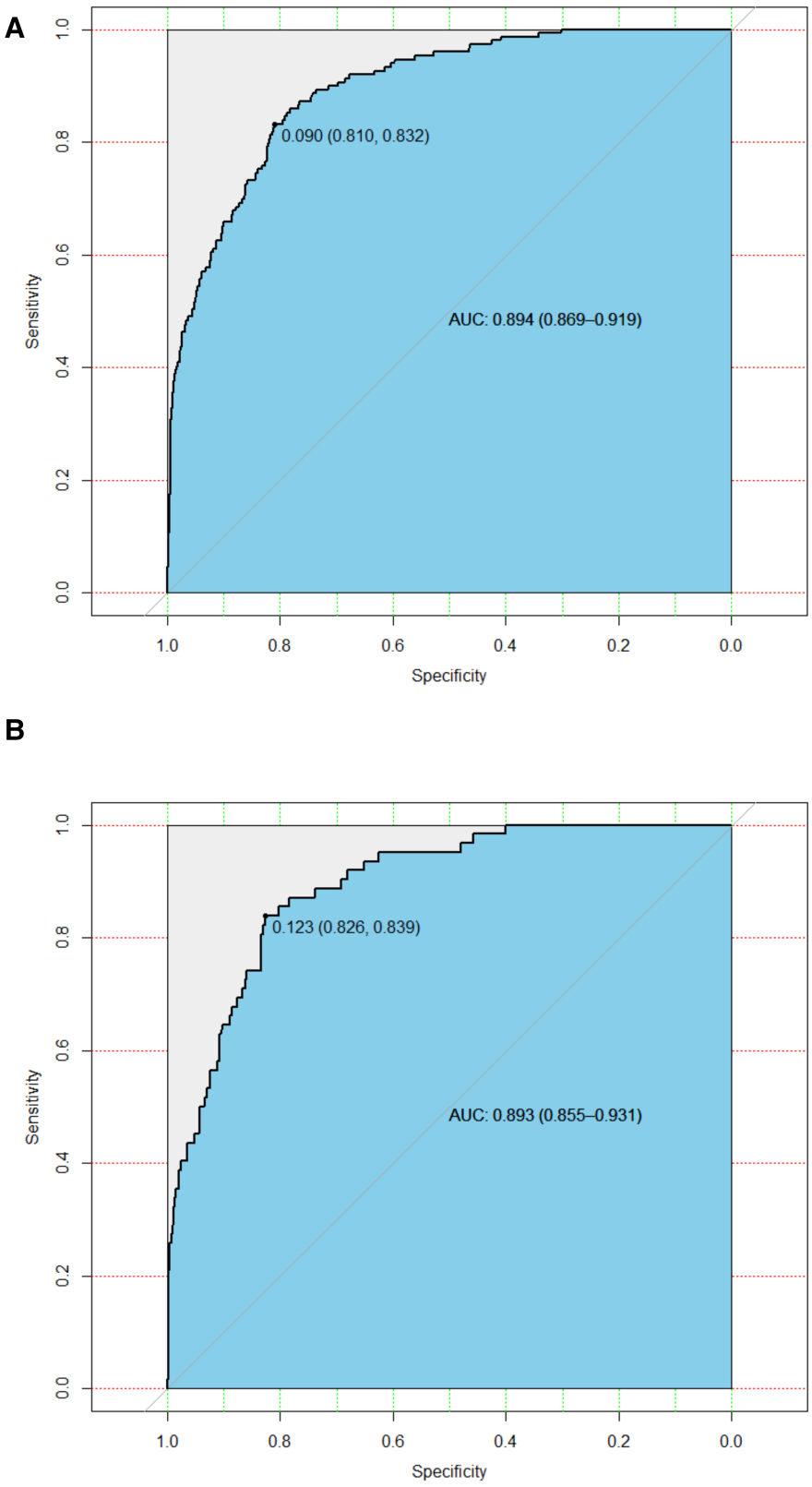

FIGURE 3
ROC curves. (A) Training cohort. (B) Validation cohort. ROC, receiver operating characteristic; AUC, area under the ROC curve.

number of points in the nomogram and the higher the probability of msBPD. BPD is a chronic lung disease secondary to the critical stages of lung morphological development (tubules and vesicles). Prematurity leads to the early termination of alveolar development and a lack of lung surfactants (20); for this reason, premature infants receive medical intervention immediately after

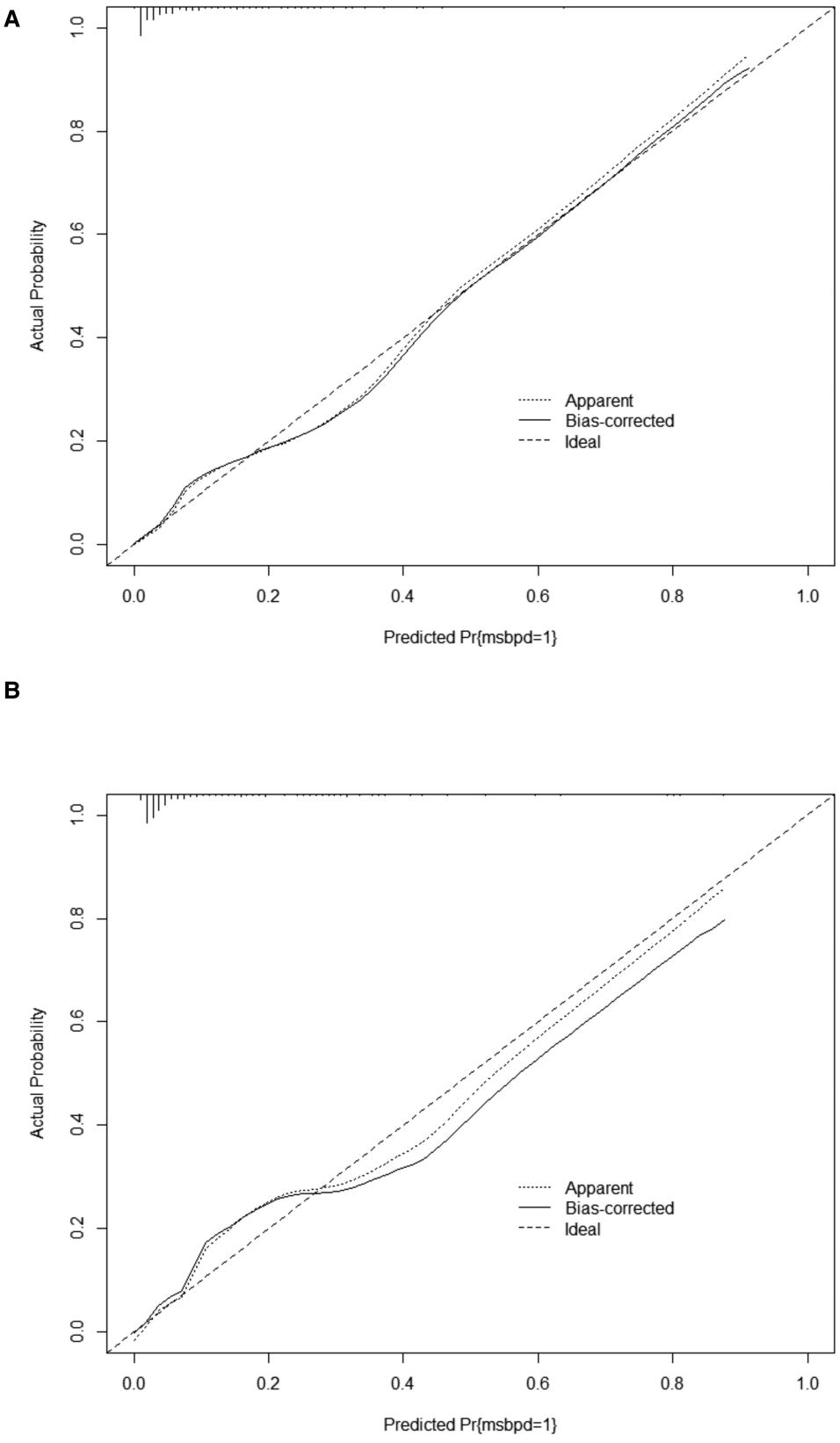

FIGURE 4
Calibration curve for predicting probability of msBPD. (A) Training cohort. (B) Validation cohort.

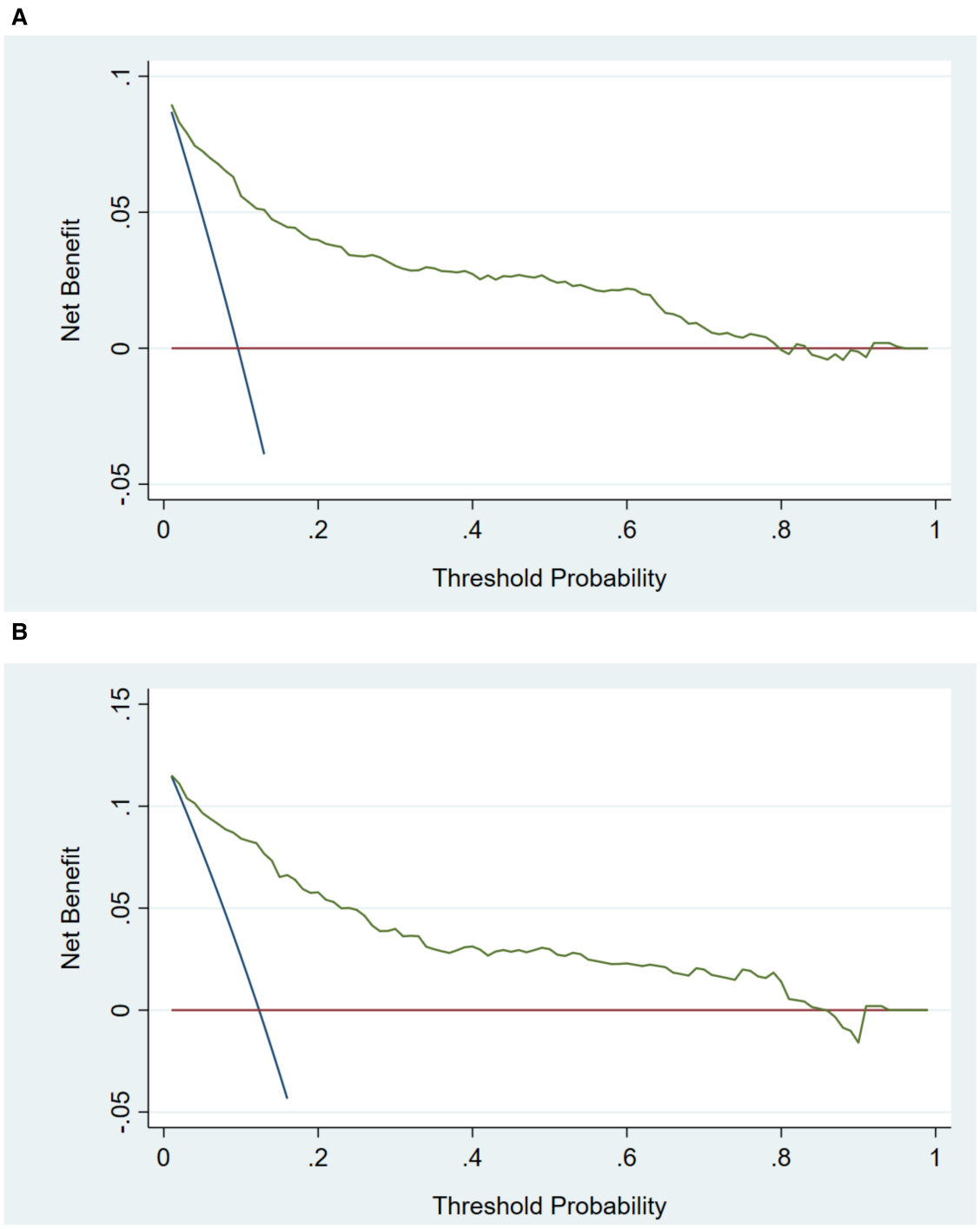

FIGURE 5
Decision curve analysis in prediction of msBPD. (A) Training cohort. (B) Validation cohort. The threshold probability for msBPD was indicated by X-axis. And Y-axis indicated the net benefit.

birth, which interferes with normal lung development. Even after prophylactic administration of pulmonary surfactant, low tidal volume, and reduced oxygen concentration, alveolarization and pulmonary vascularization in premature infants are still significantly hindered, making GA one of the most relevant factors for BPD (21).

The Apgar score is one of the most intuitive and convenient tools for assessing the clinical status of newborns immediately after birth. A low Apgar score at 5 min may be the first indication of a poor birth outcome. Barzilay et al. (22) reported that the Apgar score at 5 min, combined with GA, birth weight, and prenatal steroids, can be significant prognostic predictors of

outcomes in very low birth weight infants. Our study also showed that the Apgar score at 5 min was negatively correlated with nomogram points. This indicates that the lower the Apgar score at 5 min, the higher the risk of developing msBPD. Low Apgar scores are associated with a higher risk of preterm mortality (23). A low Apgar score indicates that the newborn has poor ability to adapt to the extrauterine environment.

EOS is also considered to be related to intrauterine infection/intra-amniotic inflammation (24), which should be treated with antibiotics. However, most intrauterine infections are difficult to detect. The diagnosis of EOS is definitive, indicating an intrauterine or parturient infection. Inflammation is an important mechanism of BPD (25). Infection/inflammation can cause lung damage and remodeling, which can increase the levels of the inflammatory factors interleukin (IL)-1β and IL-6 (26). Systemic inflammation occurs early in neonates and is associated with BPD, particularly msBPD. Inflammatory cytokines were significantly associated with BPD, especially on day 1 (27). Pathogens colonizing the respiratory tract and the use of antibiotics in preterm infants are risk factors for BPD (28). Inflammation caused by EOS leads to alveolar remodeling and fibrosis in preterm infants. Antibiotics alter the gut microbiota of preterm infants, and dysregulation of the intestinal microbiota can affect the pulmonary immune response through the intestinal-lung axis, thus increasing the risk of BPD (29). In this study, the EOS rate was significantly different between the msBPD and non-msBPD groups ($P < 0{\cdot}05$). EOS was included in the predictive model. As the diagnostic criteria for EOS are clear and the diagnostic indicators are easy to obtain, EOS is more suitable as a factor for early prediction of BPD.

Our study also included SGA as an independent predictor of msBPD in the predictive model. Our results showed that the number of SGA preterm births in the msBPD and non-msBPD groups was significantly different. Jensen et al. conducted a prospective study showing that SGA was associated with an increased risk of BPD among infants with GA < 32 weeks (30). A multicenter cohort study in China also showed that the incidence of BPD in the SGA group was 3. 1-fold higher than that non-SGA group (31). Most SGA preterm infants have intrauterine growth restriction (IUGR). IUGR is also a major risk factor for BPD. In animal models, studies have demonstrated that IUGR decreases alveolarization and abnormal pulmonary vascular development (32, 33). The SGA is easy to obtain and objective. The inclusion of SGA in the prediction model was also consistent with previous findings that SGA is related to the occurrence of msBPD.

Finally, the duration of invasive ventilation was also included in the predictive model for msBPD. Invasive mechanical ventilation is known to be significantly associated with an increased incidence of BPD (7, 34). Hyperoxia and barotrauma from mechanical ventilation can lead to inflammation, which is a significant risk factor for BPD (35). The Spanish Bronchopulmonary Dysplasia Research Group reported that the length of invasive mechanical ventilation is the most important risk factor associated with type 2/3 BPD (17). Our study also indicated that invasive mechanical ventilation was an independent predictor of msBPD. The longer the ventilation time, the higher the probability of msBPD and the higher the number of points in the nomogram.

In this study, we assessed the perinatal predictors of msBPD in preterm infants with GA < 32 weeks and built a dynamic nomogram for early risk prediction. Our validation confirmed the high specificity and accuracy of the prediction model. A dynamic nomogram provides clinicians with an intuitive and simple tool for practical prediction. Additionally, BPD is a chronic lung disease caused by multiple factors, including inappropriate clinical management. Therefore, we chose perinatal factors for BPD prediction to remove the factors of clinical management. However, our study has some limitations. First, some potential predictors, such as C-reactive protein, procalcitonin, or umbilical cord blood gas, were not assessed due to the lack of data. Second, this multicenter study only included some perinatal centers in a province of China; therefore, additional data from other perinatal centers and specialized children's hospitals are warranted for future analysis.

In conclusion, we found that GA, Apgar 5-min score, EOS, SGA, and duration of invasive ventilation were predictors of msBPD in very preterm infants. We built a dynamic nomogram for the early prediction of msBPD. The higher the total number of points in the dynamic nomogram, the higher the risk of msBPD. The intuitive, personalized, and convenient model of perinatal predictors provides clinicians with a visual and personalized tool for the early identification of msBPD, which may be of significance in reducing complication and mortality rates.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics review board of the First Affiliated Hospital of Shandong First Medical University. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization: KM, JZ. Data analysis and draft the manuscript: JZ. Review and editing: KM. The co-first authors, namely JZ, LWe, CF, RZ, LWa: participated in the design of the study, the collection and interpretation of the data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Jia-Yuh Chen,
Chung Shan Medical University, Taiwan

REVIEWED BY
Jonathan Michael Davis,
Tufts University, United States
Janneke Dekker,
Leiden University, Netherlands
San-Nan Yang,
E-Da Hospital, Taiwan

*CORRESPONDENCE
Vincent D. Gaertner
✉ vincent.gaertner@usz.ch

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Early prediction of pulmonary outcomes in preterm infants using electrical impedance tomography

Vincent D. Gaertner[1,2]*, Tobias Mühlbacher[1], Andreas D. Waldmann[3], Dirk Bassler[1] and Christoph M. Rüegger[1]

[1]Newborn Research Zurich, Department of Neonatology, University Hospital and University of Zurich, Zurich, Switzerland, [2]Department of Neonatology, Dr von Hauner University Children's Hospital, Ludwig-Maximilian-University, Munich, Germany, [3]Department of Anesthesiology and Intensive Care Medicine, Rostock University Medical Center, Rostock, Germany

Introduction: Electrical impedance tomography (EIT) allows assessment of ventilation and aeration homogeneity which may be associated with respiratory outcomes in preterm infants.
Methods: This was a secondary analysis to a recent randomized controlled trial in very preterm infants in the delivery room (DR). The predictive value of various EIT parameters assessed 30 min after birth on important respiratory outcomes (early intubation <24 h after birth, oxygen dependency at 28 days after birth, and moderate/severe bronchopulmonary dysplasia; BPD) was assessed.
Results: Thirty-two infants were analyzed. A lower percentage of aerated lung volume [OR (95% CI) = 0.8 (0.66–0.98), $p = 0.027$] as well as a higher aeration homogeneity ratio (i.e., more aeration in the non-gravity-dependent lung) predicted the need for supplemental oxygen at 28 days after birth [9.58 (5.16–17.78), $p = 0.0028$]. Both variables together had a similar predictive value to a model using known clinical contributors. There was no association with intubation or BPD, where numbers were small.
Discussion: In very preterm infants, EIT markers of aeration at 30 min after birth accurately predicted the need for supplemental oxygen at 28 days after birth but not BPD. EIT-guided individualized optimization of respiratory support in the DR may be possible.

KEYWORDS

neonatology, bronchopulmonary dysplasia, electrical impedance tomograghy, preterm infants, surfactant nebulization, respiratory support, prediction

Introduction

Bronchopulmonary dysplasia (BPD) is among the major morbidities of very preterm infants (1, 2), and it is associated with adverse long-term neurodevelopmental outcomes as well as recurrent hospitalizations which is a large burden to infants and parents (3–5). Accurate prediction of a developing BPD may be important to guide early therapeutic interventions.

Currently, there are various tools to predict BPD, all of which lack accuracy (6). Recently, lung ultrasound has gained attention as a potentially novel tool to predict short- (surfactant need, intubation) and long-term (oxygen dependency, BPD) respiratory outcomes as early as 24 or 72 h after birth (7–10). However, at this timepoint, lung ultrasound adds only little additional predictive value to known clinical parameters such as gestational age (11). Also, lung ultrasound can only detect intrapulmonary consolidations and fluids close to the chest-wall and does not allow to draw conclusions on regional lung aeration.

Electrical impedance tomography (EIT) is a novel, noninvasive tool measuring regional ventilation distribution and overall lung aeration in a cross-sectional slice of the lung (12). Recently, ventilation inhomogeneity in stable preterm infants approximately four weeks after birth was associated with worse oxygenation and a later diagnosis of BPD (13). Using EIT, we aimed to describe whether parameters of inhomogeneous aeration and ventilation measured as early as 30 min after birth may already predict respiratory outcomes in very preterm infants and may add to currently known clinical predictive parameters.

Methods

This is a secondary analysis of a prospective, parallel, randomized controlled trial conducted at the University Hospital Zurich comparing prophylactic surfactant nebulization (SN) immediately after birth to positive distending pressure alone (14). The original trial was registered with clinicaltrials.gov (NCT04315636) and approved by the local ethics committee (KEK-2020-00890). All parents provided antenatal written informed consent.

Population and intervention

The setup of the original study has been described previously (14). In short, infants between 26 and 32 completed weeks of gestation at birth were randomized to either positive distending pressure alone after birth or positive distending pressure with additional SN (200 mg/kg Curosurf®, Chiesi Farmaceutici, Parma, Italy) via the eFlow® Neo Nebulizer (PARI Pharma, Starnberg, Germany) starting simultaneously with the initial application of a face mask. Infants were initially supported on continuous positive airway pressure support (CPAP) with a positive distending pressure of 8 mbar using the EVE NEO® ventilator (Fritz Stephan GmbH, Gackenbach, Germany) and a face mask (ComfortStar®, Dräger Medical System, Lübeck, Germany). Escalation of pressure levels and change of respiratory support mode or interface were possible at any time during stabilization at the clinician's discretion. To allow comparability, infants who had received intratracheal surfactant (via endotracheal tube or via thin catheter) within the first 30 min after birth were excluded.

Data collection

Complete methods of data collection have been described previously (14). A researcher was present for each delivery to fasten a textile EIT belt at nipple level as soon as the infant reached the resuscitaire. The LuMon™ device (SenTec AG, Landquart, Switzerland) was used to record EIT data at a frame rate of 51 Hz (15, 16). The EIT belt remained on the infant's thorax for the initial 90 min or until the first chest x-ray was performed. Thirty minutes after birth, data were extracted for 30 s of artefact-free tidal ventilation using ibeX (version 1.1, SenTec AG, Landquart, Switzerland). Infants were lying in supine position during all measurements. Thus, dorsal lung regions were considered gravity-dependent (17, 18).

Predictors

We aimed to show the early predictive value of EIT parameters on short- and long-term pulmonary outcomes. Therefore, we chose to evaluate data at 30 min after birth as infants are largely transitioned at this timepoint while it still allows early prediction.

EIT data were analyzed using Matlab software (version 2019a, Mathworks, Nantick, MA, USA). Recently, heterogeneous ventilation in stable preterm infants was associated with a later diagnosis of BPD (13). Building on this finding, we pre-defined the following parameters which may influence pulmonary outcomes: The percentage of overall aerated lung volume (Aer%; as indicator of overall aeration), the aeration homogeneity ratio (AHR; as indicator of aeration homogeneity), the coefficient of variation (CV; as indicator of ventilation homogeneity), as well as the gravity- and non-gravity-dependent silent spaces (SS_{GD} and SS_{NGD}; as indicators of atelectasis and overdistension, respectively).

To obtain these predictors, the following steps of analysis were necessary: first, data was prepared by projecting predefined anatomical lung regions based on the vendor-provided human model chest atlas into the EIT image, excluding EIT signals outside of these regions and normalizing the signal for body weight (17–19). Second, the net EIT signal at end-expiration (end-expiratory lung impedance; EELI) was isolated in arbitrary units (AU) in the entire lung and the percentage of aerated lung tissue was calculated (Aer%). Then, aeration in the non-gravity-dependent and the gravity-dependent lung ($EELI_{NGD}$, $EELI_{GD}$) was isolated and the aeration homogeneity ratio (AHR) was calculated by dividing the weighted values of the non-gravity-dependent half of the EIT signal by the gravity-dependent half (18, 20). A higher value indicates more aeration in the non-gravity-dependent lung (18). Third, the CV was calculated by dividing the standard deviation (SD) of impedance changes in all pixels by the mean value of impedance (18, 21). The CV correlates with ventilation homogeneity and lower values indicate improved homogeneity. Finally, silent spaces were calculated in the gravity- and non-gravity-dependent lung [corresponding to atelectasis (SS_{GD}) and overdistension (SS_{NGD})] (12, 18, 22). As percentages, SS were not normalized for body weight.

Outcomes and timepoints

The following outcomes were assessed: First, early respiratory failure, defined as either endotracheal intubation (with subsequent surfactant application) or less invasive surfactant application (LISA) within the first 24 h after birth, as both manipulations would severely change ventilation and aeration parameters. Second, oxygen dependency at 28 days, irrespective

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of the mode of respiratory support. And third, bronchopulmonary dysplasia (BPD), defined as oxygen need or any pressure support at 36 weeks postmenstrual age.

Statistical analysis

Averages of each EIT recording were computed for subsequent analyses. Normally distributed data are presented as mean with standard deviation (SD) or 95% confidence interval (CI). Non-parametric data are presented as median and interquartile range (IQR).

Logistic regression was performed for each predictor to assess the effect of EIT variables on respiratory outcomes. The area under the receiver operating curve (ROC) was then calculated for significant predictors of respiratory outcomes using the pROC package in R statistics. Finally, significant predictors were included into a model predicting adverse outcomes using three known clinical contributors (gestational age, sex and birth weight) to assess whether there is an improvement in the prediction. *P*-values < 0.05 were considered statistically significant. All analyses were performed using R statistics (version 3.6.2) (23).

TABLE 1 Demographic characteristics of the study population.

Patient characteristics	Included patients (*N* = 32)
Demographic	
Gestational age at birth (weeks)	29.6 (28.7–31.1)
Birth weight (g)	1140 (889–1368)
Male, *n* (%)	16 (50%)
Prenatal	
Completed antenatal steroids, *n* (%)	23 (72%)
PPROM, *n* (%)	9 (28%)
Chorioamnionitis, *n* (%)	5 (16%)
Oligo- or anhydramnios, *n* (%)	6 (19%)
Preeclampsia, *n* (%)	10 (31%)
IUGR, *n* (%)	8 (25%)
Delivered by CS, *n* (%)	30 (94%)
Postnatal	
Time of cord clamping (s)	60 (49–60)
Apgar score at 5 min	8 (7–9)
Umbilical artery pH	7.32 (7.28–7.35)
Doses of surfactant	1 (0–1)
Sepsis, *n* (%)	2 (6%)
Respiratory outcomes	
Respiratory failure (intubation/LISA)	
In the delivery room, *n* (%)	1 (1/0; 3%)
Within 24 h, *n* (%)	7 (2/5; 22%)
Within 72 h, *n* (%)	11 (6/5; 34%)
During hospitalization, *n* (%)	11 (6/5; 34%)
Bronchopulmonary dysplasia (Walsh) (24)	
Any, *n* (%)	15 (47%)
Mild, *n* (%)	14 (44%)
Moderate or severe, *n* (%)	1 (3%)

Median (IQR) are shown except where otherwise specified. PPROM, prolonged premature rupture of the membranes; IUGR, intrauterine growth retardation; CS, cesarean section.

Results

Population

Overall, 35 infants were randomized in the original study, two of which were intubated before 30 min and 1 had a faulty EIT recording, leaving 32 infants for data analysis. Demographic characteristics can be found in **Table 1**.

Prediction of respiratory parameters by EIT variables

None of the pre-specified EIT parameters predicted early intubation within the first 24 h after birth or moderate/severe bronchopulmonary dysplasia (**Table 2**). A lower percentage of overall aerated lung [OR (95% CI) = 0.8 (0.66–0.98), ROC = 0.761, $p = 0.027$, **Figure 1A**] as well as a higher aeration homogeneity ratio (AHR) and thus, more aeration in the non-gravity-dependent lung predicted the need for supplemental oxygen at 28 days after birth [OR (95% CI) = 9.58 (5.16–17.78), ROC = 0.898, $p = 0.0028$, **Figure 1B**].

Prediction models including clinical parameters

Predicting oxygen need at 28 days after birth, the two EIT variables combined had a similar predictive value [area under the

TABLE 2 Prediction of respiratory outcomes by pre-specified EIT parameters.

	Early intubation		O_2 dependency at 28 days		Moderate/severe BPD	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Aer%	0.86 (0.72–1.03)	0.1252	**0.8 (0.66–0.98)**	**0.0272**	1.13 (0.79–1.6)	0.4856
AHR	0.85 (0.01–68.74)	0.9424	**9.58 (5.16–17.78)**	**0.0028**	11.0 (0.01–17.29)	0.3407
CV	2.92 (0.08–109.51)	0.5636	1.57 (0.07–34.7)	0.7756	31.2 (0.01–1084.0)	0.4084
SS_{NGD}	1.02 (0.79–1.32)	0.8483	0.97 (0.78–1.2)	0.7549	1.02 (0.57–1.84)	0.9566
SS_{GD}	0.76 (0.43–1.33)	0.3257	1.23 (0.83–1.83)	0.3013	3.13 (0.48–20.52)	0.2383

Bold values indicate significant differences.
CV, coefficient of variation; SS_{GD}, gravity-dependent silent spaces; AHR, aeration homogeneity ratio; SS_{NGD}, non-gravity-dependent silent spaces; Aer%, percentage of overall aerated lung tissue; OR, odds ratio; CI, confidence interval.

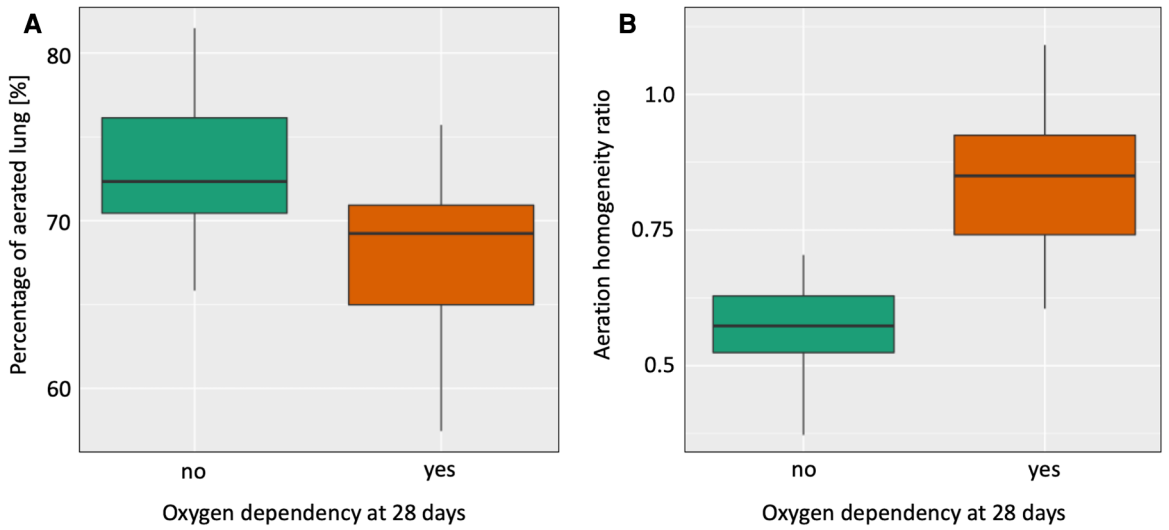

FIGURE 1
Differences in the percentage of overall lung aeration (Aer%, A) and the aeration homogeneity ratio (AHR, B) between infants who did vs did not receive supplemental oxygen at 28 days after birth.

receiver operating curve (ROC) = 0.925] as a model including known contributing clinical parameters (sex, weight and gestational age: ROC = 0.910). Adding the two EIT variables to the model of clinical parameters alone improved the ROC only slightly (ROC = 0.929). All models provided a near perfect prediction of oxygen dependency at 28 days (**Figures 2A–C**).

Discussion

In a small sample of very preterm infants, EIT markers of aeration and ventilation at 30 min after birth could not predict early intubation or bronchopulmonary dysplasia. However, EIT markers of aeration, i.e., a lower percentage of lung aeration as well as a higher aeration homogeneity ratio, measured 30 min after birth predicted the need for supplemental oxygen at 28 days after birth. The predictive value of EIT parameters was similar to a model including clinical parameters.

Electrical impedance tomography is a relatively novel tool which provides functional images of ventilation and aeration (12), and is representative for the entire lung in ventilated preterm infants (24). Recently, heterogeneous ventilation, measured by EIT, was associated with poor oxygenation and a later diagnosis of BPD in stable preterm infants approximately four weeks after birth (13). In this small pilot analysis, we did not see an association of EIT parameters with early intubation and subsequent surfactant application. However, we saw that the percentage of aerated lung as well as a higher aeration homogeneity ratio (AHR) predicted the need for oxygen at 28 days after birth. Early intubation is mostly performed in case of poor respiratory drive or insufficient oxygenation which may be compensated initially, e.g., by the use of noninvasive positive

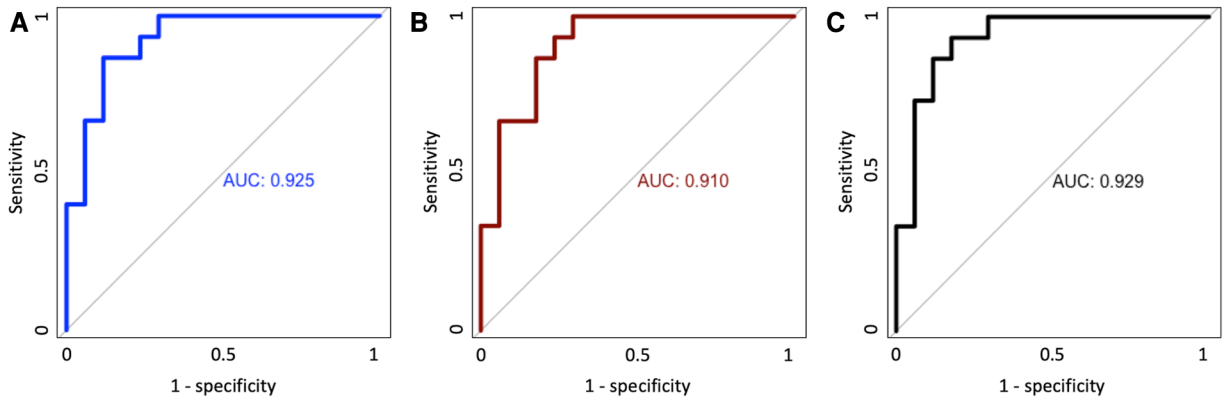

FIGURE 2
Area under the receiver operating curve (ROC) for clinical variables alone (A), EIT variable alone (B) and the combination of clinical and EIT variables (C).

pressure ventilation and/or increased pressure levels. Infants who were not intubated within the first 24 h could still go on to require oxygen at 28 days after birth due to inflammatory processes which may explain why we only saw an association of EIT parameters with oxygen dependency.

It is conceivable that infants with an improved overall aeration at 30 min, indicated by a large percentage of aerated lung, may require less respiratory support. This in turn is associated with an improved respiratory outcome (25), and may explain the association with oxygen dependency at 28 days. At the same time, a higher AHR, which corresponds to more aeration in the non-gravity-dependent part of the lung when compared with the gravity-dependent part, was associated with a higher likelihood of requiring oxygen 28 days after birth. Thus, overdistension of the lung (indicated by an increase in AHR) may be injurious to the lung which may explain the inverse association of the AHR with oxygen dependency. Finding the optimal balance between too little aeration and overdistending the lung remains challenging. Targeting the ideal PEEP level is of utmost importance to guide early respiratory interventions during the transitional period of extremely preterm infants. Accordingly, currently ongoing trials are targeting an individualized approach to pressure support immediately after birth (26). We speculate that EIT may be a helpful tool to guide respiratory support in the delivery room and beyond but future prospective studies need to evaluate this before a recommendation can be made.

Interestingly, these two EIT variables predicted oxygen dependency at 28 days after birth similarly good as clinical parameters (27). While EIT added little predictive value to clinical parameters, our finding underlines the importance of establishing functional residual capacity after birth not only for the immediate adaptation after birth but also for medium-term respiratory outcomes. However, our data is based on a limited number of infants in a single centre and results may differ in other settings.

While oxygen dependency in very preterm infants is largely explained by gestational age and birth weight, BPD is influenced by more factors than basic demographic characteristics and thus, early prediction of BPD by clinical parameters is still inaccurate (6). However, a late prediction is not as useful because therapeutic options are more effective in the first days after birth (28). While we saw an association of EIT parameters on medium-term respiratory outcome, we did not find an association with the development of BPD in this small study. Thus, it remains unclear whether EIT can add to the existing tools to predict BPD. While there was no clear association, the effect direction was similar to the effects found on oxygen dependency at 28 days after birth. Factors associated with mild BPD may also be associated with moderate or severe BPD in a larger cohort. It is important to note that we only had one patient who developed moderate or severe BPD and subsequent lack of power precluded meaningful analyses. Recently, lung ultrasound has gained attention as a potentially novel tool to predict BPD as early as 24 or 72 h after birth (7–10). However, at this timepoint, there are various clinical parameters allowing a fairly accurate prediction of BPD and lung ultrasound adds only little additional predictive value (11). While lung ultrasound only detects intrapulmonary consolidations and fluids, EIT could add to this knowledge by describing lung aeration (12). We speculate that the combination of lung ultrasound and EIT may allow a good prediction of BPD even as early as 30 min after birth but prospective clinical studies with a larger sample size are needed to evaluate this hypothesis. As BPD is a multifactorial disease, an all-encompassing approach may be needed which includes clinical and visualization (EIT, lung ultrasound) parameters as well as biomarkers (29) and machine-learning (30) to accurately predict this important outcome for patients and their families.

This study has limitations: First, it was a single-center study and it is unclear whether different approaches to airway management in other centres may have yielded different results. Second, it is a secondary analysis of a prospective trial on the effect of surfactant nebulization which is not standard of care and may have skewed the data. Third, it was a small trial with only 32 infants included in the analysis. This precluded meaningful analysis of the most important respiratory outcome in the NICU, BPD. However, this is the largest prospective trial with EIT measurements in preterm infants immediately after birth and it provides a first idea of the predictive value of EIT parameters after birth. Larger prospective trials, possibly including lung ultrasound as well as EIT, are needed to accurately describe their additional predictive value.

Conclusion

In very preterm infants, EIT markers of aeration and ventilation at 30 min after birth could not predict early intubation or bronchopulmonary dysplasia. However, EIT markers of aeration, i.e., a lower percentage of lung aeration as well as a higher aeration homogeneity ratio at 30 min after birth, accurately predicted the need for supplemental oxygen at 28 days after birth. In fact, these two EIT parameters had a similar predictive value to a model including known contributing clinical parameters. Our results highlight the importance of an individualized approach to respiratory support in very preterm infants after birth, and EIT may be useful to guide respiratory support in this situation.

Data availability statement

Deidentified individual participant data will be made available from three months to three years following publication to researchers who provide a methodologically sound proposal, with approval by an independent review committee ("learned intermediary"). Data requestors will need to sign a data access or material transfer agreement approved by USZ. Proposals should be submitted to VG, vincent.gaertner@usz.ch.

Ethics statement

The studies involving human participants were reviewed and approved by Cantonal Ethics Committee of Zurich. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

VG conceptualized and designed the study, recruited patients and collected data for the original study, analyzed the data, wrote the initial draft of the manuscript and reviewed and revised the manuscript. TM recruited patients and collected data for the original study and critically reviewed the manuscript for important intellectual content. DB helped to conceptualize and design the study, and critically reviewed the manuscript for important intellectual content. CR conceptualized and designed the study, recruited patients and collected data for the original study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The EIT monitor and belts were provided by SenTec AG. The company had no role in study planning, data collection, data analysis, data interpretation or manuscript writing.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Shahana Perveen,
Cohen Children's Medical Center, United States

REVIEWED BY
Chung-Ming Chen,
Taipei Medical University, Taiwan
Kristen Leeman,
Harvard Medical School, United States
Barry Weinberger,
Cohen Children's Medical Center, United States
Xueyu Chen,
Shenzhen Maternity and Child Healthcare Hospital, China

*CORRESPONDENCE
Stefano Ghirardello
✉ s.ghirardello@smatteo.pv.it

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Association between the development of bronchopulmonary dysplasia and platelet transfusion: a protocol for a systematic review and meta-analysis

Roberto Chioma[1], Stefano Ghirardello[2]*, Krzysztof Włodarczyk[3], Joanna Ulan-Drozdowska[4], Antonio Spagarino[5], Marta Szumska[4], Klaudia Krasuska[4], Joanna Seliga-Siwecka[4], Roy K. Philip[6], Niazy Al Assaf[6] and Maria Pierro[7]

[1]Department of Woman and Child Health and Public Health, Neonatology Unit, Università Cattolica del Sacro Cuore, Rome, Italy, [2]Department of Woman and Child Health and Public Health, Neonatology Unit, Ospedale San Matteo, Pavia, Italy, [3]Main Library, Medical University of Warsaw, Warsaw, Poland, [4]Neonatal and Intensive Care Department, Medical University of Warsaw, Warsaw, Poland, [5]Neonatal Intensive care Unit, University of Ferrara, Ferrara, Italy, [6]Division of Neonatology, Department of Pediatrics, University Maternity Hospital Limerick and Medical School University of Limerick, Limerick, Ireland, [7]M. Bufalini Hospital, AUSL Romagna, Cesena, Italy

Background: There is a lack of consensus on the management of thrombocytopenia in preterm infants, and the threshold for prophylactic platelet transfusion varies widely among clinicians and institutions. Reports in animal models suggested that platelets may play a relevant role in lung alveolarization and regeneration. Bronchopulmonary dysplasia (BPD) is a severe respiratory condition with a multifactorial origin that affects infants born at the early stages of lung development. Recent randomized controlled trials on the platelets count threshold for prophylactic transfusions in preterm infants with thrombocytopenia suggest that a higher exposition to platelet transfusion may increase the risk of BPD. Here, we report a protocol for a systematic review, which aims to assist evidence-based clinical practice and clarify if the administration of platelet products may be associated with the incidence of BPD and/or death in preterm infants.

Methods: MEDLINE, Embase, Cochrane databases, and sources of gray literature for conference abstracts and trial registrations will be searched with no time or language restrictions. Case–control studies, cohort studies, and nonrandomized or randomized trials that evaluated the risk for BPD and/or death in preterm infants exposed to platelet transfusion will be included. Data from studies that are sufficiently similar will be pooled as appropriate. Data extraction forms will be developed *a priori*. Observational studies and nonrandomized and randomized clinical trials will be analyzed separately. Odds ratio with 95% confidence interval (CI) for dichotomous outcomes and the mean difference (95% CI) for continuous outcomes will be combined. The expected heterogeneity will be accounted for using a random-effects model. Subgroup analysis will be performed based on *a priori*-determined covariate of interest. In case of sufficient homogeneity of interventions and outcomes evaluated, results from subgroups of studies will be pooled together in a meta-analysis.

Discussion: This systematic review will investigate the association of BPD/death with platelet components administration in preterm infants, and, consequently, it will provide reliable indications for the evidence-based management of premature patients with thrombocytopenia.

KEYWORDS

preterm infant, NICU, platelet transfusion, bronchopulmonary dysplasia, chronic lung disease of prematurity

1. Introduction

Bronchopulmonary dysplasia (BPD), also known as chronic lung disease (CLD) of prematurity, is a severe respiratory condition with a multifactorial origin that affects infants born at the early stages of lung development. BPD is the commonest respiratory morbidity among very premature infants, leading to short- and long-term pulmonary and non-pulmonary complications. The arrest in lung development, due to prematurity itself, is considered a prerequisite to the development of lung damage (1, 2). In addition, the role of several pro-inflammatory prenatal and postnatal pathogenic noxae is well-known, all driving to lung injury through chronic inflammation (3).

Recently, a new insight into the possible interaction between platelet transfusion and impairment of lung parenchyma development was provided by the pivotal PlaNeT-2 trial (4). In this study, a more liberal platelet transfusion threshold (50×10^9/L) for prophylactic use increased the risk of BPD, compared to a more restrictive threshold (25×10^9/L). The authors hypothesized a possible role of pro-inflammatory injury mediated by platelet product-derived bioreactive components (5), augmenting oxidative stress and aberrant angiogenesis. However, the PlaNeT-2 trial was not primarily designed to evaluate the relationship between platelet transfusions and BPD, as the main outcome was a composite of death or major bleeding. Moreover, the results for secondary outcomes could not be adjusted for multiplicity. Given the lack of strong evidence, it is a matter of priority to synthesize the available data from the literature through a systematic review and meta-analysis.

While thrombocytopenia is frequently encountered among preterm infants, there is a lack of consensus on its management, and the threshold for prophylactic platelet transfusion varies widely among clinicians and institutions (6–8). Several studies suggest that platelet products may have systemic pro-inflammatory consequences and damage various organs, including the lung (4, 5). There is a growing body of research about the interaction between platelet biogenesis and pulmonary development (9, 10). Animal studies suggest that up to 50% of circulating platelet biogenesis could be in the lungs in mammals (11). Recent reports in animal models also suggested that platelets may play a relevant role in lung alveolarization and regeneration (9, 10).

Here, we report a protocol for a systematic review, which aims to assist evidence-based clinical practice and clarify if the administration of platelet products could be associated with the development of BPD and/or death in preterm infants.

2. Methods and analysis

2.1. Protocol and registration

The reporting guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis for Protocols 2015 (PRISMA-P) were followed for the development of the present protocol. The filled PRISMA-P checklist is available as **Supplementary material**. This protocol is registered in the PROSPERO International Prospective Register of Systematic reviews (no. CRD42021279329). The resulting review will follow the updated PRISMA statement and will report important amendments to the original protocol.

2.2. Population, intervention, comparison, and outcomes questions

This project aims to answer two population, intervention, comparison, and outcomes (PICO) questions:

1. Are preterm infants receiving platelet transfusions at higher risk of BPD compared to those who did not?
2. Does a liberal threshold for prophylactic platelet transfusion increase the risk of developing BPD in preterm infants?

2.3. Study selection

Studies will be selected by the following criteria.

2.3.1. Population

2.3.1.1. Inclusion criteria

(1) Preterm babies born <32 weeks of gestational age (GA).

2.3.1.2. Exclusion criteria

(1) Infants with lung malformations, or related lung morbidity not related to prematurity.
(2) Infants with inborn platelets disorders.
(3) In case of relevant studies including populations with mixed GA, we will contact the study authors to obtain data from all patients with a GA below 34 weeks. If the

authors cannot provide this information, the study will be excluded.

2.3.2. Type of studies

2.3.2.1. Inclusion criteria

This systematic review will include case–control studies, cohort studies, and nonrandomized or randomized trials that evaluated platelet transfusion in preterm infants. Both prospective and retrospective studies will be included. This data analysis will not combine randomized controlled trials (RCTs) with observational and nonrandomized studies.

2.3.2.2. Exclusion criteria

This systematic review will not include case series, qualitative thematic analysis, narrative reviews, editorials, systematic reviews, or expert opinions.

2.3.3. Type of intervention

2.3.3.1. Inclusion criteria

We will include preventive and rescue platelet transfusion regardless of the initial platelet count. Preventive platelet treatment refers to transfusion in the case of increased risk of bleeding, while rescue treatment refers to transfusion in the case of active bleeding. We will not limit preventive transfusion by platelet threshold, although the threshold and the pre-transfusion platelet count will be recorded and serve as items for subgroup analysis. We will not limit the inclusion of the study based on reason for platelet transfusion. Therefore, any indication will be admissible. Whether or not the indication for platelet transfusion is described in the study, it will be recorded and assessed in subgroup analysis or meta-regressions, if a sufficient number of studies are found.

2.3.3.2. Exclusion criteria

Studies evaluating other interventions in case of risk of bleeding or active bleeding will be excluded.

2.3.4. Type of comparator

We will include studies having control groups assigned to placebo or no intervention. The comparator may also be represented by different thresholds of platelet count in the case of preventive transfusion.

2.3.5. Timeframe

The primary outcome (BPD incidence) will be assessed at 36 weeks of post-menstrual age (PMA). Transfusion-related adverse events will be considered if they occurred up to 6 h from the platelet product administration. The secondary medium-term outcomes will be regarded as starting from birth to discharge from the neonatal intensive care unit (NICU). Long-term outcomes will be considered up to 6 years of age.

If the timeframe of the listed outcomes is not specified, we will contact the authors of the study to obtain the specific data. If the authors will not be able to provide this information, the study will be excluded.

2.3.6. Setting

The study setting will be NICU stay.

2.3.7. Language and publication time

We will not apply any time or language restrictions.

2.4. Outcome measurements

2.4.1. Primary outcome

Primary outcome extracted from articles will focus on the incidence of BPD, defined as oxygen dependency at 36 weeks PMA and further classified by disease severity categories according to the most adopted definitions (12, 13). However, we will not select the studies based on BPD definition. We will include any BPD definition and then run subgroup analysis based on that.

2.4.2. Secondary outcomes

The secondary outcomes are grouped in immediate transfusion-related complications and medium-term outcomes, as follows.

Immediate transfusion-related complications

(1) Incidence of any adverse event possibly related to platelet transfusion. Adverse events will be classified as serious or nonserious; expected or unexpected; and study-related, possibly study-related, or not study-related.
(2) Pulmonary hypertension (14).
(3) Incidence of lung-related transfusion complications, transfusion-associated lung injury (15), defined as acute respiratory distress during or within 6 h of blood component, in the absence of temporally associated risk factors for respiratory distress syndrome.
(4) Mortality within 24 h from platelet transfusion.

Medium-term outcomes:

(1) Duration of ventilation: invasive, noninvasive, and total duration of ventilator dependency.
(2) Duration of low-flow oxygen dependency: days spent on low-flow supplemental oxygen (below 3 L/min); can be administered through nasal cannula or oxygen hood.
(3) Postnatal steroids treatment, including, but not limited to, betamethasone, hydrocortisone, dexamethasone, methylprednisolone, and prednisolone.
(4) Duration of hospital stay.
(5) Discharge with home oxygen or home ventilation with or without tracheostomy.
(6) Incidence of retinopathy of prematurity, classified in five stages, ranging from mild (stage I) to severe (stage V). Aggressive-posterior ROP will be also recorded (16).
(7) Incidence and type of treatment for retinopathy of prematurity.
(8) Incidence of intraventricular hemorrhage, defined and classified according to either to Papile or Volpe grading (17, 18).

(9) Incidence of periventricular leukomalacia, defined as either focal ("cystic PVL") or diffuse ("non-cystic PVL") injury (19).
(10) Incidence and treatment of necrotizing enterocolitis defined according to Bell staging or modified Bell staging (20, 21).
(11) Incidence and treatment of patent ductus arteriosus, defined as hemodynamically significant according to echocardiographic criteria chosen by the authors (22).
(12) Incidence of early-onset and late-onset sepsis, defined as clinical deterioration with positive cultures (23).
(13) Mortality during the NICU stay.
(14) Mortality during the NICU stay due to respiratory morbidity, in case it is separately described by the authors.

Long-term outcomes (up to 6 years):

(1) Somatic growth.
(2) Admissions to the hospital.
(3) Number and type of infections.
(4) Neurological development defined by Bayley-Ill scale at 2 years of corrected age (24).
(5) Need for transfusion.
(6) Survival.

We aim to obtain a comprehensive evaluation of possible complications or medium-term outcomes deriving from platelet transfusion. However, as recommended, studies not respecting the present definitions or reporting different outcomes will not be excluded. Eventually, we will specify in the review secondary outcomes added to the original list.

If the information is provided, subgroup analysis will evaluate the possible association of volume and rate of platelet transfusion with the different outcomes.

2.5. Search strategy

The MEDLINE, Embase, and Cochrane databases have been searched for this systematic review, following a standardized strategy developed using a standardized set of keywords and operators, with the consult of a research librarian (KW). We have not applied any other filtering or restrictions to the search strategy.

Additional strategies included manually reviewing reference lists from key articles that met our eligibility criteria and use of PubMed's "related articles" feature. Studies included in relevant systematic reviews may also be used if they satisfy our eligibility criteria.

The search query is as follows:

(((platelet[tiab] OR platelets[tiab] OR plasma[tiab] OR plasmas[tiab] OR FFP[tiab]) AND (transfusion[tiab] OR transfusions[tiab] OR infusion[tiab] OR infusions[tiab])) OR "Platelet Transfusion"[Mesh] OR "Plasma"[Mesh]) AND (infant [tiab] OR infants[tiab] OR newborn[tiab] OR newborns[tiab] OR neonate[tiab] OR neonates[tiab] OR neonatal[tiab] OR postnatal[tiab] OR "Infant"[Mesh] OR "Intensive Care Units, Neonatal"[Mesh]) NOT (review[pt] OR "systematic review"[pt] OR "meta-analysis"[pt] OR "case reports"[pt] OR editorial[pt] OR letter[pt] OR comment[pt]) NOT ("animals"[mesh] NOT "humans"[mesh])

2.6. Data management

The results of the search will be uploaded to an Internet-based software program facilitating the study selection process (DistillerSR®, Ottawa, Canada). Based on the inclusion and exclusion criteria, screening questions and forms will be developed and tested for levels 1 (title and abstract screening) and 2 (full-text screening). The full-text articles for level 2 screening will be uploaded with screening questions to DistillerSR. We will conduct a calibration test before each screening step to pilot and refine the screening questions.

2.7. Study selection process

For feasibility reasons, the references will be divided into two sequential groups. Two authors will independently assess each group for titles and abstracts, for a total of four reviewers.

In level 1, articles' titles and abstracts will be screened by the two independent authors using an initial screening questionnaire. In level 2, all the references retained will undergo full-text screening to select those matching our eligibility criteria. To ensure adequate inter-reviewer agreement, calibration exercises will be conducted on 20 random articles for each screening level. An agreement between authors will be needed to include a reference to the following level. When consensus cannot be reached, a third author (MP) will intervene to resolve the conflict. Where necessary, study authors will be contacted to obtain additional information to resolve eligibility issues. In the case of exclusion of trials in level 2, the reasons will be recorded. The review authors will be unblinded to the study authors or institutions or the journal titles.

2.8. Data extraction

Forms for the data extraction will be designed *a priori* and pilot-tested using a standardized extraction form on DistillerSR® by our team. The data extraction will be performed by two independent reviewers using DistillerSR®'s quality control function. To ensure that the approach to data charting will be consistent with the review questions and aim, the extraction forms will be piloted on five random articles. Each reviewer will chart half of these articles and audit the other half, consulting a third independent reviewer in case of conflicts. Results will be discussed by the team, and the data extraction forms will be updated throughout the process, to include other aspects of the intervention not considered *a priori*.

Table 1 will report the following extracted data:

(1) Lead author, year of publication, and country of origin.
(2) Sample size (total and per group).
(3) Design of the study.
(4) Inclusion and exclusion criteria.
(5) Setting.
(6) Definition of BPD.

Tables 2 (observational studies), 3 (interventional, nonrandomized studies), and 4 (interventional, randomized studies) will report the following extracted data:

(1) Purpose of study/study aims.
(2) Characteristics of the population (including the starting respiratory support).
(3) Details of platelet transfusion (rescue or preventive, threshold for prophylactic transfusion, number of interventions, and exact postnatal day of platelet transfusion).
(4) Results reported (including raw numbers, summary statistics, and adjusted analysis where available).
(5) Outcomes of interest, as defined above.

The corresponding author will be contacted a maximum of three times for these articles in which data cannot be extracted. The study will be excluded if the author will not be able to provide this information.

2.9. Risk of bias assessment

Two authors will independently assess the methodological quality in cohort and case–control studies, using the ROBINS-I (Risk Of Bias In Non-randomized Studies—of Interventions) Scale. According to ROBINS-I, after the evaluation of six different bias domains (i.e., bias due to confounding, bias in the selection of participants, etc.), studies will be classified as low, moderate, serious, critical, and unknown risk of bias. In case of conflicts on individual and total scores of the ROBINS-I scale, a third author will intervene for resolution. The risk of bias of randomized controlled trials will be assessed by the Cochrane risk-of-bias tool. The risk of bias will be evaluated as low, high, or unclear in each domain (allocation sequence, allocation concealment, blinding of participants and outcome assessors, incomplete outcome data, selective outcome reporting, and other potential sources of bias). Any possible discrepancy during the data extraction process and evaluation of risk bias will be discussed and solved among all reviewers.

2.10. Data analysis

Means and SDs or frequency and percentages will be used to present summary data for each study, as appropriate. The mean difference [95% confidence interval (CI)] or standardized mean differences (95% CI), if different scales of measurement are used, will be calculated for quantitative outcomes. The method of Wan et al. will be utilized to estimate the mean and SD in studies reporting median and range or interquartile range for quantitative variables (25). The odds ratio (OR) with 95% CI will be calculated for nominal outcomes from the data obtained from the studies. Data about ORs adjusted for potential confounders will be extracted from studies performing this analysis. For this meta-analysis, OR was chosen for all types of articles, including RTCs. Using the same effect size measure for all the types of studies may help evaluate the effect of the study design on the association. For example, we may see the influence of the study design on the result comparing the OR of randomized controlled trials' meta-analysis and the OR of cohort meta-analysis.

Meta-analysis will be performed if we find at least two suitable studies, using comprehensive meta-analysis software (Biostat, Inc., Englewood, CO, United States). A narrative description of the study results will be provided if the number of studies is sufficient to carry out a meta-analysis, pooling data from sufficiently similar studies.

Meta-analysis will be performed separately for observational studies and nonrandomized trials and randomized controlled trials. Moreover, the rescue and the preventive administration of platelet transfusions will be analyzed separately, since the underlying clinical settings are too dissimilar to be combined.

A random-effects model will permit us to account for the expected heterogeneity, between studies as well as within studies. We chose our model *a priori*, as a formal test for homogeneity based on the Q and I^2 statistics may not always be fully appropriate for choosing the analysis method (26, 27). We will not adopt the fixed-effects model, since we cannot assume that there will only be sampling error (28, 29). Furthermore, the random-effects model is more suitable for generalizing the analysis to other populations.

To evaluate statistical heterogeneity, we will use Cochran's Q statistic and the I^2 statistic, derived from Q and explains the proportion of variation that is due to heterogeneity beyond chance. I^2 greater than 50% would indicate significant heterogeneity, requiring the following analysis. Univariate random-effects meta-regression (method of moments) will be executed, in the case that more than 10 studies will be included, to explore differences among studies that might be expected to influence the effect size. We will consider statistically significant a probability value inferior to 0.05 (0.10 for heterogeneity). Subgroup analysis will be performed as well, based on the *a priori*-determined covariate of interest. In order to better interpret heterogeneity, we will also report the prediction intervals, so that treatment effects in future settings can be predicted.

The predicted sources of variability defined to drive the subgroup analysis and/or meta-regression will be: (i) disease of pregnancy (chorioamnionitis, placental dysfunction, diabetes, etc.); (ii) gestational age; (iii) birth weight; (iv) sex; (v) platelet initial count; (vi) reason, number, and timing of transfusion; (vii) ongoing treatments to prevent BPD (i.e., postnatal steroids, diuretics, bronchodilators, pulmonary vasodilators, vitamin A); (viii) site of bleeding (lung, intraventricular of gastrointestinal); (ix) neonatal morbidity (complication of prematurity, respiratory infections, late-onset sepsis, pulmonary hypertension, poor growth, difficulty feeding, developmental delay); (x) the oxygen saturation target defined as low target (85%–89%) or high target (91%–95%); (xi) threshold for platelet transfusion; and (xii) definition of established BPD and severity of BPD (moderate vs. severe). The mixed-effects model will guide the subgroup analysis: a random-effects model will be used to combine studies within each subgroup, and a fixed-effects model will be used to combine subgroups and yield the overall effect. We do not assume that the study-to-study variance is the same for all subgroups, and its value is not pooled across subgroups but computed within subgroups.

Egger's regression test and funnel plots will be utilized to assess publication bias. We defined the most likely subgroup analysis. In case some other variables may show a significant difference, they will be used for subgroup analysis.

In order to assess the impact of a specific study on the overall conclusion, we will also perform a cumulative meta-analysis.

2.11. Data synthesis

The characteristics and findings of the included studies will be summarized and described with a systematic narrative synthesis, with the information presented in the text and tables. Moreover, a narrative synthesis of the studies that cannot be integrated in the meta-analysis will be offered. The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) working group methodology will be used to estimate the quality of evidence for all outcomes (30–32). The domains of risk of bias, consistency, directness, precision, and publication bias will be used to judge the quality of evidence. It will be assessed as high (further research is very unlikely to change our confidence in the estimate of effect), moderate (further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate), low (further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate), or very low (very uncertain about the estimate of effect).

2.12. Missing data

We will not exclude studies because of missing summary data. We will include the study in the review, and then discuss the potential implications of its absence from the meta-analysis. If the details of the study-level characteristics needed for subgroup analysis or meta-regressions are not available, we will contact the study authors for more information. In case the authors are not willing or not able to provide the information required, we will discuss that as a limitation.

3. Discussion

The impact of platelet transfusion on the short- and long-term outcome, including BPD, is yet to be determined. Similarly the best threshold for platelets transfusion in non bleeding infants has not yet be defined. In 2020, a protocol for a systematic review on platelet transfusion for neonates was published (33). However, the primary objective differs in assessing evidence concerning the best threshold for platelet transfusion to reduce mortality, bleeding, and major morbidity among neonates with thrombocytopenia. The study has not been published yet. Our study differs from the one proposed by Liu et al. in the scope and the methods. Our aim is to evaluate a possible impact of platelet transfusion on the incidence of BPD in preterm infants. Hematological triggering as a contributor in the development of BPD has already been demonstrated through red cell concentrate (RCC) transfusions increasing the incidence of BPD in preterm infants, including its cumulative impact (34). While platelet transfusions are less frequent compared to RCC among preterm infants, demonstrating an association of platelet transfusion in the etiopathogenesis of BPD would be of considerable clinical value.

Author contributions

MP and SG contributed to the conception and design of the study and wrote the first draft of the manuscript. RC wrote the draft of the manuscript. AS, JU-D, KK, and MS participated in drafting the manuscript or revising for intellectual content. RKP, NAA, and JS-S contributed to the manuscript revision. KW developed and wrote the search strategy. MP supervised the writing and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1049014/full#supplementary-material

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EDITED BY
Shahana Perveen,
Cohen Children's Medical Center, United States

REVIEWED BY
Kari Roberts,
University of Minnesota Children's Hospital, United States
Yuan Shi,
Children's Hospital of Chongqing Medical University, China

*CORRESPONDENCE
Tae Hyun Kim
✉ taehyunkim@hanyang.ac.kr
Hyun-Kyung Park
✉ neopark@hanyang.ac.kr

†These authors have contributed equally to this work and share the first authorship

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Two-stage learning-based prediction of bronchopulmonary dysplasia in very low birth weight infants: a nationwide cohort study

Jae Kyoon Hwang[1†], Dae Hyun Kim[2†], Jae Yoon Na[1†], Joonhyuk Son[3], Yoon Ju Oh[2], Donggoo Jung[2], Chang-Ryul Kim[1], Tae Hyun Kim[4*] and Hyun-Kyung Park[1*]

[1]Department of Pediatrics, Hanyang University College of Medicine, Seoul, Republic of Korea, [2]Department of Artificial Intelligence, Hanyang University, Seoul, Republic of Korea, [3]Department of Pediatric Surgery, Hanyang University College of Medicine, Seoul, Republic of Korea, [4]Department of Computer Science, Hanyang University, Seoul, Republic of Korea

Introduction: The aim of this study is to develop an enhanced machine learning-based prediction models for bronchopulmonary dysplasia (BPD) and its severity through a two-stage approach integrated with the duration of respiratory support (RSd) using prenatal and early postnatal variables from a nationwide very low birth weight (VLBW) infant cohort.

Methods: We included 16,384 VLBW infants admitted to the neonatal intensive care unit (*N*ICU) of the Korean Neonatal Network (KNN), a nationwide VLBW infant registry (2013–2020). Overall, 45 prenatal and early perinatal clinical variables were selected. A multilayer perceptron (MLP)-based network analysis, which was recently introduced to predict diseases in preterm infants, was used for modeling and a stepwise approach. Additionally, we applied a complementary MLP network and established new BPD prediction models (PMbpd). The performances of the models were compared using the area under the receiver operating characteristic curve (AUROC) values. The Shapley method was used to determine the contribution of each variable.

Results: We included 11,177 VLBW infants (3,724 without BPD (BPD 0), 3,383 with mild BPD (BPD 1), 1,375 with moderate BPD (BPD 2), and 2,695 with severe BPD (BPD 3) cases). Compared to conventional machine learning (ML) models, our PMbpd and two-stage PMbpd with RSd (TS-PMbpd) model outperformed both binary (0 vs. 1,2,3; 0,1 vs. 2,3; 0,1,2 vs. 3) and each severity (0 vs. 1 vs. 2 vs. 3) prediction (AUROC = 0.895 and 0.897, 0.824 and 0.825, 0.828 and 0.823, 0.783, and 0.786, respectively). GA, birth weight, and patent ductus arteriosus (PDA) treatment were significant variables for the occurrence of BPD. Birth weight, low blood pressure, and intraventricular hemorrhage were significant for BPD ≥2, birth weight, low blood pressure, and PDA ligation for BPD ≥3. GA, birth weight, and pulmonary hypertension were the principal variables that predicted BPD severity in VLBW infants.

Conclusions: We developed a new two-stage ML model reflecting crucial BPD indicators (RSd) and found significant clinical variables for the early prediction of BPD and its severity with high predictive accuracy. Our model can be used as an adjunctive predictive model in the practical NICU field.

KEYWORDS

machine learning—ML, bronchopulmonary dysplasia (BPD), prediction, very low birth weight infants (VLBWI), nationwide cohort

1. Introduction

Despite the advances in respiratory care, the incidence of bronchopulmonary dysplasia (BPD) is increasing with the increase in the survival rate of extremely premature infants born at immature stages of lung development (1–3). As survivors with BPD undergo longer hospitalization with an increase in readmission after discharge and a high risk of poor pulmonary and neurodevelopmental outcomes (4, 5), the early identification of the risk of developing BPD is imperative for preventive interventions.

The commonly used National Institute of Child Health and Human Development (NICHD) criteria cannot determine the BPD severity until the postmenstrual age of 36 weeks (6). Thus, several models for predicting BPD have been established using birth weight (BW), gestational age (GA), sex, patent ductus arteriosus (PDA), sepsis, artificial ventilation, etc. to estimate the probability of BPD occurrence and optimize BPD treatment strategies (3, 7, 8). The majority of the existing models use traditional statistics (multiple logistic regression) or commercial machine learning (ML) methods, pay little attention to BPD severity, and are based on small sample populations (7–9). In addition, the fact that deceased patients were excluded from prediction model development was pointed out as a limitation.

Recently, artificial intelligence (AI) models have become promising prediction tools and have been used in several clinical applications (10–12); however, the use of machine learning (ML) algorithms is still limited in the field of neonatology. In previous studies, we showed efficient performance of the PDA prediction tasks of machine learning models (11), and then developed our new artificial neural networks (ANNs) for predicting intestinal perforation using very low birth weight (VLBW) infant data from a nationwide cohort (12). Therefore, we intend to apply our multilayer perceptron (MLP)-based experience and enhance its performance using a two-stage approach with one of the imperative variables for BPD occurrence to maximize clinical feasibility.

This study aimed to develop new ML models for the early prediction of BPD and its severity (BPD prediction model, PMbpd) using prenatal and early perinatal clinical variables obtained from a nationwide VLBW infant cohort and to compare their performance with that of classic ML models. Furthermore, we optimized the prediction model by building a two-stage approach through the first step of prediction using the duration of respiratory support (RSd), which is closely related to the BPD risk (13–15).

2. Materials and methods

2.1. Patients and data collection

This study investigated the database provided by the Korean Neonatal Network (KNN), a nationwide prospective registry of VLBW infants. The KNN consists of 77 tertiary hospitals, covering approximately 75%–80% of VLBW infants born in South Korea. It has enrolled VLBW infants, preterm infants born with birth weights of less than 1,500 g, or those transferred within 28 days after birth to registered neonatal intensive care units (NICUs) since 2013. This study was approved by the Hanyang University Institutional Review Board (IRB No. 2013-06-025-043). The inclusion criteria for this analysis was all VLBWIs from KNN data. Exclusion criteria are those that are more than 32 weeks old, or infants with major congenital anomalies, or has unclear data. It also excluded cases of death prior to 36 weeks, but deaths from BPD were included in the severe BPD.

2.2. Clinical variables and definition

In the KNN database, each chief neonatologist of the participating NICUs provided information regarding the data. The KNN network collects demographic, environmental, and clinical variables of VLBW infants from the prenatal period to 36 months of corrected age. The neonatologists reviewed the published literature and selected the potential variables. Overall, 45 variables were selected and modified from the database and classified as continuous or discrete (categorical or ordinal).

We used the BPD definition based on the 2001 NICHD criteria (6). No BPD (BPD 0) was defined as <28 days of supplemental oxygen intake. Mild BPD (BPD 1) included infants who received oxygen or respiratory support for >28 days but were on room air at 36 weeks postmenstrual age (PMA). Infants with moderate BPD (BPD 2) required supplemental oxygen and a <30% fraction of inspired oxygen concentration at 36 weeks PMA. Finally, severe BPD (BPD 3) was classified as the use of >30% oxygen or positive pressure at 36 weeks' PMA or death before 36 weeks' PMA from BPD.

RSd was defined as the duration of invasive ventilation in days. PDA treatment was defined as PDA with any treatment, and low blood pressure (BP) was defined as hypotension with medication within the first week of age. Sepsis was defined as a confirmed infection within the first week of life. Intraventricular hemorrhage (IVH) was defined using Papile's criteria and cranial ultrasonography (16). The majority of IVHs develop within 3 days, and PDA also affects the early postnatal period; therefore, these factors were included. Pulmonary hypertension (PHT) was defined as whether PHT was suspected or confirmed by echocardiography or clinically and was treated with medication within 1 week of age. The complete list of the 45 variables used in the analysis is shown in the **Supplementary (Table S1)** and was selected from the database based on the existing literature using the KNN database (12, 17).

2.3. Statistical analysis

The chi-square test and one-way analysis of variance (ANOVA) were used to compare the demographic and clinical characteristics among the four BPD severity levels. A *P*-value

<0.05 were considered significant for all statistical analyses. Statistical analyses were performed using SPSS, version 26.0 (IBM, Armonk, New York, USA).

2.4. Machine learning prediction model development

2.4.1. Classic machine learning algorithms

Several classic ML algorithms can handle disease prediction problems. Therefore, we chose several algorithms to confirm the diagnostic performance of classic ML algorithms for comparison with our proposed models. Predictions were conducted using the linear Support Vector Machine (SVM), radial SVM, logistic regression, k-Nearest Neighbor (k-NN), decision tree, Extreme Gradient Boost (XGBOOST), and Light Gradient Boost Machine (GBM) methods. We used the *xgboost* library for XGBOOST and the *lightgbm* library for Light GBM, and the remaining algorithms were obtained from the *Scikit-Learn library*.

2.4.2. Data preprocessing

The data preprocessing step before training is essential for improved training and performance in data-limited situations. First, among the 416 variables that can be obtained from KNN, we excluded variables in which more than half of the missing value. Forty-five variables were determined by selecting and processing prenatal and early postnatal variables related to BPD. To fill in the missing values, we divided the variables into three types: continuous, nominal, and ordinal. The remaining missing values were filled with the means for the continuous type of variables and the modes (nominal or ordinal variables). Before training, with min-max normalization, we scaled the data between 0 and 1. Finally, the preprocessed data was divided into 0.9 and 0.1 ratios for training and validation data, and additionally, to prevent data bias, the dividing process was conducted class-wise.

2.4.3. Training

With respect to training in the traditional and proposed approaches, the same preprocessed data were used for fairness. Traditional models were trained and evaluated using the *Scikit-Learn library,* and training was performed using default hyperparameter settings. When training MLP models, it is necessary to set hyperparameters, optimizers, and loss functions. Therefore, the proposed models were trained with the Adam optimizer (18), with a batch size of 128, a learning rate of 1e−3, and a dropout rate of 0.2. In addition, we used the mean squared error (MSE) loss for training instead of cross-entropy loss or binary cross-entropy loss, as setting the MSE loss for the objective function showed better performance in experiments. Although entropy-type losses are commonly used in training classification models, MSE losses are occasionally more effective (19). The training was conducted until the loss value of the evaluation did not decrease 10 times in a row, instead of fixing the training epoch. The parameters of the models were updated by backpropagating the MSE loss for PMbpd and TS-PMbpd. To aid the optimization process, we added dropout (20) and batch normalization (21) at each layer, except for the last one. All the settings aimed to improve the area under the receiver operating characteristic curve (AUROC) values, and to clarify each case's results, we added AUROC values and precision, recall, and f1-score. Our MLP models were implemented using the *PyTorch* library, and evaluations were performed using *the Scikit-Learn library*.

2.4.4. Prediction model development (PMbpd, Ts-PMbpd)

Traditional methods exist for accurate forecasts of BPD; however, improvements are required for accurate forecasts. In this study, we designed an ANN model for precise diagnoses, expecting neural networks to analyze extensive data on BPD more accurately. Because diagnosing BPD is a binary- or multi-classification problem that predicts the occurrence of BPD and types of BPD in infants from a given set of 45 variables, we started modeling from a simple MLP architecture that is widely used as a classifier. PMbpd is a one-dimensional input ANN model with a hidden layer (**Figure 1A**). The number of hidden layers was experimentally selected, and it exhibited high accuracy and stability during training. Our simple MLP model (PMbpd) showed excellent performance compared with traditional algorithms; however, we further developed PMbpd. The developed model is a two-stage model (TS-PMbpd) that uses information from the RSd to predict BPD severity. The TS-PMbpd consists of two MLP models and can be divided into two steps. In the first step, an MLP model predicted the RSd. In the next step, the final model uses input variables and a feature vector from the MLP model that predicts RSd by concatenation (12) at the hidden layer to forecast the BPD severity, as shown in **Figure 1B**. Because the feature vector from the first step MLP model contains information about RSd, which is a disease relevant to BPD, it helps to predict BPD severity. This could be confirmed by the improvement in performance for the BPD multi-classification problem, which is a problem with a small number of cases.

2.4.5. Shapley additive exPlanation (SHAP)

The Shapley value was calculated to determine the input variables that significantly affected the judgment of the model (22). Although there are other approaches (e.g., permutation feature importance and coefficients as feature importance) to determine the contribution of variables to the predictive model, it is often difficult to apply them to ANNs for interpretability. The Shapley value is an approach based on cooperative game theory that can check the degree of positive or negative impact on all variables. To obtain convenient computations and explainable results, a calculation method called SHAP was used (23).

3. Results

3.1. Study population and data selection

The study flowchart is shown in **Figure 2**. In total, 16,384 VLBW infants were enrolled in this prospective cohort. We

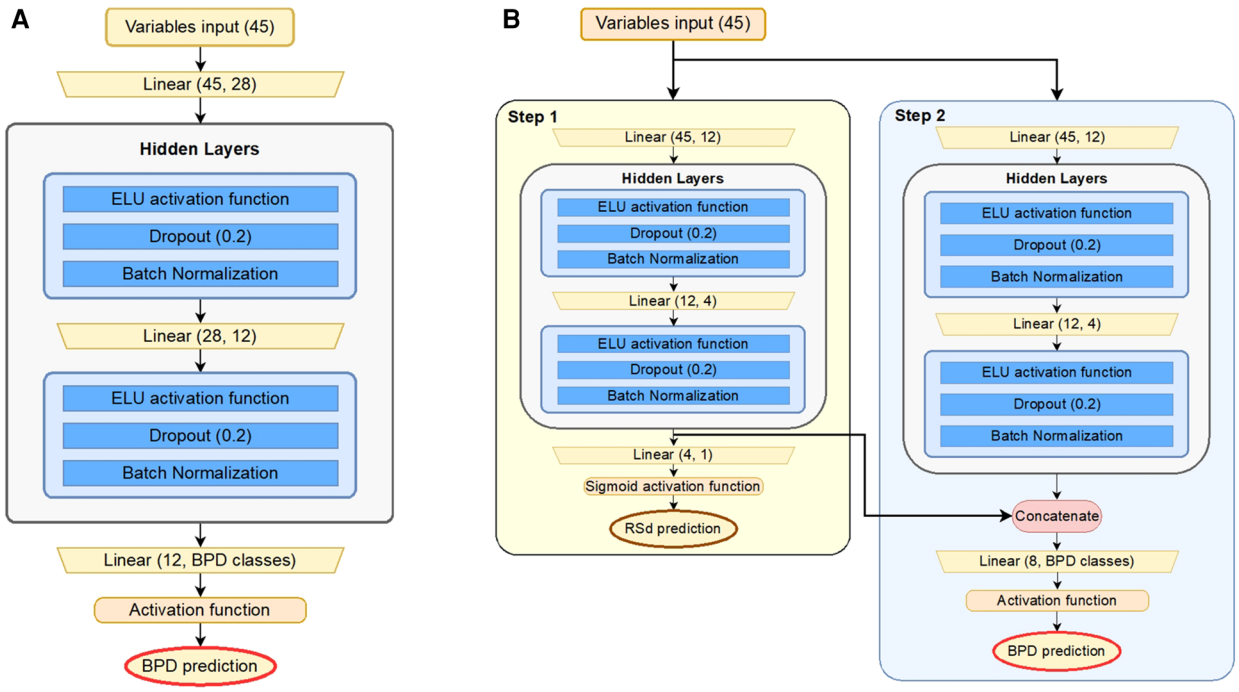

FIGURE 1
Structure of models: (A) pMbpd is a baseline neural network based on the conventional MLP architecture. (B) TS-PMbpd is composed of two different MLPs. In Step 1, one MLP predicts RSd, and in Step 2, the other MLP predicts BPD. The feature vectors from the second layer of the network for RSd are concatenated with the second layer of the MLP for BPD. The activation function of the last layer is sigmoid function for binary classification and softmax function for multi-classification. MLP, multilayer perceptron; BPD, bronchopulmonary dysplasia.

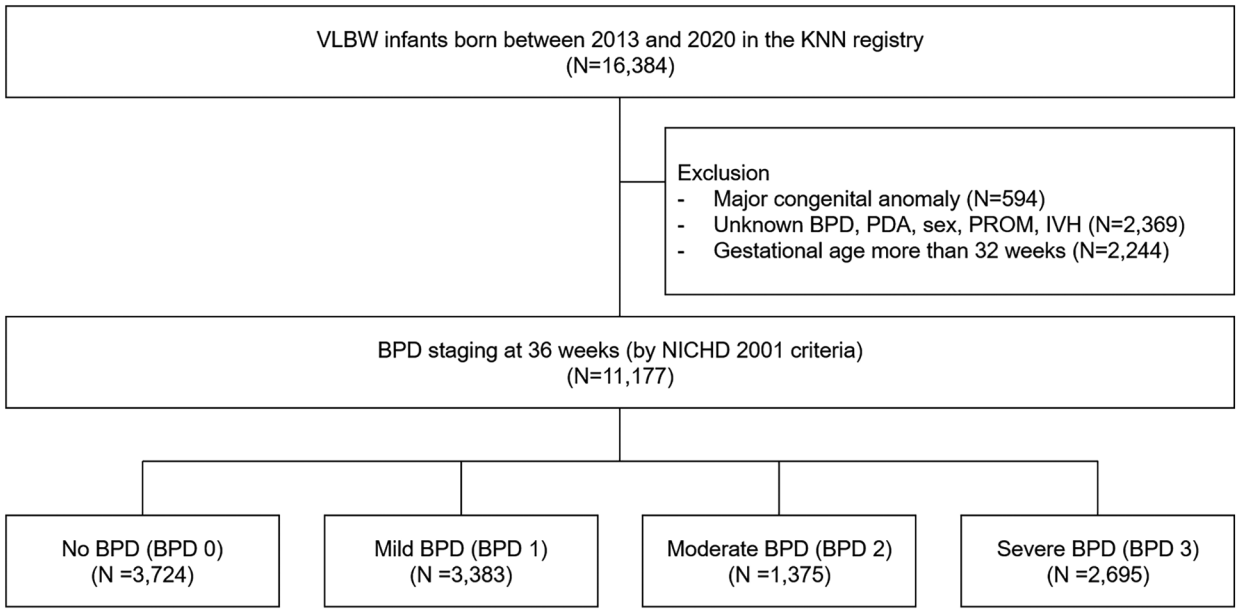

FIGURE 2
Flow diagram of the study population. KNN, Korean Neonatal Network; BPD, bronchopulmonary dysplasia; PDA, patent ductus arteriosus; PROM, premature rupture of membranes; IVH, intraventricular hemorrhage; NICHD, National Institute of Child Health and Human Development.

excluded infants who were diagnosed with major congenital anomalies ($N = 594$), cases whose presence of BPD, PDA, sex, premature rupture of membrane, IVH was not specified ($N = 2,369$), and gestational age >32 weeks ($N = 2,244$). Patients who died before being diagnosed with BPD were excluded, but those who died due to BPD were included in BPD 3. The baseline demographic characteristics of the subjects are summarized in Table 1. All variables except maternal overt diabetes mellitus,

maternal chronic hypertension and chorioamnionitis showed significant differences among the subgroups ($P < 0.001$). All the 45 variables stated in **Supplementary (Table S2)**.

3.2. Prediction performance between new PMbpd and classic ML models

The performance evaluation of the newly proposed BPD prediction model (PMbpd, TS-PMbpd) and other ML models applied in our study is summarized in **Table 2**. TS-PMbpd demonstrated outperformance about AUROC value and accuracy in the analysis of the diagnosis of BPD (0.8966, 0.8199, respectively). PMbpd demonstrated outperformance about F1-score and accuracy, and TS-PMbpd about AUROC value in the analysis of the diagnosis of BPD ≥2 (0.7754, 0.7764, 0.8253 respectively). PMbpd demonstrated outperformance about F1-score and AUROC values in the analysis of the presence of BPD 3 (0.7793, 0.8277, respectively). TS-PMbpd demonstrated

TABLE 1 Demographic and clinical characteristics of the study participants.

Variables	BPD 0 (*n* = 3,724)	BPD 1 (*n* = 3,383)	BPD 2 (*n* = 1,375)	BPD 3 (*n* = 2,695)	*P*-value
Sex (male)	1,718 (46.1)	1,673 (49.5)	743 (54.0)	1,465 (54.4)	<0.001
GA, weeks	30.04 ± 1.17	27.90 ± 1.65	27.61 ± 1.99	27.00 ± 2.12	<0.001
AS1	5.57 ± 1.79	4.53 ± 1.87	4.06 ± 1.83	3.95 ± 1.81	<0.001
AS5	7.61 ± 1.37	6.80 ± 1.66	6.37 ± 1.76	6.29 ± 1.79	<0.001
BW, gram	1,259.19 ± 183.51	1,064.39 ± 224.15	1,010.93 ± 245.57	885.79 ± 250.51	<0.001
BW_z	−0.01 ± 0.01	0.01 ± 0.54	0.06 ± 1.19	0.15 ± 1.84	<0.001
DM					
O_DM	48 (1.3)	48 (1.4)	21 (1.5)	44 (1.6)	0.709
A_DM	449 (12.1)	394 (11.6)	153 (11.1)	219 (8.1)	<0.001
HTN					
C_HTN	92 (2.5)	79 (2.3)	36 (2.6)	66 (2.4)	0.95
A_HTN	899 (24.1)	526 (15.5)	241 (17.5)	539 (20.0)	<0.001
CA	917 (24.6)	1,139 (33.7)	500 (36.4)	997 (37.0)	0.05
pH1h	7.27 ± 0.09	7.27 ± 0.10	7.26 ± 0.10	7.26 ± 0.11	<0.001
BE1h	−4.73 ± 3.16	−5.15 ± 3.38	−5.25 ± 3.47	−5.51 ± 3.62	<0.001
RDS	2,576 (69.2)	3,121 (92.3)	1,282 (93.2)	2,565 (95.2)	<0.001
SFT	2,543 (68.3)	3,161 (93.4)	1,291 (93.9)	2,575 (95.5)	<0.001
SFTnu	0.77 ± 0.61	1.20 ± 0.63	1.31 ± 0.79	1.38 ± 0.76	<0.001
PDATx	735 (19.7)	1,493 (44.1)	686 (49.9)	1,626 (60.3)	<0.001
PDALg	53 (1.4)	325 (9.6)	231 (16.8)	778 (28.9)	<0.001
PHT	21 (0.6)	78 (2.3)	76 (5.5)	453 (16.8)	<0.001
lowBP	195 (5.2)	652 (19.3)	412 (30.0)	1,237 (45.9)	<0.001

Values are expressed as numbers (%) or means (standard deviations). BPD, bronchopulmonary dysplasia; BPD 0, no BPD; BPD 1, mild BPD; BPD 2, moderate BPD; BPD 3, severe BPD; AS1, 1-minute Apgar score; AS5, 5-minute Apgar score; BW, birth weight; BW_z, birth weight z-score; O_DM, maternal overt diabetes mellitus; A_DM, maternal all types of diabetes mellitus; C_HTN, maternal chronic hypertension; A_HTN, maternal all types of hypertension; CA, histologic chorioamnionitis; pH1h, hydrogen ion concentration in the blood within 1 h after birth; BE1h, Base excess within 1 h after birth.
RDS, respiratory distress syndrome; SFT, need for surfactant; SFTnu, Number of administered surfactants; PDATx, patent ductus arteriosus with any treatment; PDALg, Patent ductus arteriosus with surgical closure; PHT, pulmonary hypertension with treatment within 1 week of age; low BP, hypotension with medication within 1 week of age.

TABLE 2 Comparisons of the performance in BPD and severity prediction.

	BPD 0 vs. BPD 1,2,3					BPD 0,1 vs. BPD 2,3					BPD 0,1,2 vs. BPD 3					BPD 0 vs. BPD 1 vs. BPD 2 vs. BPD 3				
	Precision	Recall	F1-score	AUROC	Accuracy	Precision	Recall	F1-score	AUROC	Accuracy	Precision	Recall	F1-score	AUROC	Accuracy	Precision	Recall	F1-score	AUROC	Accuracy
Linear SVM	0.8507	0.8632	0.8569	0.7801	0.8078	0.7475	0.5675	0.6452	0.7301	0.7728	0.6927	0.6309	0.6604	0.6309	0.7792	0.5052	0.5303	0.5174	0.7772	0.5803
Radial SVM	0.8405	0.8619	0.8511	0.768	0.7989	0.7249	0.5503	0.6256	0.7152	0.7602	0.777	0.6459	0.7054	0.6479	0.8096	0.4998	0.5741	0.5343	0.7721	0.5741
Logistic Regression	0.8518	0.8632	0.8575	0.7815	0.8087	0.7401	0.5945	0.6594	0.7375	0.7763	0.7444	0.667	0.7036	0.667	0.8051	0.5073	0.5803	0.5413	0.7842	0.5803
k-NN	0.8122	0.8351	0.8235	0.7245	0.7613	0.6726	0.5503	0.6054	0.6985	0.7388	0.6922	0.6411	0.6657	0.6411	0.7792	0.4757	0.4919	0.4836	0.6778	0.4919
Decision Tree	0.8083	0.8029	0.8056	0.7057	0.7417	0.5394	0.5381	0.5387	0.6433	0.6645	0.6644	0.6688	0.6666	0.6615	0.7524	0.4387	0.4375	0.4381	0.6053	0.4509
XGBOOST	0.8533	0.8579	0.8556	0.7815	0.8069	0.7005	0.6093	0.6517	0.7301	0.7629	0.7266	0.6686	0.6964	0.6686	0.798	0.5408	0.575	0.5573	0.7563	0.575
Light GBM	0.8626	0.8672	0.8649	0.7955	0.8194	0.7122	0.6019	0.6524	0.7313	0.7665	0.7328	0.6747	0.7026	0.6747	0.7533	0.5699	0.583	0.5764	0.7712	0.583
PMbpd	0.9441	0.7466	0.8338	0.8952	0.8019	0.7008	0.6732	0.7754	0.824	0.7764	0.7082	0.7591	0.7793	0.8277	0.7668	0.5045	0.5776	0.5672	0.7825	0.5777
TS-PMbpd	0.952	0.7185	0.8189	0.8966	0.8199	0.6651	0.7076	0.7653	0.8253	0.7639	0.7039	0.7523	0.7764	0.823	0.7641	0.5223	0.5912	0.5534	0.7855	0.5912

BPD, bronchopulmonary dysplasia; BPD 0, no BPD; BPD 1, mild BPD; BPD 2, moderate BPD; BPD 3, severe BPD; SVM, support vector machine; k-NN, k-Nearest Neighbor; XGBOOST, Extreme Gradient Boost; GBM, Gradient Boost Machine.

outperformance about AUROC value and accuracy in the analysis of the diagnosis of each BPD severity (0.7855, 0.5912, respectively). Detailed performance results, including prediction, recall, F1-score, and accuracy, are summarized in **Table 2**, and the receiver operating characteristic (ROC) curves are shown in **Figure 3**.

3.3. Importance analysis by SHAP

After producing ANN models for each binary and multi-classification, we sorted the top 20 variables that contributed the most to predicting the outcomes. **Figure 4** depicts the importance matrix plot and the SHAP summary plot designated for the ANN models. The principal variables that contributed to the diagnosis of BPD in VLBW infants were GA, BW, PDA treatment, and low BP (**Figure 4A**). Among the principal variables for the presence of BPD ≥2 and 3 in VLBW infants, the first two variables in the top six were BW and low BP, in the same order. The latter four were IVH, sex, PHT, and PDA ligation, which had the same composition but different orders (**Figures 4B,C**). The principal variables that helped the diagnosis of each BPD severity in VLBW infants were GA, BW, PHT, PDA ligation, low BP, and sex (**Figure 4D**). In particular, BW, low BP, and sex were in the top six in all classifications, and PDA ligation and PHT were in the top six in three out of four classifications. In general, we found that low BP, male sex, PHT, PDA ligation, and PDA treatment were positively correlated with BPD severity, and GA and BW were negatively correlated.

4. Discussion

This national cohort study developed new ML models enhanced with a complementary MLP network (PMbpd) and an additional stepwise approach (TS-PMbpd) using antenatal and early postnatal clinical variables and compared their predictive power for the prediction of BPD and its severity using antenatal

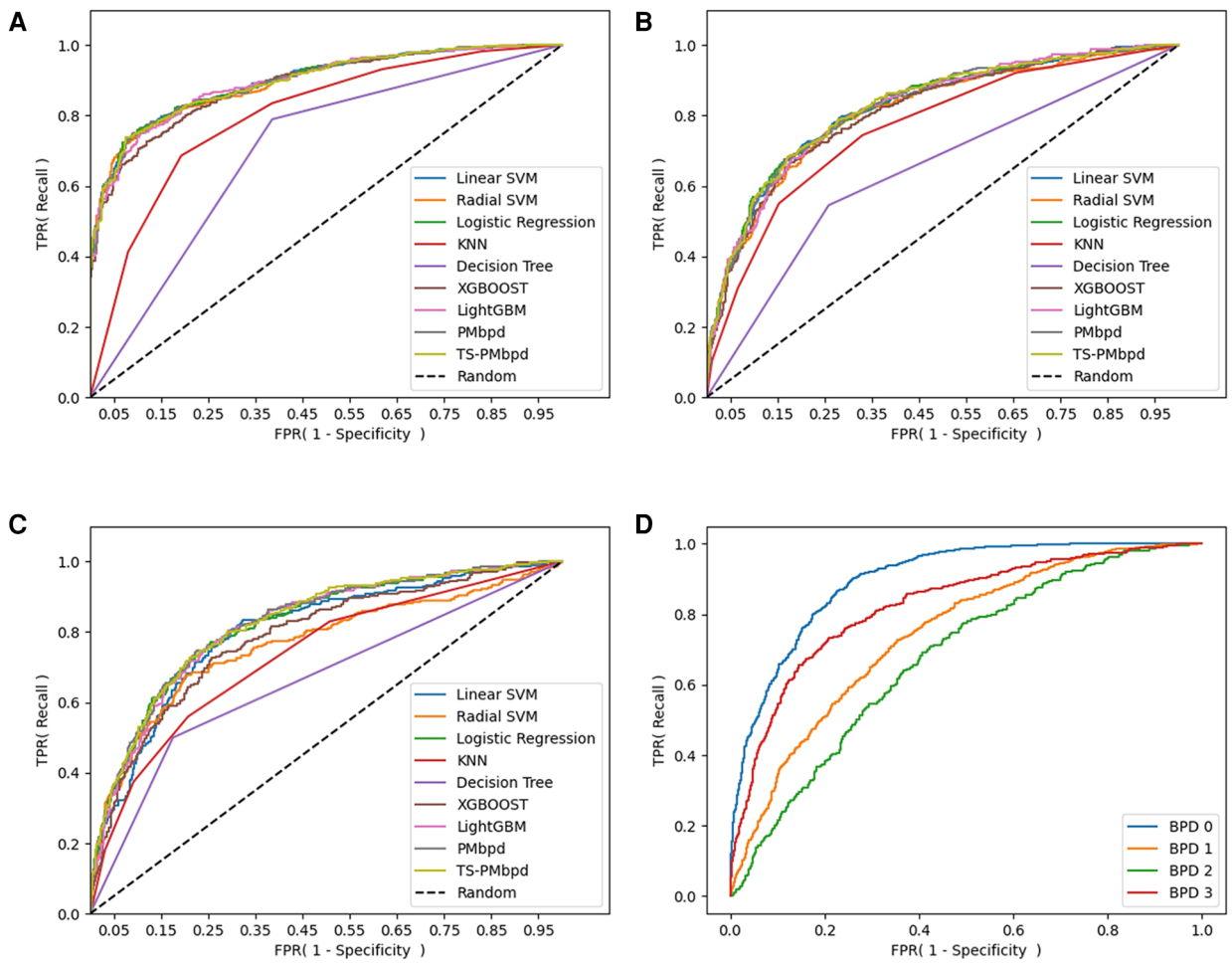

FIGURE 3
ROC curve of BPD prediction ML models: (**A**) ROC curve of BPD prediction ML models in binary classification (BPD 0 vs. BPD 1,2,3). (**B**) ROC curve of BPD prediction ML models in binary classification (BPD 0,1 vs. BPD 2,3). (**C**) ROC curve of BPD prediction ML models in binary classification (BPD 0,1,2 vs. BPD 3). (**D**) ROC curve of the TS-PMbpd model in multi-classification (BPD 0 vs. BPD 1 vs. BPD 2 vs. BPD 3). BPD, bronchopulmonary dysplasia; BPD 0, no BPD; BPD 1, mild BPD; BPD 2, moderate BPD; BPD 3, severe BPD.

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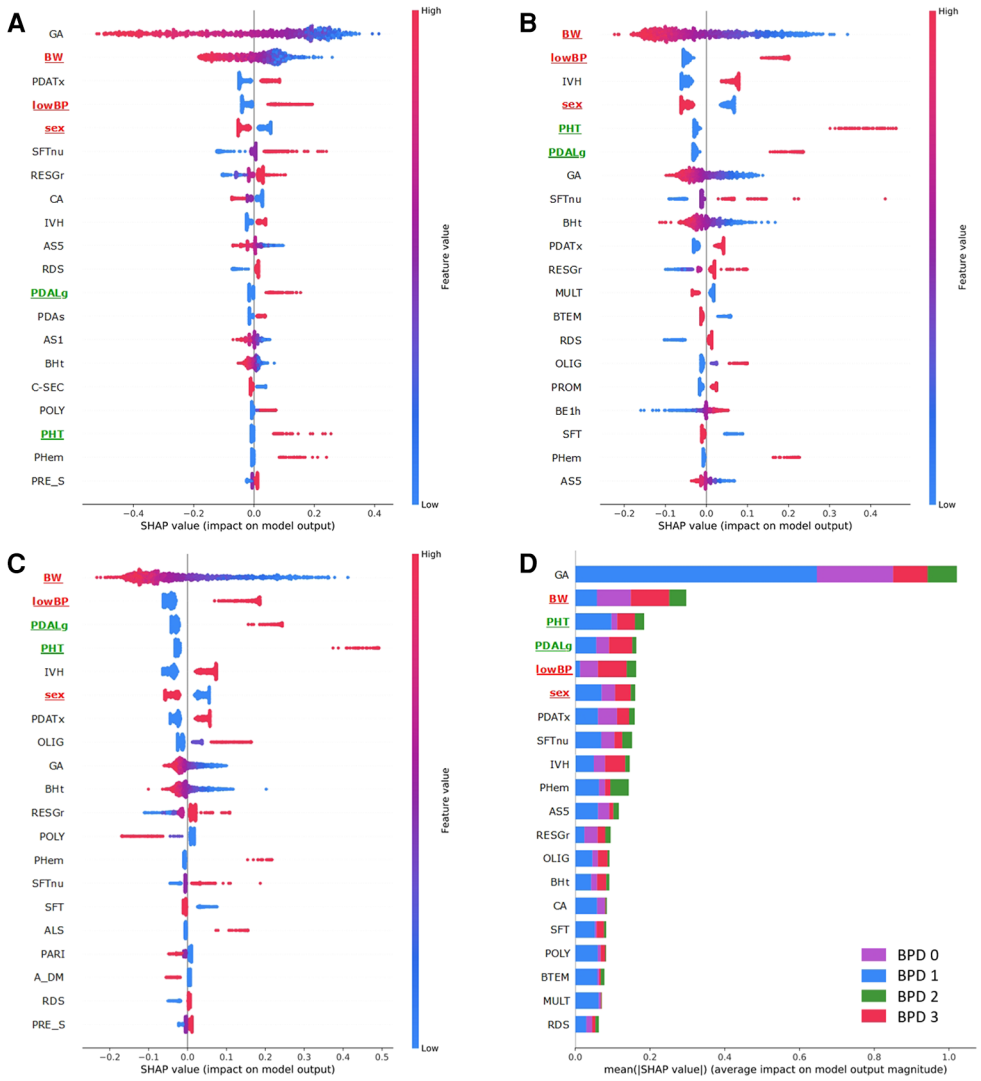

FIGURE 4
Top 20 variables contributions for BPD prediction by SHAP. (**A**) SHAP summary and importance matrix plots of the TS-PMbpd model in binary classification (BPD 0 vs. BPD 1,2,3). (**B**) SHAP summary and importance matrix plots of the TS-PMbpd model in binary classification (BPD 0,1 vs. BPD 2,3). (**C**) SHAP summary and importance matrix plot of the PMbpd model for binary classification (BPD 0,1,2 vs. BPD 3). (**D**) SHAP summary and importance matrix plot of the TS-PMbpd model in multi-classification (BPD 0 vs. BPD 1 vs. BPD 2 vs. BPD 3). In the dotted plot on the left, each dot represents one patient per feature, where red represents a higher value in continuous and ordinal variables (or positive correlations in categorical variables) and blue represents a lower value in continuous and ordinal variables (or negative correlations in categorical variables). The bar plot on the right presents the importance of each clinical variable in predicting the severity of BPD in VLBW infants in each model. The variable names in the top six for all classifications are highlighted in red, and top six in three out of four classifications are highlighted in green.

and early perinatal clinical variables. Our prediction model outperformed conventional logistic regression and other ML methods (SVM, k-NN, decision tree, XGBOOST, and Light GBM). We identified that GA, BW, and PDA treatment were significant variables for the occurrence of BPD and BW, and low BP for both BPD ≥2 and 3. Moreover, GA, BW, and PHT were the most important variables that predicted BPD severity in VLBW infants. Notably, BW, low BP, and sex were in the top six in all classifications, and PDA ligation and PHT were in the top six in three out of four classifications.

Although the SHAP value cannot be an absolute risk criterion, it is known to provide a common sense of the importance of each variable for individual predicted values (22). Therefore, it is possible to indirectly find out which variables affect the severity of BPD through the SHAP value. Antenatal Steroids, chorioamnionitis, fetal growth restriction, gestational age, birth weight, and sex are well-known prenatal risk factors for BPD. Postnatal factors include mechanical ventilation, postnatal steroids, patent ductus arteriosus, supplemental oxygen, and sepsis (24). In addition to well-known risk factors, in this study, low BP (25) and PHT (26), which required treatment within one week, were important as early factors for BPD. Previous studies (25, 26) have shown that the above variables play a role in the risk factors of BPD, but their importance is higher than expected, so attention should be paid to premature care and additional studies are needed in the future.

Several investigations have been intensively conducted to predict BPD and various severities in recent years, as poor short- and long-term outcomes have occurred in patients with BPD, especially in those with moderate and severe BPD (27). Most existing prediction tools have implemented traditional statistical methods for predicting BPD risk, mainly from smaller datasets or a single center, and have focused on the early prediction of BPD. Few have focused on its severity, although the three levels of severity have different outcomes (7, 27). Additionally, such models often have variable accuracy and yield inconsistent findings, leading to confusion or uncertainty among healthcare providers regarding the model to be used.

Recently, ML models have been promising prediction tools and have been used in numerous clinical applications, with the advantage of being able to minimize the error between predicted and observed outcomes. They applied ML algorithms such as logistic regression, XGBOOST, gradient-boosting decision trees, and random forests (27). Our study takes a step forward in identifying the clinical risk factors and developing effective early prediction models for BPD and its severity by securing a large number of BPD patients from a nationwide cohort registry and implementing a new deep learning technique. Although classic ML models are used for prediction, neural approaches have shown remarkable results in solving complex problems with big data. In addition, owing to their flexibility in designing the architecture of models and their nonlinearity, ANN often surpasses other ML methods in treating big data with complex distributions. Therefore, we first attempted to create a simple MLP model (PMbpd) that predicts BPD with factors that may occur prenatally and early after birth (usually within 1 week). Subsequently, we developed TS-PMbpd, which can provide various interpretations for input variables by creating the architecture of the model in two stages. Concatenating as our two-stage method is often used to improve performance in the computer vision field (28, 29) and is further used for classification problems (30) with insufficient data for securing diversity in feature interpretation, such as each severity prediction in the paper.

The KNN database is a nationwide cohort registry that includes approximately 75%–80% of VLBW infants born in South Korea and contains antenatal, postnatal, and long-term neurodevelopmental data. Our study sought to determine which factors were significant predictors of BPD in the NICU. Specifically, we selected the top 20 out of 45 variables to select the appropriate features. It is thought that it would be beneficial to reduce the variables a little more in future research to create compact modeling and go through the validation process. Additionally, by pooling VLBW infants from 77 different NICUs with a wide range of clinical conditions, neonatologists' preferences, and therapeutic protocols, we assume that our enhanced models could be feasible as BPD prediction tools in tertiary NICU settings that manage VLBW infants.

Respiratory support, especially for ventilator-induced lung injury, plays an important role as an independent risk factor in BPD development (13–15, 31). Therefore, in the first step of modeling, we chose variables to predict this factor and subsequently developed a two-stage model (TS-PMbpd) in a stepwise fashion to improve the predictive power. This could be confirmed by the improvement in performance for the BPD multi-classification problem, which is a problem with a small number of cases. This TS-PMbpd model showed its strength in predicting the severity and presence of BPD.

This study had a few limitations. BPD-related data, such as biomarkers, clinical symptoms, vital signs, and radiologic findings, could not be included because only data were collected from the KNN. Second, it was difficult to apply several clinical parameters to the model development because the exact timing of the occurrence was not recorded in a nationwide registry. Third, the longitudinal follow-up of variables during the NICU stay was not included in this model because each NICU has a different decision-making policy. Finally, it is difficult to determine the meaning of each parameter in the models and how ML methods generate results because of the nature of self-extracted data from large datasets.

Future developments with our BPD prediction models should reflect the changing BPD definition of these years and the great importance of predicting severe BPD patients, who have a worse prognosis and require more intensive care and follow-up. Moreover, we are considering developing a scoring system that is easy to use in clinical practice by inferring and assigning weights to the top compact variables.

5. Conclusions

Using a nationwide VLBW infant cohort, we developed new ML models incorporating crucial BPD indicators (RSd) into a two-step analysis and found significant clinical variables to predict early BPD and its severity with high predictive accuracy. In particular, our TS-PMbpd model showed the best performance in predicting the multi-classification of BPD for each severity; therefore, it can be used as an adjunctive predictive tool in clinical NICU practice for the early stratification of BPD.

Data availability statement

The datasets generated and/or analyzed during the current study are not publicly available due to the Korean Neonatal Network's (KNN) publication ethics policy. All information about patients is confidential; however, it is available from the corresponding author upon reasonable request. Requests to access the datasets should be directed to Hyun-Kyung Park, neopark@hanyang.ac.kr.

Ethics statement

This study was approved by the Hanyang University Institutional Review Board (IRB No. 2013-06-025-043). Hanyang University Seoul Hospital, 222-1 Wangsimni-ro, Seongdong-gu, Seoul 04763, Korea. Written informed consent to participate

in this study was provided by the participants' legal guardian/next of kin.

Author contributions

JH, DK, JN, JS, CK, TK, and HP had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. JH, DK, JS, and HP: Study concept and design. DK, YO, DJ, and TK: acquisition, analysis, and interpretation of the data. JH, DK, JN, and HP: manuscript drafting. DK, YO, and DJ: statistical analysis. TK and HP obtained funding, and park administrative, technical, or material support. JN, CK, TK, and HP supervised the study. All the authors critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1155921/full#supplementary-material.

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EDITED BY
Chung-Ming Chen,
Taipei Medical University, Taiwan

REVIEWED BY
Dinesh Upadhya,
Manipal Academy of Higher Education, India
Giuseppina Milano,
Centre Hospitalier Universitaire Vaudois (CHUV), Switzerland

*CORRESPONDENCE
Karen C. Young
✉ kyoung3@med.miami.edu

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Extracellular vesicles: pathogenic messengers and potential therapy for neonatal lung diseases

Shu Wu[1,2,3], Merline Benny[1,2,3], Joanne Duara[1,2,3], Kevin Williams[1,2,3], April Tan[1,2,3], Augusto Schmidt[1,2,3] and Karen C. Young[1,2,3,4]*

[1]Department of Pediatrics, University of Miami Miller School of Medicine, Miami, FL, United States, [2]Batchelor Children's Research Institute, University of Miami Miller School of Medicine, Miami, FL, United States, [3]Holtz Children's Hospital, Jackson Memorial Medical Center, Miami, FL, United States, [4]Interdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine, Miami, FL, United States

Extracellular vesicles (EVs) are a heterogeneous group of nano-sized membranous structures increasingly recognized as mediators of intercellular and inter-organ communication. EVs contain a cargo of proteins, lipids and nucleic acids, and their cargo composition is highly dependent on the biological function of the parental cells. Their cargo is protected from the extracellular environment by the phospholipid membrane, thus allowing for safe transport and delivery of their intact cargo to nearby or distant target cells, resulting in modification of the target cell's gene expression, signaling pathways and overall function. The highly selective, sophisticated network through which EVs facilitate cell signaling and modulate cellular processes make studying EVs a major focus of interest in understanding various biological functions and mechanisms of disease. Tracheal aspirate EV-miRNA profiling has been suggested as a potential biomarker for respiratory outcome in preterm infants and there is strong preclinical evidence showing that EVs released from stem cells protect the developing lung from the deleterious effects of hyperoxia and infection. This article will review the role of EVs as pathogenic messengers, biomarkers, and potential therapies for neonatal lung diseases.

KEYWORDS

extracellular vesicle, neonatal lung disease, bronchopulmonary dysplasia, mesenchymal stem cell (MSC), biomarkers

1. Introduction

Airway cells are often exposed to microbes, environmental insults such as hyperoxia, hypoxia, and mechanical stimuli. These ecological cues induce airway injury, inflammatory responses, and repair processes in the respiratory system. Coordinated intercellular communication is required to maintain lung homeostasis. However, constant exposure to these environmental insults can damage the epithelial barrier leading to excessive inflammatory responses and lung pathology. In the last decade, extracellular vesicles (EVs) have been recognized as important mediators of lung homeostasis and disease (1).

EVs are nano-sized particles characterized based on their physical properties such as size (small EVs are <200 nm and large or medium EVs are >200 nm) or density (low, middle or high), biochemical composition ($CD63^+/CD81^-$ EVs, Annexin A5 EVs, etc.) and description of conditions or cells of origin (lung epithelial cell-derived EVs, podocyte-derived EVs, hypoxia-induced EVs, etc.) (2). EVs contain a cargo of cell-specific lipids, proteins,

metabolites, and nucleotides that influence the molecular and functional properties of neighboring and distant target cells (2).

EVs are also categorized based on how they are generated (2). EVs generated by directly budding of the cell plasma membrane have been termed microvesicles, and these are typically 100–1,000 nm in size (3). On the other hand, exosomes (30–100 nm in diameter) are formed from exocytosis of intraluminal vesicles (ILVs). ILVs are generated by endocytosis of cellular cargo (proteins, lipids, metabolites, nucleotides), forming endosomes and subsequently multivesicular bodies (MVBs). MVBs are transported to the plasma membrane through the cytoskeletal and microtubule network. They undergo fusion with the plasma membrane and secretion of ILVs into the extracellular space as exosomes (4). This is regulated by various signaling mechanisms and stimuli, including receptor activation by adenosine triphosphate (ATP) and lipopolysaccharide (LPS) (5, 6). The process also involves the assembly of SNAREs (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors) complexes, which draw opposing membranes together to create the energy required for membrane fusion (7). Microvesicles are released through the outward budding and fission of the plasma membrane; this is calcium dependent and associated with cytoskeleton remodeling (3, 8–10).

Specific combinations of proteins and lipids such as tetraspanins, adhesion molecules, glycoproteins, cholesterol, sphingomyelin, and antigen presenting molecules are present on the surface of EVs (2). The exact composition is however dependent on the EV cellular origin, pathogenic conditions, and the mechanism of biogenesis (2). These proteins and lipids influence cellular transport, target cell identification and reception, cargo sorting, and cell programming (8).

EVs are produced by almost all cell types in the respiratory tract (11). Cell types already studied include alveolar type II pneumocytes, pulmonary vascular endothelial cells (PVECs), macrophages, mast cells, and fibroblasts. Under stress such as infection, oxidative stress, and mechanical stress, EVs released by injured lung cells contribute to the development of lung pathologies (12). In addition, lung cell-derived EVs may serve as biomarkers for lung disease risk and severity (11). We will review the mechanisms by which EVs induce lung pathology, the role of EVs as biomarkers in both adult and neonatal lung diseases, and the potential of EVs as vehicles for drug delivery (**Figure 1**).

2. EV isolation

The EV membrane is composed of a phospholipid bilayer containing major histocompatibility complex molecules and tetraspanins. A major challenge of EV research however is achieving high purity EVs while maintaining their integrity and biological activity. **Table 1** summarizes common methods of EV isolation.

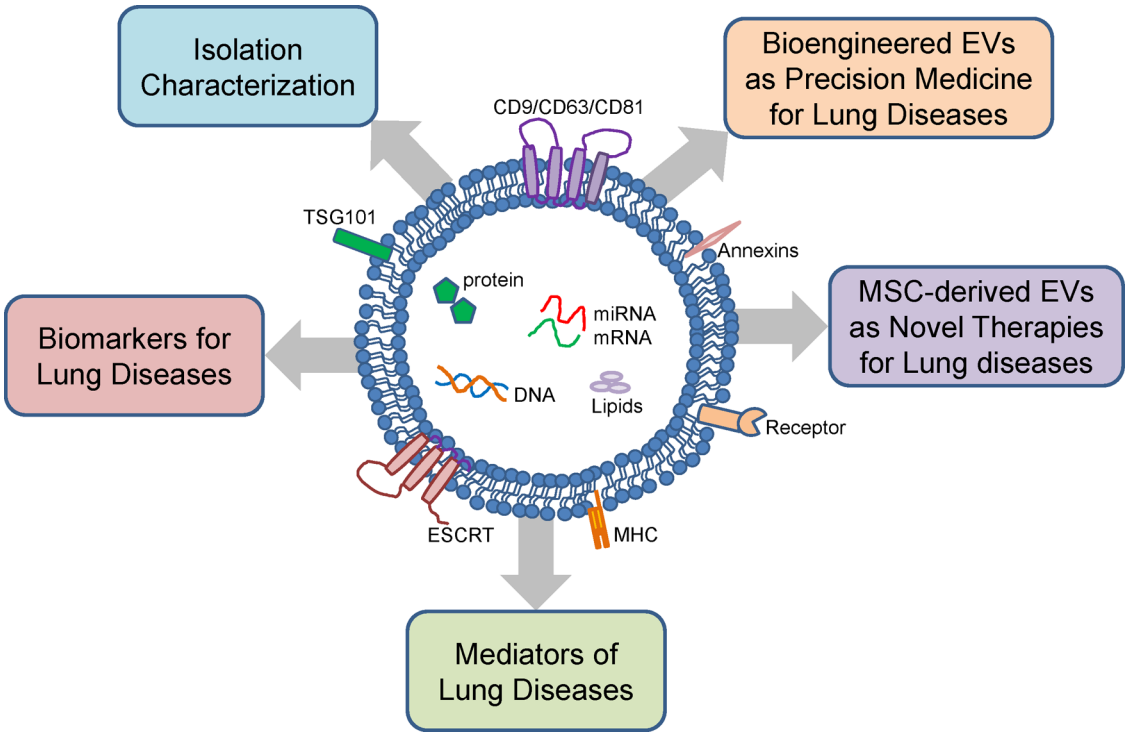

FIGURE 1
Structure, cargo and function of extracellular vesicles. Extracellular vesicles (EVs) are composed of a lipid bilayer containing transmembrane proteins with cargo consisting of proteins, mRNA, miRNA, DNA, and lipids. EVs can be isolated from various body fluids and have diverse sizes ranging from 100 to 1,000 nm. EVs isolated from the lung fluids and peripheral blood can be used as biomarkers for neonatal lung diseases. EVs have also been linked to the pathogenesis neonatal lung diseases. Mesenchymal stromal cell (MSC)-derived EVs and bioengineered EVs are potential novel therapies for neonatal lung diseases.

TABLE 1 Isolation of EVs.

Method	Advantages	Disadvantages	References
Ultracentrifugation	• Gold-standard • Cost-effective • High yield	• Time-consuming • Costly • Easily contaminated • Poor preservation of EV integrity and bioactivity due to high G force	(13, 14)
Ultrafiltration	• Simple • Cheap	• Low-yield • Poor preservation of EV integrity	(15)
Size-exclusion chromatography	• Cheap • Biologically intact EVs • Consistent yield	• Time-consuming but quicker than ultracentrifugation	(15–17)
Polymer precipitation	• Quick, simple process • Cost-effective	• Only smaller volume samples • Extremely prone to contamination by precipitation with non-exosome particles • Inconsistent results	(18, 19)
Immunoaffinity	• Quick, simple process • High purity	• Costly • Only smaller volume samples	(11, 13)
Membrane-based separation	• Quick • High yield • High purity	• Specific sample types only (e.g. urine), unable to handle samples with heterogenous cell types–will affect purity • Membrane clogging	(16)
Microfluidic platforms	• High-throughput • High yield • High purity	• Lack of standardization of devices resulting in heterogenous data • Only smaller volume samples	(20)

Ultracentrifugation is considered the gold standard. It utilizes extremely high centrifugal forces to separate EVs from other biological particles, is affordable and requires little technical skill. However, ultracentrifugation as a purification method is time-consuming, prone to contamination by other particles of similar weight and density, and EV integrity and bioactivity may not be preserved after ultracentrifugation (13, 14, 21).

Ultrafiltration employs physical filters of varying pore sizes and properties like application of electric charge and transmembrane pressure. Ultrafiltration can also be combined with other techniques such as low-speed centrifugation, ultracentrifugation, or size-exclusion chromatography (15). The process of ultrafiltration is simple to perform. However, there are limitations to sample processing – low-yield, membranes clogging, damage to EVs, types of samples that can be ultrafiltered, and the process of ultrafiltration is time-consuming (9).

Size-exclusion chromatography (SEC) is an increasingly popular technique that involves running samples through porous beads leading to separation of molecules by size. EVs isolated by SEC are biologically intact, making this method ideal for functional research (16). When used in conjunction with ultracentrifugation, EV yield and purity are significantly increased (17). The polymer precipitation method utilizes reagents such as polyethylene glycol (PEG) to cause precipitation of EVs, allowing isolation of EVs by simple centrifugation, making it cost-effective and efficient, but prone to contamination (18).

The immunoaffinity technique is done by priming a medium with target antibodies to bind with specific surface antigens or receptors present on EVs of interest. This isolates EVs with high purity, but this method is costly and difficult to sustain. Membrane-based separation methods isolate EVs through binding of membrane hydrophilic phosphate of EVs to metal oxides or the negatively charged membranes to positively charged molecules. This method is high yield, efficient and has high purity rates (19).

Microfluidic platforms are sophisticated networks utilizing various methods of purification organized in a miniature device. Purification methods include immunoaffinity, membrane-based filtration, nanowire trapping, acoustic nanofiltration, deterministic lateral displacement, and viscoelastic flow sorting. Microfluidic devices can achieve high throughput, high yield and high purity EVs, but there is a lack of standardization of devices contributing to heterogeneity of results reported by multiple investigators utilizing various devices (20).

3. EV characterization

Analyzing the particle size, morphology and biocomposition of EVs by multiple, complementary techniques is critical in evaluating the likelihood that biomarkers or functions are associated with EVs and not other co-isolated materials (2).

The International Society for Extracellular Vesicles has proposed the Minimal Information for Studies of Extracellular Vesicles-2018 (MISEV2018) guidelines, which recommends that the source and preparation of the EV must be described quantitatively (2). MISEV2018 also recommends using techniques that provide images of single EVs at high resolution

such as electron microscopy, using single particle analysis techniques that estimate biophysical features of EVs, and assessing the topology of EV-associated components (2). The commonly used EV characterization techniques are listed in Table 2.

Dynamic light scattering can be used to measure particle size, but analysis is limited when EVs of various sizes are present, and this cannot be used for functional analysis (22, 23). High resolution flow cytometry is a reliable and popular technique that enables structural analysis, quantification, and functional EV characterization (26). Nanoparticle tracking is a method by which the concentration, size distribution and particle velocity of EVs are measured. In nanoparticle tracking, specific antigens can also be identified with fluorescent tagged antibodies, providing more functional information (22). Atomic force microscopy is a technique that provides outputs of EV quantity, morphology, structural and functional analysis at a molecular level. This technique also preserves the integrity and bioactivity of EVs (24). Electron microscopy (EM) can also be used for structural characterization of EVs. EVs can be visualized with transmission EM, with a characteristic cup-shaped appearance of EVs due to dehydration during sample processing (25). Cryo-EM, on the other hand, allows for visualization of intact EVs without dehydration, enabling ultrastructural analysis of EV membranes and contents (31). EV membrane and cargo components can be analyzed with techniques according to molecule type, such as Western Blot and mass spectroscopy for proteins, and microarray and next generation sequencing for DNA or RNA (27–30).

Given the wide range of techniques available for both isolation and characterization of EVs with varying qualities, and with numerous research studies focusing on EVs that have been reported and are ongoing, there is a need for standardization of research protocols and techniques to maximize knowledge-sharing and productivity of the scientific community. Efforts are being made through the International Society for Extracellular Vesicles (ISEV) to create task forces and research guidelines to overcome these challenges (32, 33).

4. EVs in the pathogenesis of lung diseases

Increasing evidence indicates that EVs play essential roles in the pathogenesis of various adult lung diseases, including acute lung injury (ALI), acute respiratory distress syndrome (ARDS), asthma, chronic obstructive pulmonary disease (COPD), and pulmonary hypertension. The involvement of EVs in neonatal lung diseases has also been reported in bronchopulmonary dysplasia (BPD), but much less is known.

4.1. EVs and adult lung diseases

ALI and ARDS are devastating and rapidly progressive respiratory disorders that are characterized by disruption of the integrity of alveolar and vascular endothelial barriers (34–36). In

TABLE 2 Characterization of EVs.

Technique	Main features	Drawbacks	References
Nanoparticle tracking analysis (NTA)	• One of the most used methods • Provides parameters of concentration and particle size (10–2,000 nm)	• Critical parameters for success of NTA are sample preparation and the correct dilution factor	(2, 22)
Dynamic light scattering (DLS)	• Used for size measurements in the range of 1–6,000 nm • Possible recovering samples after analysis	• Detection of smaller particles becomes challenging in the mixture of small and large particles	(22, 23)
Atomic force microscopy (AFM)	• Detects the morphology of the sample in three-dimensional space • Generates topographic images of the samples with a resolution limit around 1 nm	• Measures samples in their native condition, which can turn into a limitation of the method as native state of different samples can be varied	(24)
Transmission electron microscopy (TEM)	• Images of high-resolution particles • Using immunogold-labeling to further reveal EV proteins	• Loss of material during extensive sample preparation • Lack of multiparametric phenotyping and low throughput capacity	(25)
Flow cytometry	• Records both the scattering and fluorescence signals • Analyzes multiple labels on individual particles • Identifies various types and subsets	• Low sensitivity to discriminate small size EVs • Low fluorescence being emitted by labeled EVs • Limited feasibility of post-stain washing to reduce background fluorescence	(26)
Protein content of EVs	• Proteomics technology allows the creation of large-scale profiling of proteins secreted through EVs • Immunoblotting can be used to detect EV markers and target proteins	• EVs must be broken prior to analysis • Some of the makers are not present in every/each EV • No single protein or combination of proteins can be recommended as universal EV markers	(27–30)
RNA content of EVs	• High-throughput RNA-seq • Validates by RT-qPCR	• Low yield of materials often below the detection limit of the most common quantification techniques	(27–30)

response to inflammatory stimuli, EVs and microparticles (MPs) are released from circulating inflammatory cells, damaged PVECs, and epithelial cells (37). In preclinical models, PVEC-derived EVs induce significant lung injury, as demonstrated by alveolar-capillary barrier failure, lung edema, and neutrophil infiltration in mice (38). These pathological effects are linked to and presumably at least in part mediated by the detrimental effects of PVEC-derived EVs on endothelial function. In ALI models, PVEC-derived EVs induce a reduction in endothelial nitric oxide (NO) production and an increased release of lung inflammatory cytokines (39).

Alveolar macrophage derived EVs are also abundant in the bronchoalveolar lavage fluid (BALF) in animal models of ALI. They are capable of inducing inflammatory responses both in vivo and in vitro (37, 40–42). Alveolar macrophage derived EVs trigger EV release by epithelial cells and neutrophils and deliver high concentrations of TNF-α to alveolar epithelial cells, leading to increased production of keratinocyte-derived chemokine and intercellular adhesion molecule-1 (37, 40–42), inducing a vicious cycle of inflammatory injury.

Alveolar epithelial cell derived EVs are also important mediators of ALI. In hyperoxia-induced ALI, alveolar epithelial cell-derived EVs are increased in BALF and serum (43) and they activate proinflammatory responses in systemic and pulmonary macrophages leading to disease progression (44).

COPD is characterized by severe airway inflammation and subsequent lung parenchymal damage. Mononuclear/macrophage-derived EVs rich in inflammatory mediators such as cytokines, chemokines, adhesion molecules, and proteases have been linked to alveolar wall destruction and emphysema, the hallmarks of COPD (11, 45). Endothelial-derived microparticles can promote the progression of COPD by inducing apoptosis of neighboring health endothelial cells upon delivery of inflammatory cargo (46). Epithelial-derived EVs have also been linked to the pathogenesis of COPD. Cigarette smoke stimulates human bronchial epithelial cells to release EVs enriched in full-length CYR61/CTGF/NOV family 1 (CCN1) protein that not only mediates IL-18 induced inflammation but also helps maintain lung homeostasis by increasing the levels of vascular endothelial growth factor (VEGF) (47). Cigarette smoke extract-induced human bronchial epithelial cell-derived EVs promote myofibroblast differentiation of lung fibroblasts, leading to the development of fibrosis (48). Cigarette smoke-exposed lung epithelial cells also release EVs that contain pro-inflammatory cytokines and Wnt-5a into the circulation, and these EVs can reach distant cells and organs (49).

EVs are also implicated in the pathogenesis of pulmonary hypertension. Patients with pulmonary arterial hypertension (PAH) have increased endothelial-derived CD62e microparticles in their pulmonary arterial blood (50). PAH patients also have increased microparticles positive for endothelial PECAM and VE-cadherin in their plasma samples (51). In monocrotaline-induced PAH, lung- and plasma-derived small-sized EVs isolated from monocrotaline-exposed mice induce PAH in healthy mice (52). EVs from PAH mice and patients contain elevated levels of miR-19b, miR-20a, miR-20b, and miR-145, known to target bone morphogenesis protein receptor signaling, apoptosis, and cell proliferation. EVs from the lungs of PAH mice reduce apoptosis of PVECs (53). Furthermore, EVs released by PVECs from PAH mice convert healthy bone marrow-derived endothelial progenitor cells into a pathological progenitor phenotype. These cells induce pulmonary vascular remodeling when injected into the lungs of healthy mice (54).

4.2. EVs and bronchopulmonary dysplasia (BPD)

BPD is the most common adverse outcome of extreme prematurity (55). It is the result of antenatal injury to the developing lung combined with repetitive and multiple post-natal insults, including oxygen therapy and ventilation, leading to alveolar simplification and vascular rarefaction (55). Not much is, however, known about the role of EVs in BPD pathogenesis. Genschmer and collaborators compared the function of EVs derived from BALF from BPD and non-BPD infants in a murine model (56). Intriguingly, mice that received intranasal BPD-derived EVs had significant alveolar hypoplasia and right ventricular hypertrophy, suggesting a role for EVs in BPD pathogenesis (56).

Recently, Lal et al. also demonstrated that the tracheal aspirate of infants with severe BPD had higher EV particle concentrations as compared to control infants, and the majority of these EVs were derived from epithelial cells (57). EVs shed from hyperoxia and LPS-exposed epithelial cells had reduced miR-876-3p. Gain of miR-876-3p in murine models attenuated hyperoxia and LPS-induced alveolar simplification, highlighting a potential critical role of lung epithelial cell-derived EV-miRNAs in the pathogenesis of BPD (57). miRNAs are non-coding RNAs that bind to sequences in the 3′ untranslated region (3′UTR) of target mRNA, resulting in the destruction of target mRNA or its repression (58).

Recently, our laboratory investigated the critical role of circulating EVs from hyperoxia-exposed and mechanical ventilated newborn rats in inducing brain injury in healthy newborn rats (59, 60). In the hyperoxia model, newborn rats were exposed to room air or 85% oxygen for two weeks, and circulating EVs were isolated from the plasma of these rats. Fluorescence activated cell sorting (FACS) and Western blot analyses demonstrated that the EVs from hyperoxia-exposed rats contain increased levels of both surfactant C (SPC) and gasdermin D (GSDMD), a key executor of inflammasome-induced cell pyroptosis. When these EVs were adoptively transferred into healthy newborn rats by intra-tail vein injection, they were taken up by the lung and brain. In the lung, the EVs from the hyperoxia-exposed rats induced inflammation, indicated by increased inflammatory cell infiltration in the alveolar airspaces and expression of inflammatory cytokines and chemokines. Furthermore, alveolarization and vascular density were drastically reduced in the lungs that received EVs from hyperoxia-exposed rats. In vitro experiments with PVECs demonstrated reduced cell

proliferation and increased cell death when cultured with EVs from hyperoxia-exposed rats (59). Upon examining the brain, EVs from hyperoxia-exposed rats induced brain inflammation by activating microglia and increasing expression of pro-inflammatory cytokines. These changes were associated with increased cell death in the cortex, subventricular zone, and subgranular zone. Additionally, in vitro experiments showed that neural stem cells (NSC) had decreased proliferation and increased cell death when cultured with EVs from hyperoxia-exposed rats (59). EVs from cultured hyperoxia-exposed lung epithelial cells induced pyroptosis in NSC (59). This data revealed a novel lung-brain crosstalk mediated by lung epithelial-derived EVs in both lung and brain injury.

This EV-mediated lung-brain crosstalk was further investigated in mechanical ventilation-associated brain injury in newborn rat models (60). We demonstrated that injurious mechanical ventilation induced similar markers of inflammation and pyroptosis, such as IL-1β and activated caspase-1/GSDMD in both lung and brain, in addition to inducing microglial activation and cell death in the brain (60). EVs isolated from neonatal rats with ventilator-induced lung injury had increased caspase-1. Adoptive transfer of these EVs into healthy newborn rats led to neuroinflammation with microglial activation and activation of caspase-1 and GSDMD in the brain, similar to that observed in neonatal rats that were mechanically ventilated (60). Thus, circulating EVs can contribute to brain injury and possibly poor neurodevelopmental outcomes in preterm infants exposed to hyperoxia and mechanical ventilation (60).

5. EVs as biomarkers for lung diseases

The stability of EVs is a potential advantage over traditional biomarkers. Traditional biomarkers such as proteins and RNA molecules are often unstable and susceptible to degradation over time, making them less reliable for diagnostic purposes. In contrast, EVs are surrounded by a protective lipid membrane that helps to stabilize their contents, including proteins, nucleic acids, and other molecular components (61). Proteomic and phosphoproteomic studies conducted on EVs from different cell types have suggested that they transport a diverse range of biologically relevant molecules, such as lipids, carbohydrates, RNAs, and some are believed to exhibit heterogeneity in composition, which is dependent on their cellular origin (62). EVs can carry specific proteins or RNA molecules that are unique to lung diseases. For example, sputum of patients with severe asthma has elevated levels of miR-142-3p, miR-629-3p, and miR-223-3p (63), and sputum-derived EVs from idiopathic pulmonary fibrosis (IPF) patients show an aberrant expression of miR-142-3p, miR-33a-5p, and let-7d-5p compared to healthy subjects (64).

There are few reports that EV-miRNAs can be used as biomarkers for BPD (65). In the study by Lal et al., EV miR876-3p was a potential biomarker for severe BPD in preterm infants. Decreased expression of EV miR-876-3p at birth predicted the future development of severe BPD in ELBW infants (57). This study established the predictive potential and causative role of microbiota-regulated miR-876-3p in severe BPD (57).

More recently, Ransom et al. characterized tracheal aspirate EVs in preterm infants between 22- and 35-week gestational age. Across all gestational ages, the majority of tracheal aspirate EVs expressed epithelial and immune cell markers. Moreover, infants who developed BPD had increased CD14+ EVs in their first tracheal aspirate obtained within 24 h of birth (66).

6. EVs as therapies for neonatal lung diseases

Mesenchymal stromal cells (MSCs) have regenerative properties and it is increasingly known that MSC-derived EVs replicate many of the beneficial effects of MSCs. EVs may also be bioengineered for drug delivery and genetically modified to carry specific target molecules. Although these therapeutic strategies are in the early stage of development, the prospect of using them in newborn infants is encouraging.

6.1. Stem cell derived EVs for newborn lung diseases

MSCs are efficacious in neonatal lung injury models (67–69). The pleiotropic properties of these cells make them particularly attractive and given their paracrine-mediated mechanism of action, MSC-derived EVs have been investigated as potential therapies.

In an experimental model of chorioamnionitis, antenatal administration of MSC-EVs reduced placental inflammation, and preserved lung structure, suggesting that antenatal MSC-EVs are efficacious in alleviating the deleterious effects of intrauterine inflammation. In experimental pre-eclampsia, MSC-EVs restore placental vascularity and preserve neonatal lung structure (70). In experimental BPD models, MSC-derived EVs restore alveolar structure, prevent lung vascular rarefaction, and alleviate PH by altering macrophage polarization, reprogramming bone marrow myeloid cells and increasing pro-angiogenic signaling pathways (70–77).

We recently compared the therapeutic efficacy of intra-tracheal (IT) and intravenously (IV) delivered MSC-EVs in a preclinical model of BPD. We demonstrated that systemically and IT delivered MSC-EVs have similar beneficial effects in experimental BPD (78). This finding is promising as IV MSC-EVs may also have beneficial effects on the developing brain (79). Another important question which we recently sought to address is the duration of MSC-EV therapeutic effects in experimental BPD. We administered MSC-EVs to neonatal pups with hyperoxia-induced BPD on postnatal day 3 and followed the pups into young adulthood (78). We found that one dose of MSC-EVs at postnatal day 3 had persistent beneficial effects at three month follow up (78). Importantly, late administration of MSC-EVs in an established BPD model was also found to partially reverse lung injury (79, 80). Clinical trials are now on the horizon but identifying the ideal patient will be critical.

6.2. Engineered EVs

EVs are also being investigated as "drug vehicles" (81). The ability of EVs to target a particular tissue or cell could be used to deliver drugs to intended targets while avoiding off-targets selectively (81). The "drug cargo" is selectively loaded into the EVs and the EVs are engineered to have specific properties to enhance their targeting and biomimetic features (82, 83). The lower number of transmembrane proteins, such as MHC complexes on their surface, make EVs less immunogenic than their parental source (84, 85). In addition, EVs do not replicate after injection. Thus, EVs are less likely to transfer latent viral pathogens or enable tumor generation (86). Compared to synthetic drug carriers, the intrinsic ability of EVs to cross cell barriers and penetrate tissues gives them an advantage (87). Synthetic drug carriers such as polymeric micelles and lipid nanoparticles cause high toxicity and immunogenicity compared to EVs (88). As therapeutic EVs are derived from benign biological or autologous sources, they are less likely to induce adverse effects.

Harnessing these unique properties of EVs to develop smart drug delivery systems with enhanced targeting, safety and pharmacokinetics has however been challenging (89). One study showed that that after intravenous injection, EVs are rapidly distributed and retained in the liver, spleen, gastrointestinal tract and lungs (90). Another study however showed rapid clearance of plasma-derived EVs following intravenous administration, with a half-life of approximately 7 min (91). Moving forward, more studies will be needed to understand EV circulation kinetics, biodistribution, cell tropism, and intracellular trafficking routes as the cellular origin, dose and route of administration may affect EV biodistribution pattern (92).

Other obstacles such as low isolation yield, the lack of purification protocols, large-scale clinical grade production, parental cell-dependent composition, and inefficient drug payload of the EVs continue to hamper the therapeutic ability of EVs (93). To improve *de novo* EV yield and therapeutic efficacy, re-engineering of the parental cell has been done through genome modification, stimulation with exogenous biomolecules and specific environmental factors (93). Bioreactors are also being extensively used to scale up the production of cell-based therapy and EVs. Bioreactors provide well-controlled nutrients, uniform culture conditions and biomimetic stimuli to regulate cell growth, differentiation and tissue development (94). While bioengineering of the parental cell predictably loads only a small proportion of the modified content into EVs, direct modification of isolated EVs may be another strategy to enrich EVs (95). For example, hydrophobically modified small interfering RNAs efficiently load into EVs upon coincubation, without altering EV size or integrity (96). Active EV loading can also be done by electroporation, sonication, extrusion, freeze-thawing and by surfactant-assisted loading, where surfactant saponin disrupts the membrane and increases its permeability (97).

Another option currently being investigated is the development of artificial EVs, namely the top-down and bottom-up approaches. The top-down approach is based on the disruption of the cultured cells to produce membrane fragments that will be used to form vesicles, while retaining the same membrane features of the initial cell (98). The bottom-up approach starts from small components of molecular building blocks to create complex structures, namely synthetic EVs (99).

7. Conclusion

We presented the evidence for lung-derived EVs as novel biomarkers and mediators for neonatal lung diseases and the potential for MSC-derived EVs as novel therapeutic modalities for neonatal lung diseases. Many of the studies discussed in this review are preclinical investigations that require successful translation from the bench to the bedside. Given that lung diseases are among the most common complications in preterm infants, with few effective therapies, it is crucial to continue discovering and understanding how EVs contribute to neonatal lung diseases and how to harness EVs to prevent and treat neonatal lung diseases. The incredible features of EVs in terms of their biocompatibility, cargo loading, cellular uptake, and escaping the immune system make them an appealing therapeutic strategy, but determining the ideal patient, route, dosing and timing will be essential to move forward. Procurement of EVs from physiologically relevant environments, the ability to scale up their manufacturing, optimize their biodistribution, and *in vivo* kinetics will also be crucial (93). This will contribute immensely to increasing the potential of EVs as acellular nanoscale therapeutics for neonatal lung diseases.

Author contributions

SW, MB, JD, KW, AT, AS, KY: conceived, drafted and reviewed the final submitted version of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Hisanori Sobajima,
Saitama Medical University, Japan

REVIEWED BY
Kazutoshi Cho,
Hokkaido University Hospital, Japan
Euiseok Jung,
University of Ulsan, Republic of Korea

*CORRESPONDENCE
Heui Seung Jo
✉ jo-zo@ hanmail.net

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Required biological time for lung maturation and duration of invasive ventilation: a Korean cohort study of very low birth weight infants

Heui Seung Jo[1]*, Myoung Nam Lim[2] and Sung-Il Cho[3]

[1]Department of Pediatrics, Kangwon National University Hospital, Chuncheon, Republic of Korea, [2]Biomedical Research Institute, Kangwon National University Hospital, Chuncheon, Republic of Korea, [3]Graduate School of Public Health and Institute of Health and Environment, Seoul National University, Seoul, Republic of Korea

Background: We investigated the duration of invasive ventilation among very low birth weight (VLBW) infants to evaluate the current minimum time required for lung maturation to breathe without ventilator assistance after preterm birth.
Methods: A total of 14,658 VLBW infants born at ≤32^{+6} weeks between 2013 and 2020 were enrolled. Clinical data were collected from the Korean Neonatal Network, a national prospective cohort registry of VLBW infants from 70 neonatal intensive care units. Differences in the duration of invasive ventilation according to gestational age and birth weight were investigated. Recent trends and changes in assisted ventilation duration and associated perinatal factors between 2017–20 and 2013–16 were compared. Risk factors related to the duration of assisted ventilation were also identified.
Results: The overall duration of invasive ventilation was 16.3 days and the estimated minimum time required corresponded to 30^{+4} weeks of gestation. The median duration of invasive ventilation was 28.0, 13.0, 3.0, and 1.0 days at <26, 26–27, 28–29, and 30–32 weeks of gestation, respectively. In each gestational age group, the estimated minimum weaning points from the assisted ventilator were 29^{+5}, 30^{+2}, 30^{+2}, and 31^{+5} weeks of gestation. The duration of non-invasive ventilation (17.9 vs. 22.5 days) and the incidence of bronchopulmonary dysplasia (28.1% vs. 31.9%) increased in 2017–20 (n = 7,221) than in 2013–16 (n = 7,437). In contrast, the duration of invasive ventilation and overall survival rate did not change during the periods 2017–20 and 2013–16. Surfactant treatment and air leaks were associated with increased duration of invasive ventilation (inverse hazard ratio 1.50, 95% CI, 1.04–2.15; inverse hazard ratio 1.62, 95% CI, 1.29–2.04). We expressed the incidence proportion of ventilator weaning according to the invasive ventilation duration using Kaplan–Meier survival curves. The slope of the curve slowly decreased as gestational age and birth weight were low and risk factors were present.
Conclusions: This population-based data on invasive ventilation duration among VLBW infants suggest the present limitation of postnatal lung maturation under specific perinatal conditions after preterm birth. Furthermore, this study provides detailed references for designing and/or assessing earlier ventilator weaning protocols and lung protection strategies by comparing populations or neonatal networks.

KEYWORDS

invasive ventilation, lung maturation, bronchopulmonary dysplasia, very low birth weight (VLBW) infant, neonatal network

Introduction

Bronchopulmonary dysplasia (BPD) is a serious lung disease in premature babies that affects not only the lungs but also growth and development (1–3). With the development of perinatal treatment, the survival rate of premature babies has increased; however, the prevalence rate of BPD has also increased (4–6). After preterm birth, biological time is necessary for lung development and maturation, enabling very preterm infants to survive without respiratory support. Long-term ventilator treatment, which is unavoidable to overcome the anatomical and functional limitations of an immature respiratory system in most cases, can result in tissue injury and inhibit ongoing developmental processes (7, 8). Various respiratory strategies that can minimize lung injury have contributed to the reduction of the duration of invasive ventilation and BPD incidence (6–9). However, the pulmonary structural development of alveolarization is challenging to manipulate using current treatment modalities. In addition, postnatal lung development in preterm infants is not expected to be the same as originally planned during the fetal period. Therefore, predicting the time and natural course of lung maturation is a primary concern for every very low birth weight (VLBW) infant.

The minimum time required for lung maturation sufficient enough to breathe without ventilator assistance after preterm birth is unclear. The duration of ventilator care appears to be mainly affected by the lung's immaturity. Besides postmenstrual age (PMA), various perinatal conditions, ethnic differences, and epigenetic factors are thought to be related to weaning points. Practically and preferentially, improved pulmonary function results in weaning from assisted ventilation. Spontaneous respiration without a breathing apparatus is a common treatment goal in every neonatal intensive care unit (NICU). Earlier weaning from a mechanical ventilator has many advantages: reduced hospitalization days and costs, lower mortality, and decreased risk of long-term pulmonary sequela, cardiovascular impairment and growth/neurodevelopmental delay (3, 6, 9, 10).

Herein, we aimed to investigate the different duration of assisted ventilation and determine the current PMA limit for ventilator weaning for each gestational age and birth weight group. We analyzed the incidence proportion of invasive ventilator weaning over time for each gestational age and birth weight group of VLBW infants based on the Korean Neonatal Network (KNN) database, a national multicenter prospective registry of VLBW infants in Korea (11). We also compared the current (2017–20) duration of assisted ventilation with that of 2013–16. Furthermore, the effects of associated principal early perinatal risk factors on the duration of assisted ventilation were suggested in the study.

Materials and methods

Data collection

Clinical data from 70 NICUs of KNN-participating hospitals were prospectively recorded in the KNN database and analyzed retrospectively in this study. The KNN registry was approved by the institutional review board of each participating hospital, and informed consent was obtained from the parents upon enrollment in the NICUs of KNN-participating hospitals (11). This study protocol was approved by the KNN executive board, and the requested data with decoded hospital and patient information were used to submit the results using these data, which were approved by the Korea Centers for Disease Control and Prevention.

Study participants

A total of 14,658 VLBW infants born at $\leq 32^{+6}$ weeks and registered in the KNN database from January 1, 2013, to December 31, 2020, were included in this study. Infants with major congenital or chromosomal abnormalities were excluded. A total of 7,437 VLBW infants were enrolled in 2013–16 and 7,221 in 2017–20. We identified recent demographical and clinical trends by comparing the rate or distribution of perinatal characteristics among infants born in 2013–16 vs. 2017–20.

Each study variable was defined according to the KNN manual of operation. "Surfactant treatment" was limited to the case of prophylaxis or rescue for respiratory distress syndrome, not for pulmonary hemorrhage or meconium aspiration syndrome. "Air leaks" was limited to a case that required chest tube insertion or needle aspiration. "Massive pulmonary hemorrhage" was severe bleeding enough to cause a cardiovascular collapse or acute respiratory failure. BPD was defined as the need for positive pressure ventilation or oxygen supplementation at 36 weeks PMA. This definition corresponded to the moderate to severe BPD definition from the National Institute of Child Health and Human Development (12, 13).

Study outcomes

The primary study outcome was the duration of mechanical ventilator support and the estimated weaning time from invasive ventilation according to different gestational age and birth weight groups. Assisted ventilation was defined as any type of invasive or non-invasive positive- pressure ventilation (PPV). Invasive ventilation includes conventional, high-frequency oscillatory ventilation or intermittent PPV through an endotracheal tube. Non-invasive ventilation includes nasal continuous positive airway pressure (CPAP), nasal NIPPV, and high-flow oxygen therapy delivered by a nasal cannula (HFNC) > 2 L/m. When more than one type of ventilator support was used on the same day, the longest-used ventilator was regarded as the main respiratory support mode. We investigated the difference in the duration of invasive and non-invasive ventilation between 2013–16 and 2017–20. We also identified the initial perinatal factors associated with the duration of invasive and non-invasive ventilation.

The secondary outcome was the incidence proportion of ventilator weaning by invasive ventilation duration according to

the gestational age (by 2 weeks) and birth weight (by 250 g) among the infants who received invasive ventilator care. The incidence proportion was expressed using the Kaplan–Meier survival curve. We observed that the change in the curve slope or curve shift depends on the presence of specific risk factors.

Statistical analyses

Data were analyzed using the chi-square test for categorical variables and student's t-test for continuous variables. The cumulative duration of assisted ventilation was described as the median and interquartile range (IQR). Kaplan–Meier survival curves were used to determine the weaning time of invasive ventilator treatment according to gestational age and birth weight groups. Cox regression analysis was used to identify maternal and initial neonatal factors affecting the required duration of PPV support (invasive and non-invasive). $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS version 26.0 software (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA).

Results

Maternal and neonatal characteristics

Among the study population of infants ($n = 14,658$), maternal age, the rates of maternal diabetes and hypertension development during pregnancy, and cesarean section increased in the 2017–20 group than in the 2013–16 group (Table 1). The overall maternal age was 33.3 years, 36.1% of infants were born from multiple births, and 85.2% received antenatal steroid therapy between 2013 and 2020.

The mean gestational age, birth weight, and the distribution of them did not differ between the 2013–16 and 2017–20 groups (Table 1). Apgar score at 5 min was higher in the 2017–20 group than in the 2013–16 group. The frequencies of surfactant treatment, air leaks, and massive pulmonary hemorrhage were lower in the 2017–20 group. The overall survival rate was 85.2% in the 2013–20 group and was not different between the two groups.

TABLE 1 Demographics and perinatal characteristics.

Characteristics	Total ($n = 14,658$)	2013–16 ($n = 7,437$)	2017–20 ($n = 7,221$)	*P*-value
Maternal characteristics				
Maternal age (yr)	33.3 ± 4.3	32.9 ± 4.2	33.7 ± 4.4	<0.0001
Multiple gestation	5,285 (36.1)	2,606 (35.0)	2,679 (37.1)	0.009
Maternal diabetes during pregnancy	1,500 (10.2)	636 (8.6)	864 (12.0)	<0.0001
Maternal hypertension during pregnancy	2,984 (20.4)	1,382 (18.6)	1,602 (22.2)	<0.0001
Premature rupture of membrane	5,610 (38.3)	2,872 (38.6)	2,738 (37.9)	0.38
Antenatal steroid therapy	12,483 (85.2)	6,065 (81.6)	6,418 (88.9)	<0.0001
Cesarean section	11,478 (78.3)	5,660 (76.1)	5,818 (80.3)	<0.0001
Neonatal characteristics				
Gestational age (wk)	$28^{+2} \pm 2^{+3}$	$28^{+2} \pm 2^{+3}$	$28^{+2} \pm 2^{+4}$	0.61
<26	2,990 (20.4)	1,526 (20.5)	1,464 (20.3)	0.71
26–27	3,203 (21.9)	1,643 (22.1)	1,560 (21.6)	0.47
28–29	4,338 (29.6)	2,191 (29.5)	2,147 (29.7)	0.72
30–32	4,127 (28.2)	2,077 (27.9)	2,050 (28.4)	0.54
Birth weight (g)	1,050 ± 290	1,050 ± 280	1,050 ± 290	0.14
<750	2,633 (18.0)	1,295 (17.4)	1,338 (18.5)	0.08
750–999	3,535 (24.1)	1,794 (24.1)	1,741 (24.1)	0.99
1,000–1,249	3,996 (27.3)	2,050 (27.6)	1,946 (26.9)	0.40
1,250–1,499	4,494 (30.7)	2,298 (30.9)	2,196 (30.4)	0.52
Males	7,432 (50.7)	3,771 (50.7)	3,661 (50.7)	0.99
Apgar score at 5 min	6.73 ± 1.88	6.67 ± 1.84	6.78 ± 1.91	<0.0001
Surfactant treatment	12,299 (83.9)	6,385 (85.9)	5,914 (81.9)	<0.0001
Air leaks	853 (5.8)	463 (6.2)	390 (5.4)	0.03
Massive pulmonary hemorrhage	955 (6.5)	525 (7.1)	430 (6.0)	0.007
Postnatal steroid therapy	3,744 (25.5)	1,827 (24.6)	1,917 (26.5)	0.006
Duration of hospitalization (d)	69.2 ± 44.0	67.0 ± 42.3	71.5 ± 45.6	<0.0001
Survival rate	12,494 (85.2)	6,318 (85)	6,176 (85.5)	0.44

Values are expressed as numbers (%).

BPD incidence at 36 weeks of gestation

The overall BPD incidence was 29.9%. The BPD rate increased from 28.1% in 2013–16 to 31.9% in 2017–20 ($P = 0.0001$). Also, the rate of death before 36 weeks of gestation and severe BPD increased in 2017–20 compared to 2013–16 (Table 2). The rate of surviving infants without BPD decreased from 33.5% in 2013–16 to 29.7% in 2017–20.

Among the 12,545 infants that survived, the respiratory support status at 36 weeks PMA was as follows: 29.6% needed supplemental oxygen, 18.3% required non-invasive ventilation, and 6.7% needed invasive ventilation. The rate of non-invasive ventilation and the need for supplemental oxygen at 36 weeks PMA were higher in 2017–20 than in 2013–16 (Table 2).

Duration of respiratory supports

The duration of non-invasive ventilation increased from 17.9 days in 2013–16 to 22.5 days in 2017–20 (Table 2). Also, the proportion of non-invasive ventilation to total PPV (invasive + non-invasive ventilation) was 0.59 ± 0.37, which increased from 0.55 ± 0.37 in 2013–16 to 0.63 ± 0.36 in 2017–20. However, the overall duration of invasive ventilation did not change between 2013–16 and 2017–20. The overall duration of invasive ventilation was 16.3 ± 28.6 days (95% confidence interval, 15.8–16.7), and the median duration (25–75 IQR) was 4.0 (1.0–21.0) days. The median (25–75 IQR) estimated minimal required time for weaning from invasive ventilation, calculated as the corrected gestational age, was 30^{+1} (28^{+5}–31^{+5}). The mean duration of invasive ventilation according to the gestational age groups was as follows: 35.8 days at <26 weeks of gestation, 23.0 days at

TABLE 2 Duration of respiratory supports and the incidence of bronchopulmonary dysplasia.

Variables	Total ($n = 14,658$)	2013–16 ($n = 7,437$)	2017–20 ($n = 7,221$)	*P*-value
Duration of respiratory supports (d)				
A Invasive ventilation	16.3 ± 28.6	15.9 ± 27.7	16.6 ± 29.5	0.16
B Non-invasive ventilation	20.2 ± 21.9	17.9 ± 20.6	22.5 ± 22.9	<0.0001
C Supplemental oxygen	6.8 ± 12.6	7.7 ± 13.4	5.9 ± 11.7	<0.0001
B / A + B	0.59 ± 0.37	0.55 ± 0.37	0.63 ± 0.36	<0.0001
A′ Minimal required maturation time (PMA) of weaning from invasive ventilation (wk)	$30^{+4} \pm 3^{+6}$	$30^{+4} \pm 3^{+5}$	$30^{+4} \pm 5^{+0}$	0.07
BPD at 36th postmenstrual weeks				
Non-BPD	4,659 (31.8)	2,489 (33.5)	2,153 (29.8)	<0.0001
Mild BPD	3,498 (23.9)	1,816 (24.4)	1,682 (23.3)	0.11
Moderate BPD	1,472 (10.0)	809 (10.9)	663 (9.2)	0.001
Severe BPD	2,916 (19.9)	1,278 (17.2)	1,638 (22.7)	<0.0001
Death < 36 weeks	2,113 (14.4)	1,045 (14.1)	1085 (15.0)	0.06
Respiratory support at 36th postmenstrual weeks[a]	($n = 12,545$)	($n = 6,409$)	($n = 6,136$)	
A Invasive ventilation	837 (6.7)	407 (6.4)	430 (7.0)	0.14
B Non-invasive ventilation	2,302 (18.3)	932 (14.5)	1,370 (22.3)	<0.0001
C Supplemental oxygen	3,710 (29.6)	1,774 (27.7)	1,936 (31.6)	<0.0001

Values are expressed as numbers (%); PMA, postmenstrual age; BPD, bronchopulmonary dysplasia; Non-BPD, surviving infants without BPD
[a]among the surviving 12,545 infants at 36th postmenstrual weeks.

26–27 weeks, 9.2 days at 28–29 weeks, and 4.3 days at 30–32 weeks (Table 3). The median duration (25–75 IQR) of invasive ventilation was 28.0 (9.0–51.0), 13.0 (3.0–31.0), 3.0 (1.0–8.0), and 1.0 (0.0–3.0) days at <26, 26–27, 28–29, and 30–32 weeks of gestation, respectively. With respect to the invasive ventilation duration in the years 2013–16 and 2017–20, there was no difference except in the <26 weeks of gestation group. The median estimated minimal required time for weaning from invasive ventilation was calculated as the corrected gestational age: 28^{+6}, 28^{+6}, 29^{+4}, and 31^{+3} weeks for <26, 26–27, 28–29, and 30–32 weeks, respectively. The mean duration of invasive ventilation according to the birth weight groups was as follows: 35.9 days for <750 g, 23.8 days for 750–999 g, 9.7 days for 1,000–1,249 g, and 4.6 days for 1,250–1,499 g (Table 4). The median duration (25–75 IQR) of invasive ventilation was 27.0 (7.0–51.0), 14.0 (3.0–35.0), 3.0 (1.0–10.0), and 1.0 (0.0–4.0) days for <750 g, 750–999 g, 1,000–1,249 g, and 1,250–1,499 g, respectively. With respect to the invasive ventilation duration in the years 2013–16 and 2017–20, there was no difference except in the <750 g birth weight group. The median estimated minimal required time for weaning from invasive ventilation was 29^{+4}, 29^{+5}, 29^{+6}, and 30^{+5} weeks for <750 g, 750–999 g, 1,000–1,249 g, and 1,250–1,499 g, respectively. Adjusted *p*-values for multiple comparisons between each gestational age or birth weight group are shown in Supplementary Table S1.

Perinatal risk factors associated with PPV duration

Maternal and early neonatal risk factors associated with PPV duration were investigated using Cox regression analysis (Table 5). In addition to the gestational age, the presence of

TABLE 3 Duration and distribution of assisted invasive ventilation among different gestational age groups.

	Duration of invasive ventilation (d)					Minimal required maturation time[a] of weaning from invasive ventilation (wk)				
	Mean (SD) 95% CI	Median	IQR 25th	IQR 75th	*p*-value	Mean (SD) 95% CI	Median	IQR 25th	IQR 75th	*p*-value
Gestational age (wk)										
<26 ($n = 2,990$)	35.8 (35.8) 34.6–37.1	28.0	9.0	51.0		29^{+5} (5^{+2}) 29^{+4}–29^{+6}	28^{+6}	26^{+0}	32^{+0}	
2013–16 ($n = 1,526$)	34.5 (35.0) 32.7–36.2	27.0	8.0	48.0	0.032	29^{+4} (5^{+1}) 29^{+2}–29^{+5}	28^{+5}	26^{+0}	31^{+5}	0.080
2017–20 ($n = 1,464$)	37.3 (36.6) 35.4–39.2	30.0	10.0	54.0		29^{+6} (5^{+2}) 29^{+4}–30^{+1}	29^{+1}	26^{+0}	32^{+2}	
26–27 ($n = 3,203$)	23.0 (32.4) 21.9–24.1	13.0	3.0	31.0		30^{+2} (4^{+4}) 30^{+1}–30^{+3}	28^{+6}	27^{+5}	31^{+2}	
2013–16 ($n = 1,643$)	22.9 (32.3) 21.3–24.5	12.0	3.0	32.0	0.83	30^{+2} (4^{+4}) 30^{+1}–30^{+3}	28^{+6}	27^{+5}	31^{+3}	0.81
2017–20 ($n = 1,560$)	23.1 (32.5) 21.5–24.7	13.0	3.0	31.0		30^{+2} (4^{+4}) 30^{+0}–30^{+4}	28^{+6}	27^{+5}	31^{+2}	
28–29 ($n = 4,338$)	9.2 (20.0) 8.6–9.8	3.0	1.0	8.0		30^{+2} (2^{+6}) 30^{+1}–30^{+3}	29^{+4}	29^{+0}	30^{+2}	
2013–16 ($n = 2,191$)	9.0 (19.5) 8.2–9.9	3.0	1.0	8.0	0.63	30^{+2} (2^{+6}) 30^{+1}–30^{+2}	29^{+4}	29^{+0}	30^{+2}	0.67
2017–20 ($n = 2,147$)	9.3 (20.4) 8.5–10.2	2.0	0.0	9.0		30^{+2} (2^{+6}) 30^{+1}–30^{+3}	29^{+4}	29^{+0}	30^{+2}	
30–32 ($n = 4,127$)	4.3 (15.4) 3.8–4.7	1.0	0.0	3.0		31^{+5} (2^{+2}) 31^{+4}–31^{+6}	31^{+3}	30^{+5}	32^{+2}	
2013–16 ($n = 2,077$)	4.1 (11.6) 3.6–4.6	1.0	0.0	3.0	0.42	31^{+5} (1^{+6}) 31^{+5}–31^{+6}	31^{+3}	30^{+5}	32^{+2}	0.32
2017–20 ($n = 2,050$)	4.5 (18.5) 3.7–5.3	0.0	0.0	3.0		31^{+6} (2^{+5}) 31^{+4}–31^{+6}	31^{+3}	30^{+5}	32^{+2}	

[a]Calculated values (corrected gestational age) based on the ventilation durations and gestational ages; SD, standard deviation; IQR, interquartile range; CI, confidence interval.

TABLE 4 Duration and distribution of assisted invasive ventilation among different birth weight groups.

	Duration of invasive ventilation (d)					Minimal required maturation time[a] of weaning from invasive ventilation (wk)				
	Mean (SD) 95% CI	Median	IQR 25th	IQR 75th	*p*-value	Mean (SD) 95% CI	Median	IQR 25th	IQR 75th	*p*-value
Birth weight (g)										
<750 ($n = 2,633$)	35.9 (40.3) 34.4–37.5	27.0	7.0	51.0		30^{+3} (6^{+0}) 30^{+1}–30^{+4}	29^{+4}	26^{+3}	32^{+5}	
2013–16 ($n = 1,295$)	34.3 (38.6) 32.2–36.4	25.0	6.0	48.0	0.039	30^{+1} (5^{+6}) 29^{+6}–30^{+3}	29^{+2}	26^{+3}	32^{+2}	0.053
2017–20 ($n = 1,338$)	37.5 (41.9) 35.3–39.8	28.0	8.0	53.0		30^{+4} (6^{+2}) 30^{+2}–30^{+6}	29^{+5}	26^{+4}	32^{+6}	
750–999 ($n = 3,535$)	23.8 (30.4) 22.8–24.8	14.0	3.0	35.0		30^{+4} (4^{+1}) 30^{+3}–30^{+5}	29^{+5}	28^{+0}	31^{+6}	
2013–16 ($n = 1,794$)	23.6 (31.0) 22.2–25.1	13.0	3.0	35.0	0.70	30^{+3} (4^{+2}) 30^{+2}–30^{+5}	29^{+5}	27^{+6}	31^{+6}	0.33
2017–20 ($n = 1,741$)	24.0 (29.8) 22.6–25.4	14.0	2.0	35.0		30^{+4} (4^{+1}) 30^{+3}–30^{+5}	29^{+5}	28^{+1}	31^{+6}	
1,000–1,249 ($n = 3,996$)	9.7 (20.8) 9.1–10.4	3.00	1.0	10.0		30^{+2} (3^{+0}) 30^{+2}–30^{+3}	29^{+6}	28^{+5}	31^{+2}	
2013–16 ($n = 2,050$)	10.1 (19.8) 9.3–11.0	3.0	1.0	10.0	0.25	30^{+2} (2^{+6}) 30^{+2}–30^{+3}	29^{+6}	28^{+5}	31^{+3}	0.95
2017–20 ($n = 1,946$)	9.3 (21.7) 8.4–10.3	2.0	0.0	9.0		30^{+2} (3^{+2}) 30^{+1}–30^{+3}	29^{+6}	28^{+5}	31^{+2}	
1,250–1,499 ($n = 4,494$)	4.6 (12.7) 4.2–5.0	1.0	0.0	4.0		31^{+0} (2^{+0}) 30^{+6}–31^{+1}	30^{+5}	29^{+6}	31^{+5}	
2013–16 ($n = 2,298$)	4.8 (12.4) 4.3–5.3	2.0	0.0	4.0	0.31	30^{+6} (2^{+0}) 30^{+6}–31^{+0}	30^{+5}	29^{+6}	31^{+5}	0.75
2017–20 ($n = 2,196$)	4.4 (13.1) 3.8–4.9	1.0	0.0	4.0		31^{+0} (2^{+1}) 30^{+6}–31^{+1}	30^{+5}	29^{+6}	31^{+6}	

[a]Calculated values (corrected gestational age) based on the ventilation durations and gestational ages; SD, standard deviation; IQR, interquartile range; CI, confidence interval.

surfactant treatment and air leaks increased the risk of prolonged duration of invasive ventilation. In contrast, maternal age, Apgar score at 5 min, and air leaks were associated with the duration of non-invasive ventilation.

Incidence proportion of ventilator weaning by invasive ventilation duration

The Kaplan–Meier model revealed the cumulative proportion of invasive ventilator dependency by invasive ventilation duration for the different gestational age (**A**) and birth weight (**B**) groups (**Figure 1**). The lower the gestational age or birth weight, the more delayed the successful weaning from invasive ventilation. The weaning point of 50% of the infants was marked in each subgroup.

The slope of the Kaplan–Meier survival curve slowly decreased in the presence of risk factors such as surfactant treatment or air leaks (**Figure 2**).

TABLE 5 Results of Cox regression analysis of initial perinatal factors associated with duration of invasive and non-invasive ventilation.

Variable	Invasive ventilation		Non-invasive ventilation	
	Inverse hazard ratio (95% CI)	*p*-value	Inverse hazard ratio	*p*-value
Gestational age (per week)	0.86 (0.83–0.89)	<0.001	0.89 (0.87–0.91)	<0.001
Birth weight (per 100 g)	1.00 (0.99–1.00)	0.04	1.00 (0.99–1.00)	0.62
Maternal age	1.00 (0.99–1.02)	0.64	1.01 (1.00–1.02)	0.01
Male	0.91 (0.79–1.04)	0.17	1.02 (0.94–1.11)	0.67
Cesarean section	1.00 (0.83–1.20)	1.00	0.95 (0.85–1.06)	0.34
Multiple gestation	0.93 (0.80–1.07)	0.31	0.96 (0.88–1.05)	0.35
Maternal diabetes during pregnancy	1.01 (0.78–1.30)	0.95	1.09 (0.94–1.26)	0.26
Apgar score at 5min	0.96 (0.92–1.00)	0.06	1.03 (1.01–1.06)	0.01
Surfactant treatment	1.50 (1.04–2.15)	0.03	0.98 (0.83–1.16)	0.84
Air leaks	1.62 (1.29–2.04)	<0.001	0.80 (0.67–0.94)	0.01
Massive pulmonary hemorrhage	1.08 (0.89–1.33)	0.44	0.68 (0.58–0.81)	<0.001

CI, confidence interval.

Discussion

The development of lung function after preterm birth is closely related to gestational age, birth weight, and many other factors that affect the development of alveoli and pulmonary blood vessels (14). Prolonged invasive mechanical ventilation is associated with increased mortality and morbidities related to pulmonary inflammation, developmental delay/arrest, neurodevelopmental impairment, and hospital-acquired infections (2, 8, 10). To avoid tissue injury and inhibition of the ongoing developmental process of premature lungs, various strategies have been developed to minimize invasive mechanical ventilation (4–9). The goal of treatment is to maintain invasive ventilation for only the necessary period of time and switch to non-invasive ventilation as soon as possible. The optimal extubation time implies the theoretical minimum time required for lung maturation enough to breathe without mechanical ventilation. Unfortunately, in the real world, in the clinical field of NICU, it is difficult to predict the exact point of lung maturation sufficient to wean from invasive respiratory support. Population-based PPV duration data are important to accurately predict the optimal timing of extubation and reduce trial and error. Therefore, current qualifying cohort data reflecting the up-to-date treatment strategies are necessary. From the KNN (11), the prospective

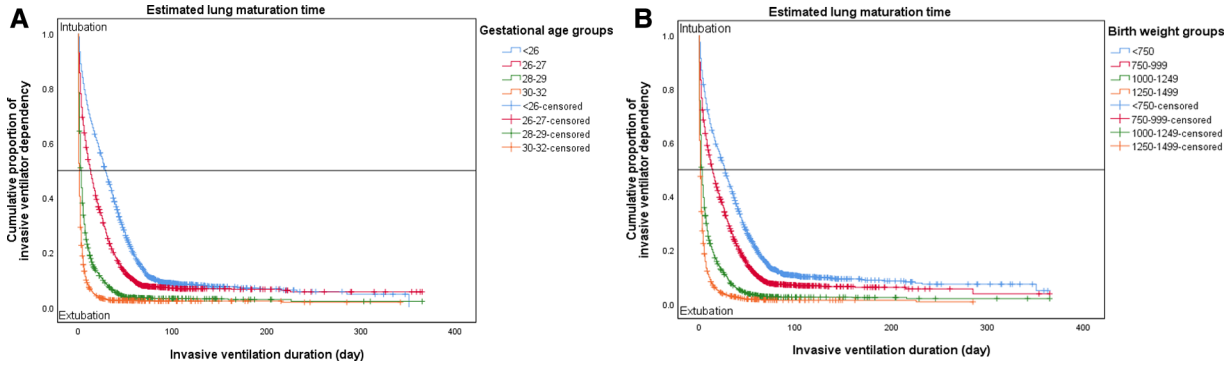

FIGURE 1
Kaplan–Meier curves showing different cumulative proportions of invasive ventilator dependency based on the duration of invasive ventilator support according to gestational age (A) and birth weight (B) groups. (A) Invasive ventilation log-rank p-value < .0001 according to gestational age groups. (B) Non-invasive ventilation log-rank p-value < .0001 according to birth weight groups.

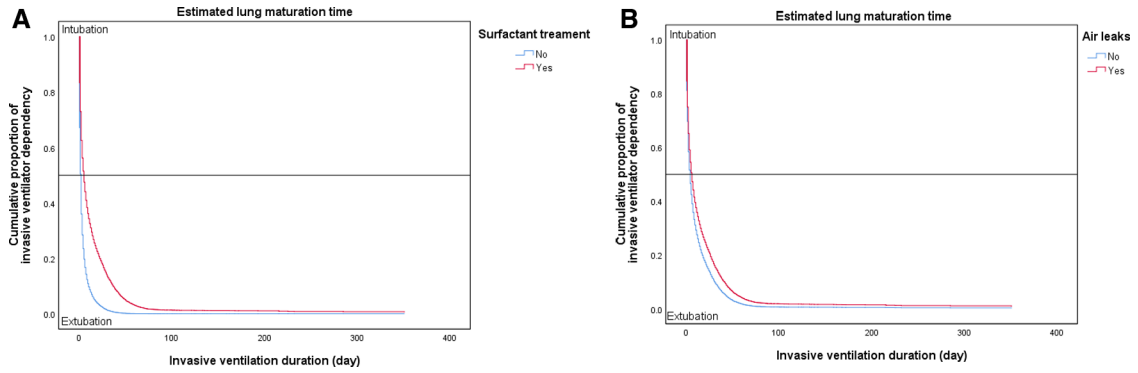

FIGURE 2
Effects of risk factors on the duration of assisted ventilation. (A) Surfactant treatment. (B) Air leaks.

cohort data of VLBW infants in Korea over 8 years, we assumed the duration of invasive ventilation as the minimal required pulmonary maturation time of weaning from invasive ventilation.

Despite various treatment strategies to prevent BPD development, BPD still remains the most serious chronic lung disease among very preterm infants. Less invasive or non-invasive assisted ventilation, such as nasal CPAP or HFNC, has been applied preferentially to minimize lung injury in VLBW infants (15). Although controversial, recent practices with active application of non-invasive ventilation, rather than invasive ventilation, has contributed to decrease the incidence of BPD and to improve long-term respiratory function (15, 16). According to the KNN data from 2013 to 2014, BPD incidence was 28.9% (17). Compared with the data from 2007 to 2008, the increased incidence of BPD in 2013–14 was probably affected by the increased survival rate of VLBW infants. In this study, the overall BPD incidence between 2013 and 2020 was 29.9%. The incidence of BPD seems to had increased in 2017–20 from 2013–16 (from 28.1% to 31.9%), even with the noticeable increased duration of non-invasive ventilator support (from 17.9 to 22.5 days) (**Table 2**). Because survival rates were not different between the two groups, it is difficult to conclude that the higher incidence of BPD was a result of more surviving premature infants. **Table 1** details recent social and demographic issues, the extremely low birth rate and the increasing proportion of high-risk newborn infants (multiple births, low birth weight, or preterm infants) among the births which are a consequence of advanced maternal age and assisted reproductive technologies (6, 18). The relationship between the incidence of BPD with these demographic changes is a possibility. Further detailed and precise analysis along with discussion are needed to clarify this discrepancy.

In the study population, the mean duration of invasive ventilation was 16.3 days, and the median duration (25–75 IQR) was 4.0 (1.0–21.0) days. The estimated median (25–75 IQR) minimum time required for weaning from invasive ventilation was 30^{+1} (28^{+5}–31^{+5}) weeks of gestation. The comparison of the duration of invasive ventilation among the gestational age subgroups showed differences except in the 28–29 and 30–32 weeks of gestation groups (**Table 3** and **Supplementary Table S1**). In contrast, the estimated minimum time required for weaning from invasive ventilation showed no differences among the <26, 26–27, and 28–29 weeks of gestation groups. Those in the three groups converged to similar corrected gestational ages:

29^{+5}, 30^{+2}, 30^{+2} corrected gestational weeks, respectively. These corrected gestational ages are considered a biologically required time for lung maturation sufficient for weaning from invasive ventilation in current NICUs in Korea (**Table 3**). This information is valuable for determining the optimal ventilator weaning time and reducing trial and error by hasty extubation.

Weisz et al. (19) reported the time of weaning from respiratory support among infants of 23–27 weeks of gestation based on the Canadian Neonatal Network in 2010–17. The corrected gestational ages were 30.4, 30.3, 29.6, 29.0, and 28.3 weeks for infants with 23, 24, 25, 26, and 27 weeks of gestation, respectively. Dassios reported the duration of mechanical ventilation and its association with BPD development using data from the UK (20). Comparison of the duration of invasive ventilation among groups, such as neonatal networks, populations, and hospitals, before and after specific treatments or strategies for lung protection, in the past and present, is a simple and intuitive method for improving quality. It can be used for BPD diagnosis to decrease the conflict between using relatively simple but limited clinical diagnostic methods and complicated diagnostic methods that reflect physiological characteristics (21).

In addition to gestational age, the presence of surfactant treatment and air leakage increased the risk of prolonged duration of invasive ventilation (**Table 5** and **Figure 2**). The Kaplan–Meier survival curve intuitively shows how long the duration of invasive ventilation increases when each risk factor associated with BPD occurrence are present (**Figures 1**, **2**). The ventilator weaning time can be predicted using the steep degree of slope according to the presence or absence of risk factors. The interpretation of risk factors associated with the duration of invasive and non-invasive ventilation requires further consideration of related various demographic phenomena (**Table 5**).

A limitation of this study is that the start and removal dates of each assisted respiratory ventilator and the exact intubation/extubation dates were not included in the data. Preterm infants born at <26 weeks of gestation or birth weight <750 g required longer invasive ventilation duration in 2017–20 compared to those in 2013–2016. Survival rates according to each gestational age group among 2013–14, 2015–16, 2017–18, and 2019–20 showed no difference including 22, 23, 24, and 25 weeks of gestation subgroups (18). Besides survival rate, more detailed factors and/or phenomena need to be clearly identified which are associated with difference of invasive ventilation duration between 2013 and 16 and 2017–20 among gestational age subgroups <26 weeks of gestation or birth weight <750 g through further analysis.

In conclusion, the present data, a population-based, multicenter cohort study based on the KNN, provided current detailed references on postnatal lung maturation under specific perinatal conditions after preterm birth. VLBW infants require additional biological time for lung maturation and further tailored treatment to overcome immature lung function, especially those with RDS or air leaks. Our results are essential for assessing the therapeutic effects or quality of care for valid comparisons among populations or neonatal networks.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

Ethics statement

The Institutional Review Board of each participating hospital including the Kangwon National University Hospital (B-2015-06-007-017) reviewed and approved the use of the KNN registry. This protocol was approved by the KNN executive board (2016-ER6307-00). Written and informed consent to participate in this study was provided by the participants' legal guardian/next of kin upon enrollment in the NICU's of KNN-participating hospitals.

Author contributions

HSJ: conceptualisation, methodology, writing (review and editing) and supervision; MNL: formal analysis and visualisation; S-IC: conceptualisation, methodology. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1184832/full#supplementary-material

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EDITED BY
Shahana Perveen,
Cohen Children's Medical Center, United States

REVIEWED BY
Bernard Thebaud,
The Ottawa Hospital, Canada
Lawrence S. Prince,
Stanford University, United States

*CORRESPONDENCE
Karen K. Mestan
✉ kmestan@health.ucsd.edu

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Development of a novel humanized mouse model to study bronchopulmonary dysplasia

Rob Birkett[1], Janu Newar[2], Abhineet M. Sharma[1], Erika Lin[2], Lillian Blank[2], Suchitra Swaminathan[3], Alexander Misharin[4] and Karen K. Mestan[1,2]*

[1]Department of Pediatrics/Division of Neonatology, Ann & Robert H. Lurie Children's Hospital of Chicago and Northwestern University Feinberg School of Medicine, Chicago, IL, United States, [2]Department of Pediatrics/Division of Neonatology, UC San Diego School of Medicine & Rady Children's Hospital of San Diego, La Jolla, CA, United States, [3]Department of Medicine/Division of Rheumatology, Northwestern University Feinberg School of Medicine, Chicago, IL, United States, [4]Department of Medicine/Division of Pulmonary & Critical Care, Northwestern University Feinberg School of Medicine, Chicago, IL, United States

Rationale: The role of circulating fetal monocytes in bronchopulmonary dysplasia is not known. We utilized a humanized mouse model that supports human progenitor cell engraftment (MISTRG) to test the hypothesis that prenatal monocyte programming alters early lung development and response to hyperoxia.
Methods: Cord blood-derived monocytes from 10 human infants were adoptively transferred into newborn MISTRG mice at p0 (1×10^6 cells/mouse, intrahepatic injection) followed by normoxia versus hyperoxia (85% oxygen × 14 days). Lungs were harvested at p14 for alveolar histology (alveolar count, perimeter and area) and vascular parameters (vWF staining for microvessel density, Fulton's index). Human CD45 staining was conducted to compare presence of hematopoietic cells. Murine lung parameters were compared among placebo and monocyte-injected groups. The individual profiles of the 10 patients were further considered, including gestational age (GA; $n = 2$ term, $n = 3$ moderate/late preterm, and $n = 5$ very preterm infants) and preeclampsia ($n = 4$ patients). To explore the monocyte microenvironment of these patients, 30 cytokines/chemokines were measured in corresponding human plasma by multiplex immunoassay.
Results: Across the majority of patients and corresponding mice, MISTRG alveolarization was simplified and microvessel density was decreased following hyperoxia. Hyperoxia-induced changes were seen in both placebo (PBS) and monocyte-injected mice. Under normoxic conditions, alveolar development was altered modestly by monocytes as compared with placebo ($P < 0.05$). Monocyte injection was associated with increased microvessel density at P14 as compared with placebo (26.7 ± 0.73 vs. 18.8 ± 1.7 vessels per lung field; $P < 0.001$). Pooled analysis of patients revealed that injection of monocytes from births complicated by lower GA and preeclampsia was associated with changes in alveolarization and vascularization under normoxic conditions. These differences were modified by hyperoxia. CD45+ cell count was positively correlated with plasma monocyte chemoattractant protein-1 ($P < 0.001$) and macrophage inflammatory protein-1β ($P < 0.01$). Immunohistochemical staining for human CD206 and mouse F4/80 confirmed absence of macrophages in MISTRG lungs at P14.

Abbreviations
BPD, bronchopulmonary dysplasia; BW, birth weight; GA, gestational age; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein-1β; MISTRG, Acronym for the 7 modified genes in humanized mouse strain: M-CSF$^{h/h}$ IL-3/GM-CSF$^{h/h}$ SIRPa$^{h/h}$ TPO$^{h/h}$ RAG2$^{-/-}$ IL2Rg$^{-/-}$; PBS, phosphate buffered saline.

Conclusions: Despite the inherent absence of macrophages in early stages of lung development, immunodeficient MISTRG mice revealed changes in alveolar and microvascular development induced by human monocytes. MISTRG mice exposed to neonatal hyperoxia may serve as a novel model to study isolated effects of human monocytes on alveolar and pulmonary vascular development.

KEYWORDS

hematopoietic stem cells, intrauterine inflammation, chorioamnionitis, preeclampsia, preterm birth, neonatal lung disease, fetal monocytes

Introduction

Bronchopulmonary dysplasia (BPD) remains the most common chronic lung complication of preterm birth. Although lower gestational age (GA) and birth weight (BW) are independent risk factors, certain endotypes of BPD appear to arise in the fetal stages of lung development. In utero events, compounded by postnatal environmental stressors such as hyperoxia, inflammation and oxidant stress account for the wide variation in pulmonary and overall health outcomes of preterm infants.

During the transition to extrauterine life, stem and progenitor cells in fetal circulation play key roles in early lung injury and repair in response to relative hyperoxia after birth. Fetal monocytes, arising from the fetal liver, give rise to lung macrophages and dendritic cells responsible for inflammatory changes and fibrosis characteristic of BPD (1, 2). The largest subpopulation of monocytes (classical, $CD14^{+}$ $CD16^{-}$) will differentiate and engraft in the lungs as alveolar macrophages (3) but this transition does not occur immediately in humans or mice (4). While it has been reported that monocytes are present in tracheal aspirates of human newborn infants (5, 6), the role of circulating monocytes in lung alveolar and vascular development and their response to hyperoxia during this period is not completely understood. More comprehensive interrogation of monocyte function in an *in vivo* experimental model is needed. Recent advances in humanized animal models have allowed us to better understand the fate and function of human monocytes in complex diseases for which human experimentation is not feasible (6). However, there are no studies reported to date on the effects of chronic neonatal hyperoxia in a humanized mouse model that specifically supports adoptive transfer of monocytes and engraftment of alveolar macrophages. The MISTRG mouse model, in which human GM-CSF and other human cytokines are knocked in to allow robust engraftment of human immune stem and progenitor cells, provides a supportive and novel genetic background upon which to study human BPD.

We recently characterized the gene expression profiles of human fetal monocytes in a large cohort of preterm infants (7). Bulk RNAseq of classical and intermediate monocyte subsets revealed distinct gene expression pathways that appear to be driven by placental inflammatory versus vascular dysfunction. Specifically, fetal monocytes exposed to intrauterine inflammatory processes such as chorioamnionitis upregulate biological pathways related to monocyte activation, chemotaxis and platelet function. Conversely, monocytes exposed to placental vascular disease such as preeclampsia downregulate these processes.

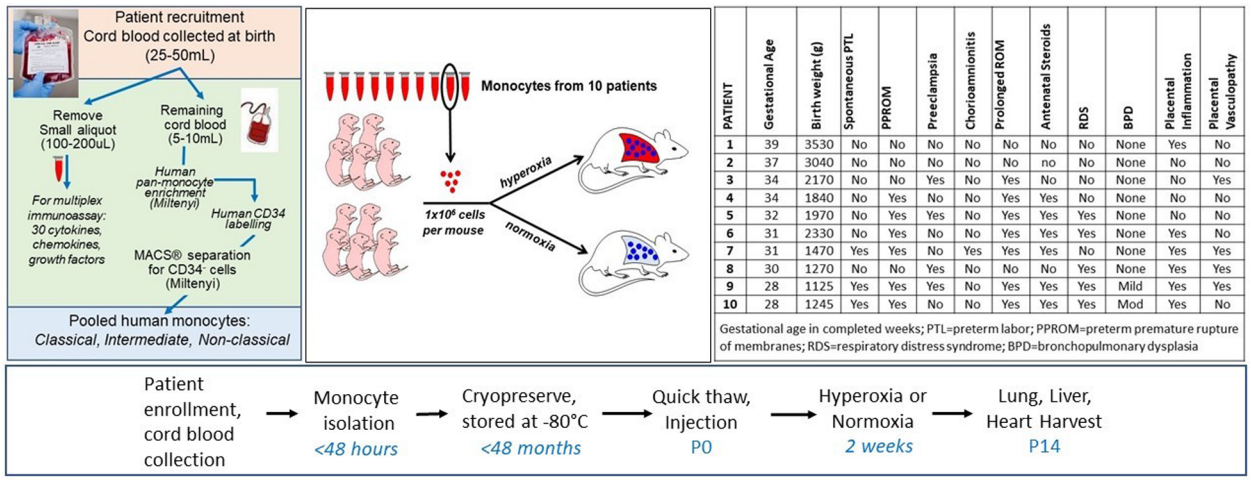
Prior studies of human monocyte transplantation in immunodeficient mice have suggested an overall protective effect of monocytes on long-term alveolarization and pulmonary function (8). Data on earlier alveolarization and vascular development is lacking, specifically at p14 to capture earlier mechanisms relevant to the fetal origins of BPD pathogenesis. This time period is important to understand the transitional influence of circulating monocytes prior to differentiation into alveolar macrophages, which typically occurs after 3 weeks (>p21). As human BPD is a highly heterogenous disease, utilization of a humanized mouse model in which fetal monocytes with distinct human profiles will allow us to identify perinatal influences that cannot be fully recapitulated in traditional animal models. These influences include perinatal processes of placental vascular dysfunction that are associated with known risk factors for BPD such as preeclampsia (9).

In this study, we investigate neonatal lung development in humanized MISTRG newborn mice upon transplantation of human monocytes derived from cord blood of infants representing a wide range of gestational ages and distinct perinatal birth characteristics. The objectives were to evaluate early influences of circulating monocytes on alveolar and pulmonary vascular development, and to test the hypothesis that fetal monocytes from births with specific perinatal profiles drive distinct lung histologic changes in response to hyperoxia.

Materials and methods

Patient enrollment

Ten mothers and their newborn infants were included in this study (**Figure 1**). Participants were prospectively enrolled through an ongoing biorepository in which all women delivering a liveborn infant at Prentice Women's Hospital (Chicago, IL) are eligible. Informed consent was obtained from all participants prior to participation. The study was approved by the Institutional Review Board of Northwestern University. The 10 births were selected based upon the availability and quality of monocytes recovered from cord blood at birth, including quantity of viable monocytes sufficient to conduct each experiment injecting 1×10^{6} monocytes per mouse pup using a single patient per litter (e.g., for 1 litter of 6–8 pups each, a



PATIENT	Gestational Age	Birth weight (g)	Spontaneous PTL	PPROM	Preeclampsia	Chorioamnionitis	Prolonged ROM	Antenatal Steroids	RDS	BPD	Placental Inflammation	Placental Vasculopathy
1	39	3530	No	No	No	No	No	No	No	None	Yes	No
2	37	3040	No	No	No	No	No	no	No	None	No	No
3	34	2170	No	No	Yes	No	Yes	No	No	None	No	Yes
4	34	1840	No	Yes	No	No	Yes	Yes	No	None	No	No
5	32	1970	No	Yes	Yes	No	Yes	Yes	Yes	None	No	No
6	31	2330	No	Yes	No	No	Yes	Yes	Yes	None	Yes	No
7	31	1470	Yes	Yes	No	Yes	Yes	Yes	No	None	Yes	Yes
8	30	1270	No	No	Yes	No	No	No	Yes	None	Yes	Yes
9	28	1125	Yes	Yes	Yes	No	Yes	Yes	Yes	Mild	Yes	Yes
10	28	1245	Yes	Yes	No	No	Yes	Yes	Yes	Mod	Yes	No

Gestational age in completed weeks; PTL=preterm labor; PPROM=preterm premature rupture of membranes; RDS=respiratory distress syndrome; BPD=bronchopulmonary dysplasia

FIGURE 1
Overview and timeline of experiments. Cord blood monocytes isolated through the workflows and protocols depicted on the far left from 10 individual patients were injected into P0 mice via intrahepatic injection. Mice were immediately placed in hyperoxia (85%) or normoxia (21%) for 14 days. Characteristics of the 10 patients are shown in the table (far right). For 7 of the 10 patients, at least 6–8 pups were injected with patient-specific monocytes. For patients #4, 5, and 7, only 2 pups injected with these monocytes were available for the normoxia group due to smaller paired litters available and lower than expected monocyte counts at the time of sample thaw. In addition, for patients #8 and 9, at least 3 pups were injected for normoxia and hyperoxia groups but all pups died in hyperoxia and 3 total died in hyperoxia before P14, leaving only 2 pups each in the hyperoxia group for lung parameter analyses at P14.

patient with at least 6–8 million monocytes available for injection into 6–8 pups was chosen, so that half of each litter could be placed in normoxia and the other half in hyperoxia) (Figure 1). In cases of smaller litters and quantity of monocytes available at the time of injection, more than or fewer than 3–4 pups per exposure group were studied. Infants with known congenital anomalies, infections and genetic syndromes were excluded.

Clinical data

Maternal and infant baseline and clinical data were obtained using standardized protocols of the larger birth cohort, with definitions as previously published by this group (7, 10). GA at birth was recorded as completed weeks and further categorized according to CDC classifications: full term birth was defined as >37 completed weeks, moderate or late preterm birth was defined as 32–36 completed weeks, and very preterm was defined as <32 completed weeks (11, 12). Preeclampsia was defined according to American College of Obstetricians and Gynecologists' (ACOG) criteria, and included other hypertensive disorders of pregnancy, such as eclampsia and hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome (13).

Cord blood collection

Delivery staff collected venous cord blood at time of birth into a cord blood bag or EDTA tube as previously described (10, 14). Cord blood specimens were stored at 4°C and monocyte isolation was performed within 48 h of delivery. Corresponding cord blood plasma from all 10 patients was separated by refrigerated tabletop centrifuge and pipetted into aliquots of 100–200 ul each, and stored at −80°C until multiplex immunoassay (see below).

Monocyte isolation, enrichment, cryopreservation and thaw

Cord blood specimens were spun down at 1,400 rpm for 10 min (Figure 1). Plasma and anticoagulant were removed. Red blood cells were lysed for 15 min at room temperature in the dark, using BD Pharm Lyse (BD Biosciences, San Jose, CA) at 10 ml lysis buffer per 1 ml cord blood. PBS was added to the tube(s) and centrifuged at 1,400 rpm for 5 min. After removing the lysis buffer solution, the cells were washed with 2% BSA in PBS to remove residual lysis buffer. Monocytes were enriched using a human pan-monocyte enrichment kit according to manufacturer's protocol (Pan Monocyte Isolation Kit, Catalog#: 130-096-537, Miltenyi Biotec, Germany). Non-monocytes were magnetically labeled with a cocktail of biotin-conjugated antibodies. The cell solution was filtered through an LS column (for up to 10^8 magnetically labeled cells) attached to a magnetic field of a MACS® Separator. This ensured a quick and gentle separation of magnetically labeled cells from monocytes. Following monocyte enrichment, the enriched monocyte solution was treated with CD34 microbeads to magnetically label CD34+ cells with a human CD34 Microbead Kit (Catalog#: 130-046-702, Miltenyi Biotec, Germany). The treated monocyte solution was filtered through an MS column (for up to 10^7 magnetically labeled cells) attached to a magnetic field of a MACS® Separator. The CD34+ cells adhered to the MS column, while the monocytes were eluted. The cells were counted to obtain cell concentration and viability (Bio-Rad, Hercules, CA).

The monocytes were frozen in 2 ml tubes using Cell Therapy Systems (CTS) Synth-a-Freeze medium (Gibco, Thermo Fisher, Waltham, MA) in Thermo Scientific™ Mr. Frosty™ freezing containers (Thermofisher, Waltham, MA). The system is designed to achieve a rate of cooling close to −1°C/min to allow an optimal rate for cell cryopreservation. Cryopreserved cells were placed at −80°C and stored until use. All cells were used within 24 months of cryopreservation (median storage time = 12 months). Given the uncertainty and variability of when the archived cells would be needed for the multiple experiments, we opted to keep all monocyte aliquots stored together in a single −80°C freezer until thaw to avoid variations in freeze methods among the patient samples. This approach was supported by past literature showing no significant difference in cell viability and function of peripheral blood stem cells stored up to 5 years using either conventional liquid nitrogen or mechanical freezer (15). A standardized "quick thaw" protocol was adopted from our own experience with monocytes and preliminary studies supporting the highest viability of monocytes upon thaw. Briefly, cryopreserved monocytes were removed from cryovials and placed in a 37°C water bath for 30 s. Media (10% FBS in RPMI + pen strep) warmed at 37°C was slowly added and the cell suspension was transferred to a 15 ml conical tube to a volume of 10 ml media. Cell viability and count was again determined upon thaw. Only patient samples with at least 80% viability were used for the adoptive transfer experiments (mean cell viability = 88%).

Mouse experimental conditions

The Institutional Animal Care and Use Committee at Northwestern University approved all animal procedures. MISTRG mice were procured from Jackson Lab (JAX 017712), and maintained on 0.27 mg/ml Baytril as previously described (6). Timed matings were conducted to ensure at least 2 litters of at least 6 pups each litter, and equal numbers of male and female pups, for each experiment using at least 2 patient samples and placebo (PBS) at a time. At p0, litters were examined for the presence of milk spots and to ensure dams were caring for the pups. Cryopreserved monocytes were thawed (see above) and resuspended at a concentration of 1×10^6 monocytes per 30 ul of PBS and administered by intrahepatic injection on p0. An equal volume of PBS was used for placebo injections (PBS group, $N =$ 17 mice). Mice were kept at room air (21% O_2) or at hyperoxia (85% O_2) in a Plexiglas chamber (Biospherix, Lacona, NY) for 14 days. Nursing dams were rotated every 24 h to prevent oxygen toxicity to adult animals.

Harvested tissues

P14 pups were euthanized according to the procedures outlined by the panel on euthanasia of the American Veterinary Medical Association. The lungs, hearts, and livers were harvested. The lungs were inflated to 25 cm H_2O with 10% formalin. Hearts were harvested at time of euthanasia and Fulton's index determined as previously described (16). Tissue processing and sectioning was performed by the Histology Core at Stanley Manne Children's Research Institute.

Lung morphometry

Lungs were sectioned and stained with hematoxylin and eosin. Images were taken with an Olympus BX40 microscope. Six nonoverlapping images were taken per animal and alveolar area and counts were measured with ImagePro (Media Cybernetics, Rockville Maryland). Mean/median values were reported for each animal. All slides were coded and randomly analyzed by a single examiner (RB) masked to the original experimental conditions, patient group assignments and treatments/exposures.

Small vessel density

Sections were incubated with von Willebrand Factor (vWF) primary antibody (Dako, Carpenteria, CA). 6–8 images/animal were randomly captured under 10× magnification. Small vessels (<100 μm) were counted and averaged per animal in masked fashion as described above for lung morphometry.

Immunohistochemistry

Anti-human CD45 (Cell Signaling, Danvers, MA) at a dilution of 1:250 and 3, 3-diaminobenzidine (DAB; Vector Labs, CA) was used to stain lung and liver tissues. CD45+ expression was observed and recorded at 10×. Immunohistochemistry staining for anti-human CD206 (Sigma-Aldrich, MO) was performed by the Northwestern Pathology Core Facility to identify presence of human alveolar macrophages in representative lung samples. Staining for mouse-specific macrophages was performed using anti-mouse F4/80 (BD Biosciences) with biotin labelled secondary antibody. Lung tissue from a non-humanized mice strain (C57BL/6J, wildtype adult mice 12 months, provided by Eniko Sajti) was used as a positive control.

Identification of human alveolar macrophages at p56

Single cell suspension of mouse lung harvested at p56 was prepared according to an established protocol (17). Briefly, the mouse lungs were perfused with PBS through the right ventricle. After mincing the lung tissue with scissors, the tissue was transferred to C-tubes (Miltenyi, Auburn, CA). The tissue was digested in HBSS with 1 mg/ml Collagenase D and 0.1 mg/ml DNase I (Roche, Indianapolis, IN) and dissociated with a GentleMACS dissociator (Miltenyi). The cell suspension was passed through a 40 um filter before being MACS enriched for human CD45 (Miltenyi) according to manufacturer's instructions. The cells were stained with HLA-DR, mCD45,

hCD206, hCD14 (BD Biosciences, Franklin Lakes, NJ), and SYTOX Green (Thermo Fisher, Waltham, MA). FACS was performed on a BD FACSAria SORP Cell Sorter at the Northwestern Robert H. Lurie Cancer Center Flow Cytometry Core Facility. HLA-DR+, hCD206+, hCD14+, mCD45- viable cells were sorted and subsequently stained for H&E following cytospin to generate the images shown in **Figure 11**.

Multiplex immunoassays

Simultaneous measurement of 30 analytes was performed by sandwich immunoassays using Luminex xMAP platform in magnetic bead format: EGF, Eotaxin, FGF-2, FLT-3l, Fractalkin, granulocyte colony stimulating factor (G-CSF), GM-CSF, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-17A, IP-10, monocyte chemoattractant protein-1 (MCP-1), MCP-3, macrophage inflammatory protein (MIP)-1α, MIP-1β, TGFα, TNFα, TNFβ, VEGFA. The multiplexed assay beads were obtained from a commercially available kit (EMD Millipore, MA). Plasma samples were thawed on ice and prepared in 1:1 dilution and analyzed according to manufacturer's instructions. All samples were run in duplicate with standard curves for each marker and controls on each plate. Only the analytes that fell within the limits of detection for at least 90% of the patient samples were included in linear regression analyses.

RT-qPCR

RNA Isolation: Total cellular RNA was isolated using the Quick-RNA Mini-prep kit (Direct-zol, R2052, ZYMO Research, Irvine, CA, USA) according to the manufacturer's instructions. The concentration of total RNA was determined by Qubit® 2.0 Fluorometer. cDNA synthesis: Complementary DNA (cDNA) was synthesized using Sensiscript RT Kit (QIAGEN) and random hexamers (QIAGEN) according to the manufacturer's recommendations. Briefly, 40 ng of total RNA was added to the master mix containing 2 µl of 10× buffer RT, 2 µl of dNTP mix (5 mM each of dNTP), 10 µm of random hexamers, and 1 µl RNase inhibitor (10 units/µl), 1 µl Sensiscript Reverse Transcriptase and volume was made up by RNase-free water in a PCR tubes. The PCR tube were incubated at 37°C for 60 min for cDNA synthesis. Quantitative RT-PCR TaqMan Assays: Real-time PCR was performed using a CFX-96 (Bio-Rad, Hercules, CA) System and TaqMan Fast Advanced Master Mix (Applied Biosystems). TaqMan® Gene Expression Assay IDs used for this assay are Human CCL2 (Hs00234140_m1), Human CCL4, (Hs99999148_m1) Human VEGFA (Hs00900055_m1), Human GAPDH (Hs02758991_g1), Mouse CCL2 (Mm00441242_m1), Mouse CCL4 (Mm00443111_m1), Mouse VEGFA (Mm00437306_m1) Mouse GAPDH (Mm99999915_g1). Real-time PCR amplification was performed using a total volume of 10 µl that contained 1 µl cDNA (40 ng), 5 µl TaqMan Fast Advanced Master Mix, 0.5 µl of each TaqMan Gene Expression Assay, and 3.5 µl ultrapure DNase-free water. The cycle parameters were as follows: UNG incubation at 50°C for 2 min, polymerase activation at 95°C for 20 s, denaturation at 95°C for 3 s and then annealing and extension at 60°C for 30 s. Ct values were calculated, defined as the number of cycles required for the fluorescent signal of a sample to cross the threshold line, and was inversely proportional to the amount of target nucleic acid in the sample (18).

Statistical analysis

Patient demographics and clinical characteristics were compared using ANOVA or Kruskal Wallis for continuous variables and X^2 or Fisher exact tests for categorical data. Lung tissue parameters from mice were reported as mean or median depending upon normality of distribution, and groups were compared using parametric or non-parametric tests where appropriate, with *post hoc* tests and adjustment for multiple comparisons with Bonferroni correction. Multivariate linear regression models were used to measure the correlations (beta-coefficients) between cord blood plasma cytokine/chemokine levels and lung parameters, with log-transformation of all continuous variables, adjustment for pertinent clinical variables, and stratification of the models by hyperoxia exposure (yes/no). $P < 0.05$ was considered statistically significant. Statistical analyses were performed using STATA/IC version 13.0 (StataCorp, College Station, TX). Graphs were prepared using GraphPad Prism 8.0 (San Diego, CA).

Results

The patient sample represented a wide range of gestational ages and clinical features

Relevant baseline demographics and clinical characteristics of the ten mothers and their infants are shown in **Table 1**. GA at birth ranged from 28 to 39 completed weeks (Mean GA: 32.9 ± 3.6 weeks). BW ranged from 1,125 to 3,530 grams (Mean BW: 1,999 ± 798 grams). Among the 10 patients, 2 were full term and 8 were born preterm: 3 were moderate to late preterm (32–34 completed weeks) and 5 were very preterm (28–31 completed weeks).

Hyperoxia exposure resulted in increased alveolar simplification and decreased pulmonary microvascular development in the MISTRG mice at P14, independent of monocyte versus PBS injection at P0

Figures 2, **3** show the individual datapoints for each of the 79 mice harvested at P14, after injection at P0 with either PBS ($n = 17$) or human monocytes from 10 patient donors ($n = 62$) followed by either 14 days of hyperoxia ($n = 43$) vs. room air ($n = 36$). Across most all histologic parameters and independent of PBS or monocyte injection, hyperoxia resulted in decreased mean alveolar count ($P < 0.001$ for both PBS and monocyte-injected

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TABLE 1 Perinatal characteristics of the patient sample.

	All births $N = 10$	Full term $N = 2$	Late preterm $N = 3$	Very preterm $N = 5$
Gestational age, weeks				
(mean ± SD)	32.9 ± 3.6	38.4 ± 1.9	33.7 ± 1.2	30.3 ± 1.8
Birth weight, grams	1,999 ± 798	3,285 ± 346	1,993 ± 166	1,488 ± 487
Birth weight-for-GA, percentile	49.3 ± 27.4	59.4 ± 10.6	37.9 ± 21.4	52.0 ± 35.7
Maternal age, years	30.5 ± 5.9	32.0 ± 1.4	26.3 ± 7.6	32.4 ± 5.4
Infant sex, *n* (%)				
Male	6 (60)	1 (50)	2 (67)	3 (60)
Female	4 (40)	1 (50)	1 (33)	2 (40)
Rupture of membranes (ROM)				
Spontaneous	6 (60)	1 (50)	2 (67)	3 (60)
Artificial	4 (40)	1 (50)	1 (33)	2 (40)
Mode of delivery				
Vaginal	8 (80)	2 (100)	3 (100)	3 (60)
Cesarean section	2 (20)	0 (0)	0 (0)	2 (40)
Antenatal steroids (complete)				
No	4 (40)	2 (100)	1 (33)	1 (20)
Yes	6 (60)	0 (0)	2 (67)	4 (80)
Spontaneous preterm labor				
No	7 (70)	2 (100)	3 (100)	2 (40)
Yes	3 (30)	0 (0)	0 (0)	3 (60)
Preterm premature ROM				
No	4 (40)	2 (100)	1 (33)	1 (20)
Yes	6 (60)	0 (0)	2 (67)	4 (80)
Prolonged ROM				
No	3 (30)	2 (100)	0 (0)	1 (20)
Yes	7 (70)	0 (0)	3 (100)	4 (80)
Chorioamnionitis				
No	9 (90)	2 (100)	3 (100)	4 (80)
Yes	1 (10)	0 (0)	0 (0)	1 (20)
Preeclampsia				
No	6 (60)	2 (100)	1 (33)	3 (60)
Yes	4 (40)	0 (0)	2 (67)	2 (40)

groups), increased alveolar perimeter ($P < 0.001$), and increased alveolar area ($P < 0.001$) (**Figure 2**). Minor changes in alveolar patterns included a decrease in alveolar area among the monocyte-injected group versus placebo ($P = 0.007$; **Figure 2C**) suggesting that the monocytes attenuated alveolar simplification due to hyperoxia. Hyperoxia also induced the expected changes of decreased microvessel counts in the lungs, as indicated by vWF staining, (see **Figure 4** for representative images) in both PBS and monocyte-injected mice (**Figure 3A**; $P < 0.001$).

Injection of mice with human monocytes at P0 resulted in increased microvessel count at P14

Figure 3A shows the median microvessel counts per high power field (hpf), as identified by vWF staining. Median vessel count was significantly increased in lungs of pups treated with monocytes as compared with PBS (26.7 ± 0.73 vs. 18.8 ± 1.7 vessels per lung field; **Figure 3A**). As mentioned above, hyperoxia exposure attenuated the differences in microvessel count between PBS and monocyte-treated mice. However, among the monocyte-treated group, there was a significant decrease in microvessel density with hyperoxia to levels similar to the PBS-treated hyperoxia group (26.7 ± 0.73–12.9 ± 0.83 vessels per lung field; normoxia to hyperoxia, respectively; $P < 0.001$). Collectively, these findings suggest that monocytes play a role in early pulmonary microvascular development, which is attenuated by hyperoxia.

Hyperoxia exposure resulted in decreased pulmonary hypertension at P14

While RV/LV + S ratios (Fulton's index) were expected to increase with hyperoxia as a measure of pulmonary hypertension in other mouse models (19, 20), the trend appeared overall reversed in the MISTRG mice (**Figure 4B**). With the exception of two outlying datapoints (Fulton index = 0.45 in one hyperoxia-exposed PBS-treated mouse and 0.44 in one room air-exposed monocyte-treated mouse) the majority of values were <0.4 overall. Regardless, either with or without these outliers included, there was a significant overall decrease in mean Fulton's index with hyperoxia when PBS and monocyte-treated mice were analyzed together (mean index = 0.29 ± 0.01 vs. 0.26 ± 0.01; normoxia versus hyperoxia, respectively; $P = 0.01$). Stratification by PBS versus monocyte-treated groups revealed that Fulton's index was significantly decreased with hyperoxia in the monocyte-treated group ($N = 62$; mean index = 0.30 ± 0.01 vs. 0.27 ± 0.01; normoxia versus hyperoxia; $P = 0.03$) but not in the PBS-treated group ($N = 17$; 0.28 ± 0.03 vs. 0.24 ± 0.03; $P = 0.24$).

Among the mice injected with human monocytes, there was variability in lung and vascular parameters at baseline and with hyperoxia that could be accounted for by distinct patient-specific features of the monocytes

Figure 1 delineates the clinical profiles data from all 10 infants of the patient sample, numbered as patient 1 through 10. **Figures 5, 6** show the humanized mouse alveolar data according to each individual patient, numbered 1 through 10 corresponding to **Figure 1**. The individual datapoints representing each mouse demonstrate that there was variability among lung and vascular parameters, suggesting that individual patient characteristics of the 10 infants played a role in these early lung developmental findings. For example, as shown in **Figure 5A** mean alveolar count was significantly decreased in mice injected with monocytes from very preterm infants (patients 7 and 10) as compared with moderate and late preterm infants (patients 3 and 4). Similarly, alveolar perimeter was increased in mice injected with monocytes from very preterm infant (patient 10) as

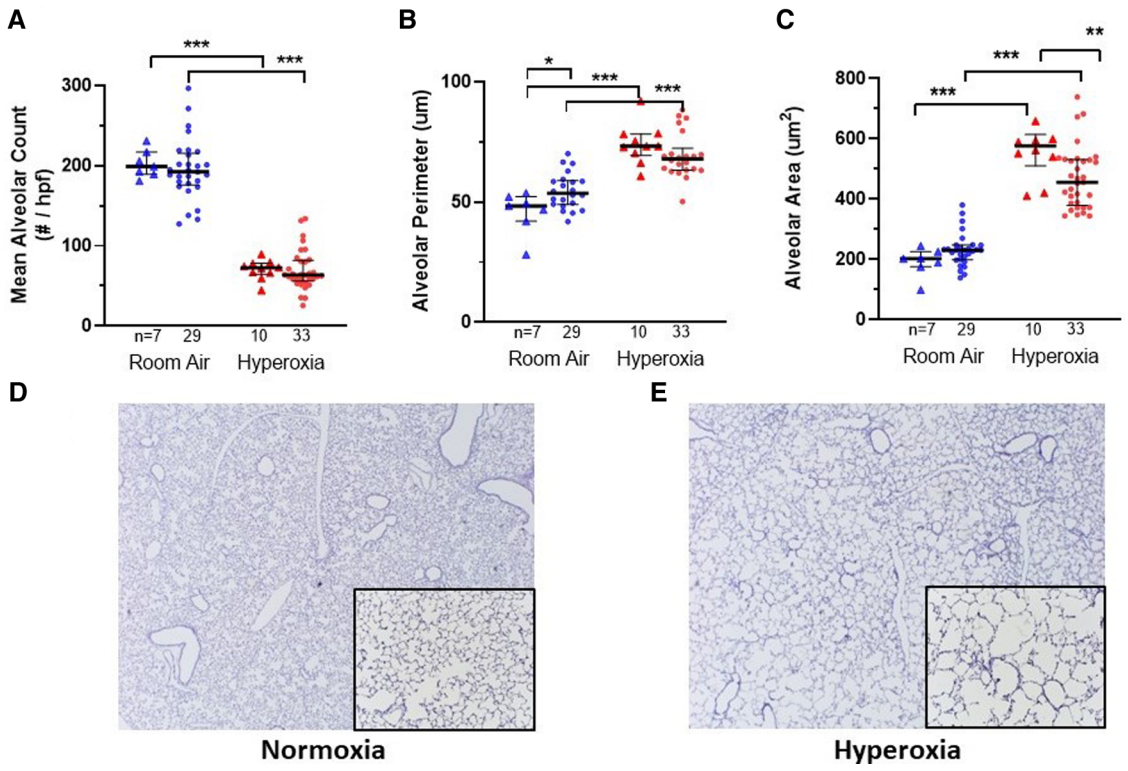

FIGURE 2
Lung alveolar morphometry and representative images at P14 according to monocyte versus placebo treatment on p0 and normoxia versus hyperoxia exposure (p0–p14). (**A–C**) Summary of alveolar count, perimeter and area, respectively. Individual datapoints represent each mouse: triangles represent PBS-injected and dots represent monocyte-injected pups. Blue indicates normoxia and red indicates hyperoxia exposure. At p14, mean alveolar area (**C**) was decreased in mice treated with human monocytes and exposed to hyperoxia. Mean alveolar perimeter (**B**) but not alveolar count (**A**) was increased with monocyte transplantation followed by room air. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. (**D–E**) Representative images of mouse lungs after 14 days of normoxia (**D**) versus hyperoxia showing typical alveolar simplification (**E**). Images stained for human CD45 with counterstaining. Images taken at 10× with 20× inlay.

compared with PBS and a late preterm infant (34 weeks) exposed to preeclampsia (patient #3). Overall, there were significant differences in alveolar count ($P = 0.001$ in normoxia group; $P = 0.007$ in hyperoxia group), perimeter ($P = 0.002$ in normoxia group) and area ($P = 0.01$ in normoxia group) when comparing parameters according to the origin of the monocytes from full term, moderately preterm and very preterm infants by ANOVA with *post hoc* testing. Table 2 summarizes the changes in lung parameters according to the 3 gestational age groups, stratified by normoxia and hyperoxia conditions.

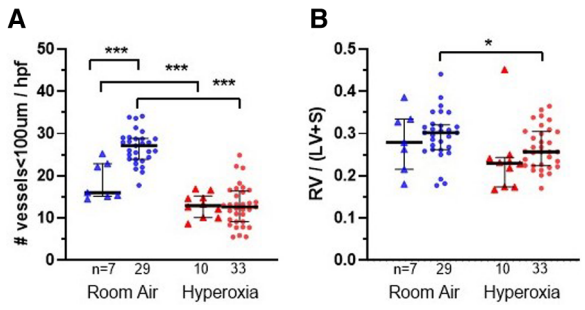

FIGURE 3
Lung vascular parameters and representative images at P14 according to monocyte versus placebo treatments and normoxia versus hyperoxia exposures. Individual datapoints represent each mouse: triangles represent PBS-injected and dots represent monocyte-injected pups. Blue indicates normoxia and red indicates hyperoxia exposure. (Panel **A**) small vessel density (measured by vonWillebrand factor stain) was increased in room air-exposed mice treated with monocytes. (Panel **B**) Fulton's index (ratio of right ventricular weight/left ventricle + septum) was decreased in monocyte-injected group with hyperoxia as compared with normoxia. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

Another prominent covariate of preterm birth in this patient sample was preeclampsia, in which 4 of the 10 infants were born to mothers with preeclampsia (patients 3, 5, 8 and 9). Mean alveolar count was increased and alveolar area was decreased in room air-exposed mice injected with monocytes transplanted from preeclamptic births as compared with non-preeclamptic births (Table 2). Comparison of parameters and all under hyperoxic conditions yielded no significant differences. Of note, the mice injected with monocytes from the very preterm infants exposed to preeclampsia (Patients 8 and 9) all died before p14 under normoxic conditions (Figures 5A,C,E), while the mice transplanted with these monocytes under hyperoxia survived to p14 (Figures 5B,D,F). The lung alveolar parameters (Figure 5), vascular findings (Figure 6) and lung CD45 counts (Figure 7) in these mice tended to be similar to other hyperoxia-exposed mice injected with monocytes from very preterm infants (patients 6, 7, and 10).

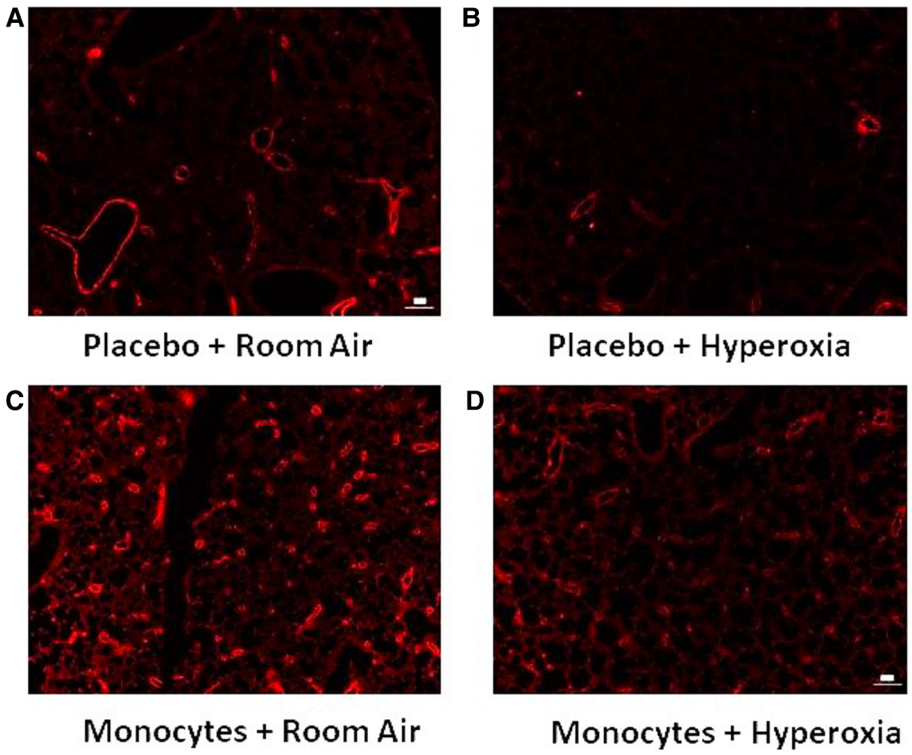

FIGURE 4
Representative immunofluorescent images with vWF identifying microvessel density. Note the relative paucity of microvessels (vessel diameter <100 um) in mice treated with placebo (A–B) versus intrahepatic injection of monocytes (C–D).

Human CD45+ hematopoietic cells are present in MISTRG lungs and liver at P14

The MISTRG mouse lungs harvested at P14 were stained for human CD45 to identify human hematopoietic cells that could have migrated to the lungs. Figure 7 shows the patient-specific profiles of human CD45+ cell counts in the MISTRG lungs and liver at P14 after injection with monocytes from the 10 patients at P0. While the median cell counts varied substantially among patients, there was a significantly decreased presence of human lung cells in mice injected with monocytes from very preterm infants (patients 7 and 10) as compared with the 34 week infant with preeclampsia exposure (patient 3). These patient-specific differences were not seen in hyperoxia-exposed mice. In room air-exposed but not hyperoxia-exposed mice, median human CD45+ cell count in the lungs was higher in mice transplanted with monocytes from preeclamptic births (Table 2). Human CD45+ counts in murine livers did not vary among patients, or with hyperoxia exposure, unlike CD45+ cell counts in the lungs which was decreased with hyperoxia (6.0 ± 0.8 vs. 28.0 ± 12.6 cells per lung field for hyperoxia versus normoxia respectively; $P = 0.005$). The differences in lung field counts were likely due to the alveolar simplification of hyperoxia exposure. When calculating the ratio of lung cell count to total CD45+ cells in lung + liver fields combined, the observed differences with hyperoxia and normoxia exposure were highly variable and no longer significant (Figures 7E,F). Figure 8 shows representative lung (Figures 8A,B) and liver (Figures 8C,D) tissues from mice exposed to hyperoxia versus normoxia.

Human plasma cytokines and chemokines define the microenvironment of the fetal monocytes

The average levels (assayed in duplicate) of all 30 analytes according to the 10 patients are shown in Table 3. Of the 30 analytes measured, 15 were consistently (≥90%) within the limit of detection. These included FGF-2, eotaxin, FLT-3l, Fractalkin, G-CSF, IL-1RA, IL-6, IL-8, IL-12p40, IL-12p70, IP-10, MCP-1, MIP-1β, TGFα, TNFα. In stepwise linear regression models adjusted for log-transformed GA, infant sex and preeclampsia status, 4 analytes had significant associations with multiple lung parameters: MCP-1, G-CSF, IL-8 and MIP-1β (Table 4). Upon further adjustment for the multiple biomarker comparisons, MCP-1 and MIP-1β were positively correlated with CD45+ lung cell counts while IL-8 was negatively correlated. Upon stratification by hyperoxia exposure (yes/no) these associations were modified: In models restricted to the normoxia group, MCP-1 was positively associated with alveolar count ($P < 0.001$), and negatively associated with perimeter and area ($P < 0.01$).

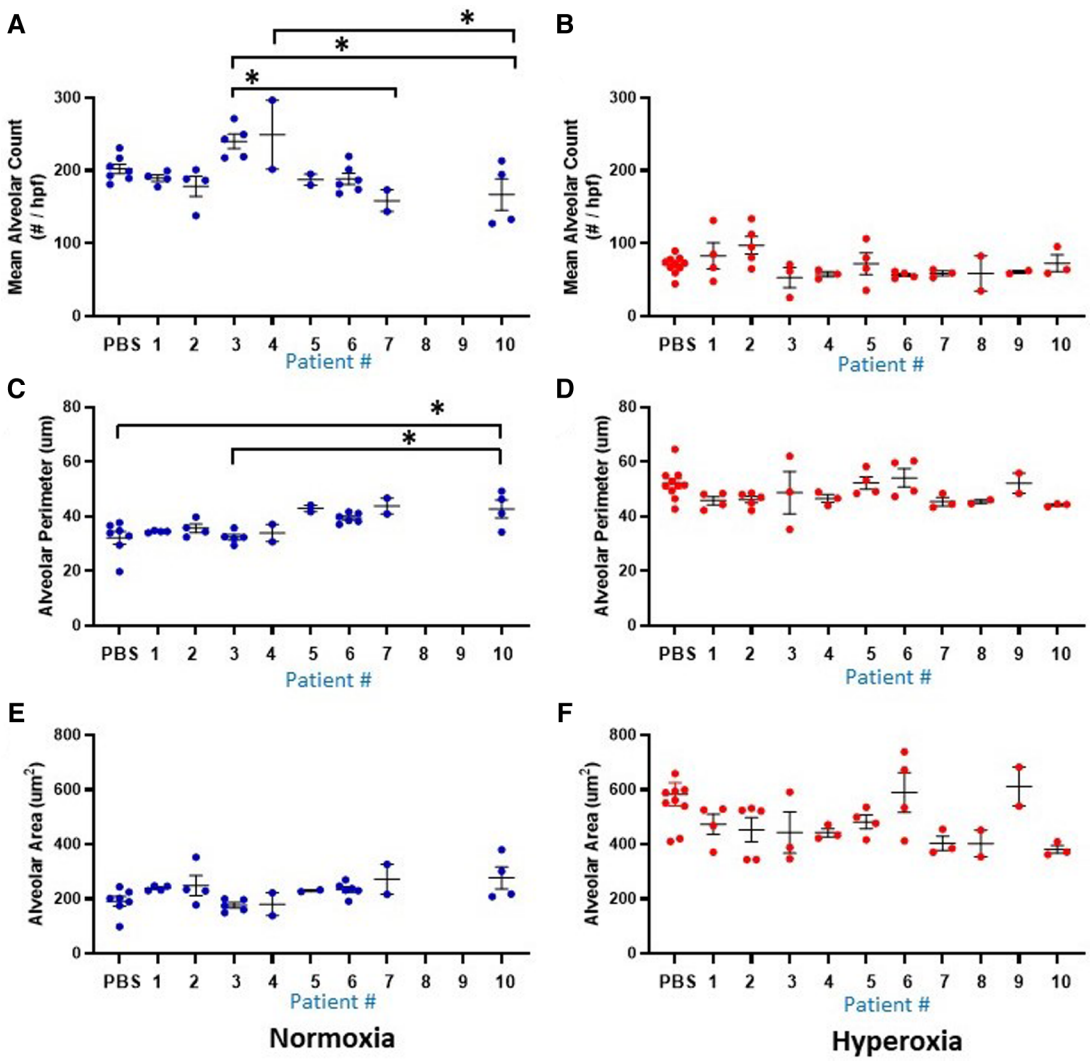

FIGURE 5
Lung alveolar morphometry data at P14 according to the 10 individual patients (1-10) and placebo (PBS). Individual datapoints represent mean/median values from 6 non-overlapping fields from each mouse for: alveolar count (A–B), alveolar perimeter (C–D) and alveolar area (E–F). Blue dots indicate normoxia and red dots indicate hyperoxia exposure. Data were analyzed using ANOVA with Bonferroni correction and post hoc analysis to identify patient-specific differences among groups. *$P < 0.05$.

Human CD206 alveolar macrophages are absent in MISTRG lungs at P14, but emerging at P28 after recovery in normoxia

Staining for anti-human CD206 antibody did not yield positively stained cells, as shown in representative samples of lungs at p14 (Figure 9A) as compared with positive human control samples (Figure 9B). Therefore, we studied a subsequent group of mice similarly transplanted with human monocytes (p0) and exposed to hyperoxia (p0–p14) and allowed to recover in room air (p15–p28). Lungs harvested at p28 revealed positively stained cells in several alveoli with morphology consistent with alveolar macrophages (Figure 9C). Additional studies of IHC staining for anti-mouse F4/80 in 10 representative mouse slides from each of the 10 patient monocyte cell lines confirmed absence of mouse macrophages in lungs at P14 (Figure 10).

To further explore the morphology and presence of human lung cells at p56, a subset of mice were allowed to recover in room air an additional 28 days, after which lung tissues were harvested at p56 and underwent flow cytometry/FACS sorting to isolate HLA-DR+, hCD206+, hCD14+, mCD45- viable cells which were stained for H&E following cytospin. This process confirmed the presence of human cells with morphology of alveolar macrophages, both in room air and hyperoxia-exposed mice at p56 (Figure 11).

RT-PCR studies of p14 lungs support paucity of human cells in the lungs expressing MCP-1, VEGF-A and MIP-1β

RT-qPCR results indicated significant levels of gene expression of mouse MCP-1, VEGF-A, and MIP-1β in all 18 representative

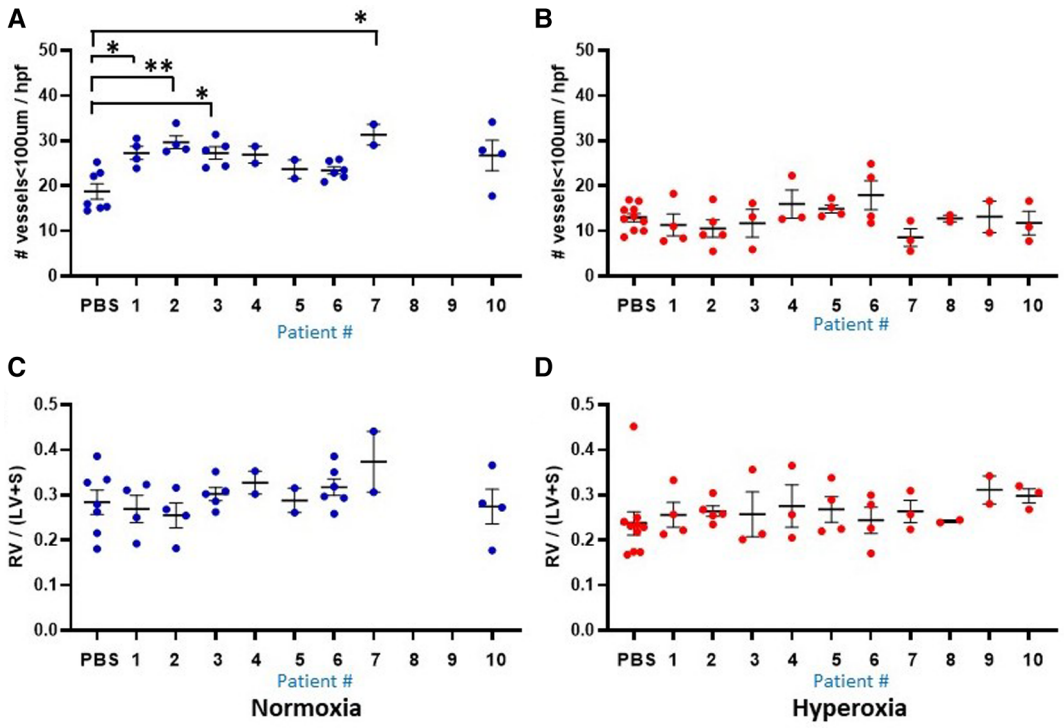

FIGURE 6
Lung vascular parameters at P14 according to the 10 individual patients (1–10) and placebo (PBS). Individual datapoints represent each mouse for: mean microvessel count (<100um) from 6-8 images (A–B), and Fulton's index (C–D). Blue dots indicate normoxia and red dots indicate hyperoxia exposure. Data were analyzed using ANOVA with Bonferroni correction and post hoc analysis to identify patient-specific differences among groups. *$P < 0.05$; **$P < 0.01$.

lung samples harvested at p14 (9 normoxia-exposed and 9 hyperoxia-exposed). There was minimal detection of human gene expression at p14 (data not shown), but upregulation of mouse gene expression for all 3 genes with hyperoxia exposure (**Figure 12A**). In addition, 4 representative lung samples harvested at p21 in additional mouse experiments were analyzed. Relative to p14, there was down-regulation (as noted by higher Ct value) of mouse MCP-1 and MIP-1β but not mouse VEGF-A after recovery in normoxia at p21 (**Figure 12B**). There was emergence of human MCP-1 and MIP-1β at p21 (**Figure 12C**).

TABLE 2 MISTRG lung parameters according to type of patient monocyte injected.

	Alveolar count	Alveolar perimeter	Alveolar area	Lung microvessel count	Lung CD45+ cell count
Normoxia exposure					
Gestational age groups					
Full term ($n = 8$ mice)	184.1 ± 20.0	35.0 ± 2.2	244.4 ± 48.9	28.5 ± 3.0	9.4 ± 5.2
Moderate preterm ($n = 9$ mice)	230.5 ± 37.8	35.1 ± 5.1	189.6 ± 35.0	26.4 ± 3.0	19.4 ± 14.1
Very preterm ($n = 12$ mice)	176.5 ± 29.7*	41.2 ± 4.3**	255.4 ± 55.3*	25.8 ± 4.9	10.3 ± 9.9
Preeclampsia					
No ($n = 22$ mice)	185.8 ± 7.5	38.3 ± 1.0	224.6 ± 11.7	26.9 ± 0.90	9.2 ± 1.7
Yes ($n = 7$ mice)	225.2 ± 12.0*	35.4 ± 2.1	192.1 ± 12.0*	26.25 ± 1.2	25.0 ± 4.6***
Hyperoxia exposure					
Gestational age groups					
Full term ($n = 9$ mice)	91.0 ± 30.0	46.0 ± 2.6	462.8 ± 84.4	10.9 ± 4.2	6.7 ± 4.6
Moderate preterm ($n = 10$ mice)	61.9 ± 22.5*	49.5 ± 7.4	458.7 ± 72.2	14.3 ± 4.1	8.5 ± 4.4
Very preterm ($n = 14$ mice)	61.5 ± 14.1*	48.6 ± 5.8	482.0 ± 131.5	13.2 ± 5.4	3.8 ± 4.3*
Preeclampsia					
No ($n = 22$ mice)	73.3 ± 5.4	47.2 ± 1.0	464.2 ± 22.0	12.7 ± 1.2	5.7 ± 1.0
Yes ($n = 11$ mice)	62.3 ± 7.1	50.0 ± 2.2	480 8 ± 31.2	13.3 ± 1.0	6.7 ± 1.6

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$ vs. full term or no preeclampsia using ANOVA or student's t-test.

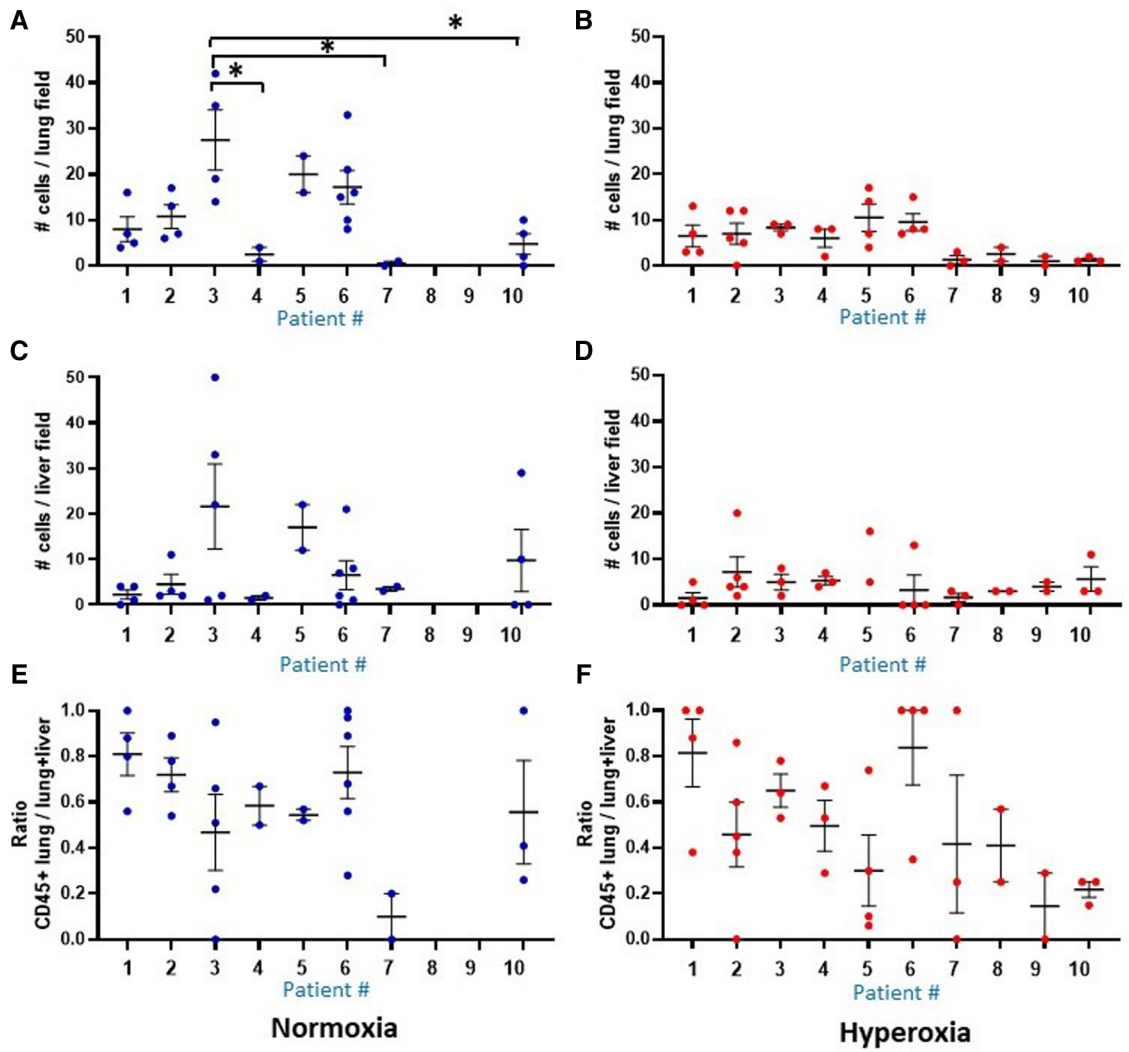

FIGURE 7
Comparison of human CD45 cell counts in MISTRG lung and liver at P14. Individual datapoints represent each mouse: blue indicates normoxia and red indicates hyperoxia exposure. Anti-human CD45 antibody staining was used to quantify human CD45+ hematopoietic cells circulating in the lungs at p14. (Panel A,B) Overall, median human cell count was decreased with hyperoxia exposure in mice treated with the human monocytes. Analysis of liver cell counts (Panel C,D) and ratio of lung to lung + liver counts per each mouse (E,F) revealed no differences in presence of human cells within the 10 patient groups, and no differences between hyperoxia versus normoxia. Missing data in normoxia (blue) for patients 8 and 9 indicate that all pups died prior to p14 and could not be harvested. *$P < 0.05$.

Discussion

In this study of a humanized mouse model of neonatal hyperoxia-induced lung injury, we found that newborn adoptive transfer of cord blood-derived fetal monocytes resulted in modest changes in alveolarization at p14. Unexpectedly, we found early signs of improved pulmonary microvessel density with human monocytes versus placebo injection at p0, suggesting a novel role of human monocytes in early pulmonary vascularization. Distinct patterns were also discovered in pooled patient analyses when GA and preeclampsia status of patient-specific monocytes were taken into account. While some of our findings support previous reports of human monocyte adoptive transfer in murine models (8), our study utilized a novel model genetically designed to support human monocyte transplantation, survival and potential for engraftment, through knock-in of human GM-CSF and other human cytokines (6). Leveraging the unique features of this humanized mouse model, we also took into account key human features of pregnancy that are associated with known perinatal risk factors for BPD. We found that monocytes derived from births complicated by preeclampsia resulted in features consistent with accelerated alveolarization (increased alveolar count, decreased area). While the effects of hyperoxia were largely independent of the pregnancy features, many of the findings in alveolarization and vascularization were in mice exposed to room air. This provides the first evidence that there are inherent features of human monocytes that may play a role in early lung development. These findings have important implications for our understanding of BPD pathogenesis.

The role of alveolar macrophages in early lung development and chronic lung diseases such as BPD has been increasingly

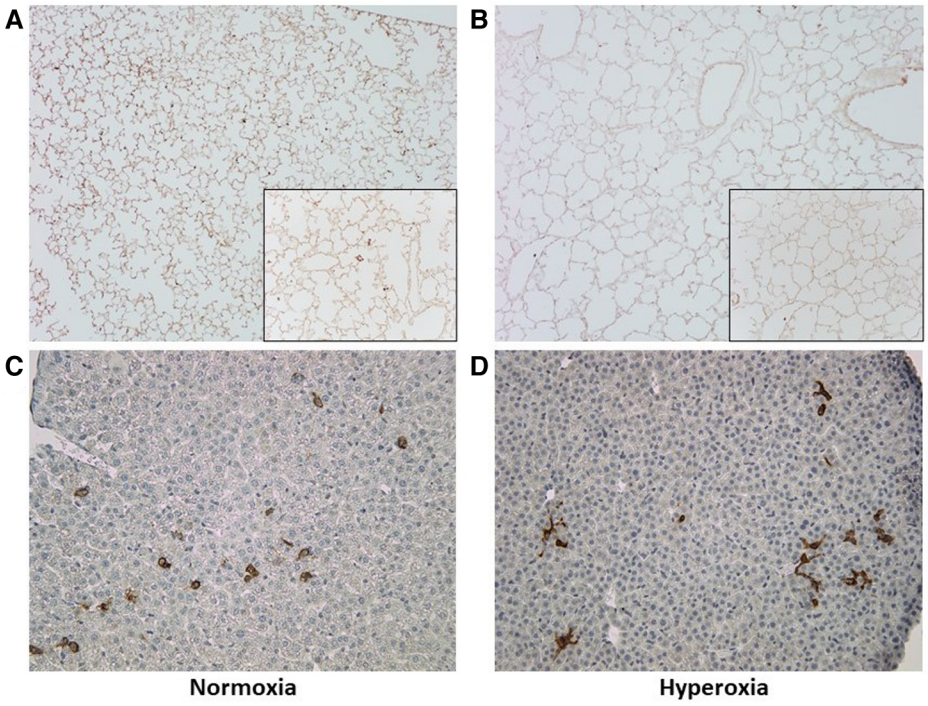

FIGURE 8
Representative lung and liver tissue sections with anti-human CD45 antibody staining. (A,B) Lung tissue sections at P14 demonstrating changes in alveolar structure and distribution of human CD45+ stained cells after normoxia (A) versus hyperoxia (B); images taken at 10× with 20× inlay. (C,D) Liver tissue section at P14 after normoxia (C) versus hyperoxia (D); images taken at 10×.

studied, with growing recognition that monocyte precursors play a pivotal role in lung injury and repair (21–23). Studies have shown a beneficial effect of human cord blood monocyte administration postnatally (p7) in a double-hit model of BPD (prenatal hypoxia followed by postnatal hyperoxia) (24), but these and other animal models of BPD have not been genetically modified to minimize human cell rejection. Mills and colleagues treated severe combined immunodeficient (SCID) mice with monocytes from cryopreserved cord blood via intravenous route and found mild improvement in alveolarization, lung compliance/elastance and decreased methacholine-induced bronchial hyperreactivity at 8 weeks (8). In contrast, we focused our investigations on a much earlier stage of alveolarization (p14) and used a mouse model (MISTRG) that specifically supports human monocyte-macrophage survival and engraftment. We administered monocytes via intrahepatic injection to more closely recapitulate the source of fetal monocytes *in vivo* and found not only robust survival of the mouse host but of circulating human monocytes at p14. Unlike typical animal models of BPD, we also describe detailed clinical characteristics of the patients from which the monocytes were derived and took these factors into consideration in our analysis. Measurement of cytokines and chemokines in human cord blood plasma from these births was also performed to understand the human immune cell microenvironment at the time of monocyte collection (i.e., birth).

We had hypothesized to find mostly changes in alveolarization with monocyte versus placebo treatment. Rather, more prominent and intriguing differences were seen in the vascular parameters. Despite only modest differences in alveolar morphology that were independent of hyperoxia exposure (**Figure 2**), mice treated with human monocytes had higher microvessel density than PBS-treated mice in room air (**Figure 3A**). This suggests that fetal monocytes may play a role in supporting early vascularization at baseline, an effect that was modified by hyperoxia such that median microvessel density in monocyte-treated mice fell to levels similar to PBS-treated mice after exposure to 85% oxygen × 14 days (**Figures 3A**, **4**).

Another unexpected finding was the baseline levels and changes in Fulton's index, a well-characterized measure of pulmonary hypertension in rodent models (25). In PBS-treated mice exposed to hyperoxia × 14 days, for example, mean Fulton's indices typically rise from 0.20 ± 0.01 to 0.35 ± 0.04 (25), indicating an elevated RV/LV + S ratio that usually accompanies a decrease in microvessel density. While the indices in our study were quite variable with some outliers >0.4, there was an overall decrease in Fulton's index with hyperoxia as compared with baseline. The decrease was statistically significant in the monocyte-treated group, supporting the hypothesis that fetal monocytes may incur a protective effect against pulmonary hypertension. We speculate that this effect may be independent of early pulmonary vascular development as the microvessel density was decreased in this group with hyperoxia. Of note, the base line Fulton's indices in the MISTRG mice were somewhat higher at baseline than other, non-humanized mouse models (19, 26), suggesting that the absence of early lung macrophages in p14 MISTRG mice (**Figure 10**) may play a role in

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TABLE 3 Cord blood plasma levels (pg/mL) of the 30 analytes measured by multiplex immunoassay.

Patient #	1	2	3	4	5	6	7	8	9	10
EGF	<3.2	22.48	21.52	<3.2	4.50	<3.2	24.10	27.56	9.73	42.59
Eotaxin	21.22	24.55	75.81	30.61	23.39	19.27	58.30	24.74	19.91	29.63
FGF-2	10.16	37.47	18.32	23.01	ND	129.11	1,201.75	251.33	73.58	55.46
FLT-3l	7.45	17.41	22.85	16.38	1.31	12.26	32.56	15.57	19.67	37.25
Fractalkine	121.24	55.20	116.94	133.02	58.17	83.89	120.86	140.14	66.45	138.27
G-CSF	87.38	<4.8	197.37	106.37	79.07	156.49	2,808.40	323.40	18,544.69	318.40
GM-CSF	ND	<2.6	<2.6	ND	<2.6	<2.6	ND	<2.6	<2.6	<2.6
IFN-γ	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
IL-1α	3.80	1.57	6.95	24.84	ND	ND	40.48	1.99	14.86	12.30
IL-1β	0.17	0.90	23.58	ND	<1.6	<1.6	7.84	0.17	2.17	3.60
IL-1RA	71.11	126.79	714.35	1,562.66	93.20	15.02	10,441.25	330.65	3,541.34	1,531.55
IL-2	0.11	<0.6	0.12	0.57	<0.6	<0.6	0.61	<0.6	<0.6	0.10
IL-3	0.60	<1.3	0.24	2.12	<1.3	<1.3	0.90	0.39	<1.3	0.28
IL-4	<0.6	<0.6	0.73	1.00	0.50	<0.6	0.56	<0.6	<0.6	0.89
IL-6	14.89	0.83	79.66	35.87	7.38	14.91	191.89	14.62	3,923.95	51.90
IL-8	0.93	0.45	43.13	14.81	2.23	7.64	275.69	43.47	59.90	69.21
IL-10	11.89	<2.6	17.58	12.62	26.96	6.21	19.39	9.47	5.82	<2.6
IL-12 (p40)	487.67	132.98	108.00	295.65	75.17	304.92	191.25	319.51	305.41	92.86
IL-12 (p70)	1.39	1.67	4.45	2.02	1.06	1.05	2.39	2.38	1.56	2.63
IL-13	<6.4	ND	19.41	ND	<6.4	<6.4	6.16	14.58	3.84	20.51
IL-17A	<1.3	<1.3	1.01	<1.3	<1.3	<1.3	<1.3	0.25	<1.3	ND
IP-10	<2.6	39.38	388.16	13.25	49.17	18.40	50.92	497.92	151.11	88.22
MCP-1	148.17	93.41	1,310.10	924.83	158.60	356.98	815.10	224.56	235.13	290.21
MCP-3	10.81	ND	25.85	14.96	<8.0	<8.0	18.70	15.08	4.47	27.26
MIP-1α	<3.2	<3.2	21.71	20.71	<3.2	<3.2	35.15	18.44	12.97	35.68
MIP-1β	12.75	44.68	66.47	43.57	12.23	23.05	38.27	29.16	59.45	123.10
TGF-α	2.26	2.31	2.19	7.97	2.26	2.57	2.99	2.17	2.33	2.89
TNF-α	19.36	20.40	29.96	24.61	13.90	17.46	29.25	45.72	76.48	33.93
TNF-β	<1.6	<1.6	1.14	<1.6	<1.6	<1.6	ND	0.94	10.73	2.54
VEGF-α	<2.6	<2.6	ND	<2.6	14.54	<2.6	48.21	59.32	171.37	338.13

Cord blood plasma levels measured by Luminex multiplex immunoassay. Values reported as picogram/ml and are the average of 2 assays performed in duplicate.
EGF, epidermal growth factor; FGF, fibroblast growth factor; FLT, Fms-related tyrosine kinase; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP, interferon gamma-induced protein; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.
ND = not available (level was not detected by the assay); levels "<" indicate the calculated value was below the limit of detection for the assay (see MILLIPLEX® Human Cytokine/Chemokine/Growth Factor Panel HCYTA-60K for reference values and ranges).

development of pulmonary hypertension. There is recent emerging evidence that alveolar macrophages may contribute to pulmonary hypertension (27). To our knowledge, the vascular findings reported here in the MISTRG model of neonatal hyperoxia-induced lung injury have not been previously reported. Thus, further investigation leveraging this model may elucidate mechanisms of distinct vascular endotypes of BPD, such as BPD-associated pulmonary hypertension (28).

MISTRG mice were first described in 2014, developed by Rongvaux and colleagues to model the human immune system in a wide range of organ systems and disease states (6). The unique features of this model include knock-in of human macrophage colony-stimulating factor (M-CSF) and GM-CSF—two cytokines essential for macrophage development (29, 30), as well as expression of human cytokines interleukin-3 (IL-3) and thrombopoietin (TPO) in a immunodeficient $Rag2^{-/-}Il2rg^{-/-}$ background, and signal regulatory protein alpha (SIRPα) to establish mouse-to-human phagocytic tolerance (6). This supports a highly permissive microenvironment for human hematopoiesis in a mouse host (3). A growing number of studies have used MISTRG mice to study various disease states (31–35). To our knowledge none have been reported to date on the effects of neonatal hyperoxia, prematurity or preeclampsia. Evren and colleagues are the first to provide a comprehensive report of the early course and fate of human $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) in MISTRG lungs (3). In a series of adoptive transfer and fate mapping studies, they showed that HSPCs migrate to lung tissue and give rise to human interstitial and alveolar macrophages. They reported that HSPCs give rise to all types of human lung monocytes and macrophages, and that classical $CD14^+CD16^-$ monocytes (the most abundant subpopulation in our cord blood samples) appear >3 weeks postnatally in the mice. In fate mapping studies, classical monocytes were shown to be the precursors of human lung macrophages—with a predominance of alveolar macrophages. However, similar to our findings upon anti-human CD206 staining, they found a paucity of CD206+ cells at <3 weeks postnatally, and an emergence of these as human alveolar macrophages at 8 weeks suggesting that $CD14^+$ monocytes are at most the precursors to adult alveolar macrophages.

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TABLE 4 Correlations in beta-coefficient (95% CI) between human cord blood plasma cytokine/chemokine levels and humanized mouse lung parameters.

	MCP-1 β-coef (95% CI)	G-CSF β-coef (95% CI)	IL-8 β-coef (95% CI)	MIP-1β β-coef (95% CI)
All ($N = 56$)				
Alveolar count	0.13 (−0.25, 0.50)	0.09 (−0.13, 0.30)	−0.28 (−0.67, 0.11)	0.56 (−0.11, 1.22)
Alveolar perimeter	−0.03 (−0.13, 0.07)	−0.01 (−0.07, 0.05)	0.03 (−0.08, 0.13)	−0.13 (−0.31, 0.05)
Alveolar area	−0.13 (−0.38, 0.12)	0.02 (−0.13, 0.17)	0.05 (−0.21, 0.31)	−0.22 (−0.67, 0.23)
Microvessel density	0.23 (−0.08, 0.54)	0.06 (−0.12, 0.24)	−0.23 (−0.56, 0.09)	0.23 (−0.32, 0.79)
Lung CD45+ cell count	1.00 (0.55, 1.45)***	0.03 (−0.23, 0.30)	−1.07 (−1.56, −0.58)***	1.08 (0.31, 1.85)**
Normoxia ($n = 26$)				
Alveolar count	0.29 (0.13, 0.45)***	0.03 (−0.09, 0.16)	−0.15 (−0.35, 0.06)	0.02 (−0.29, 0.33)
Alveolar perimeter	−0.13 (−0.23, −0.04)**	−0.05 (−0.12, 0.02)	0.12 (0.00, 0.24)*	−0.16 (−0.34, 0.02)
Alveolar area	−0.29 (−0.50, −0.09)**	−0.004 (−0.15, 0.16)	0.10 (−0.17, 0.36)	0.04 (−0.37, 0.45)
Microvessel density	−0.13 (−0.27, 0.02)	−0.07 (−0.18, 0.04)	0.18 (−0.01, 0.36)*	−0.09 (−0.38, 0.20)
Lung CD45+ cell count	0.78 (0.04, 1.52)*	0.05 (−0.50, 0.59)	−1.20 (−2.21, −0.19)*	1.57 (0.19, 2.97)*
Hyperoxia ($N = 30$)				
Alveolar count	−0.24 (−0.52, 0.04)	0.01 (−0.13, 0.15)	0.02 (−0.25, 0.29)	0.06 (−0.43, 0.55)
Alveolar perimeter	0.08 (−0.02, 0.17)	0.02 (−0.03, 0.07)	−0.08 (−0.18, −0.01)*	0.01 (−0.15, 0.18)
Alveolar area	0.11 (−0.06, 0.28)	0.09 (0.00, 0.17)*	−0.19 (−0.35, −0.03)*	0.09 (−0.20, 0.38)
Microvessel density	0.34 (0.03, 0.66)*	0.02 (−0.14, 0.18)	−0.22 (−0.52, 0.08)	−0.16 (−0.70, 0.37)
Lung CD45+ cell count	0.96 (0.43, 1.50)***	−0.05 (−0.33, 0.23)	−0.71 (−1.24, −0.18)*	0.16 (−0.74, 1.06)

MCP-1, monocyte chemoattractant protein-1; G-CSF, granulocyte colony stimulating factor; IL-8, interleukin-8; MIP, macrophage inflammatory protein 1-β (CCL4).
All parameters log transformed to approximate normal distribution. Models adjusted for gestational age in weeks (log transformed), infant sex, and preeclampsia status, and for the multiple biomarker comparisons.
*$P < 0.05$.
**$P < 0.01$.
***$P \leq 0.001$.

The $CD45^+$ cells present in lung tissue at p14 in our studies most likely represent circulating hematopoietic human cells that have migrated to the lungs, however the overall paucity of these cells in the lung and their location in vascular rather than alveolar regions suggests that the original $CD14^+$ fetal monocytes derived from cord blood and transplanted at P0 are not immediate early precursors to alveolar macrophages in the perinatal period. These findings are consistent with a more recent study by Evren, et al, in which they identified a distinct lineage of circulating $CD116^+CD64^-$ fetal macrophage precursors that originate in the fetal liver and readily migrate to the perinatal lung in the MISTRG model, populating the lungs at <7 weeks postnatally as functional alveolar macrophages (36). In conjunction with these recent findings, our data alternatively suggest a pulmonary vascular and/or paracrine effect of $CD14^+$ monocytes on lung alveolarization, in which $CD14^+$ monocytes remain in circulation and express differential gene expression according to their exposure to acute inflammation or vascular dysfunction mediated by the placenta (7).

Our data showed that hyperoxia resulted in significantly reduced median CD45+ cell counts in the lungs, but not the liver (**Figures 7**, **8**). Overall, these data and to a lesser extent the lung morphometry data were quite variable with small numbers of animals in certain groups given the limitations in timing matings, litter size and availability of cells for injection which may have been due to inherent properties of cord blood monocytes in human infants and their birth characteristics (e.g., fewer monocytes were recoverable from lower gestational age and/or preeclamptic births). Thus, pooled analysis of the patients according to these birth characteristics was necessary. As such, both individual and pooled analysis suggests that preeclampsia could be a driver of increased median cell count in the lung, and that this effect may be modified by hyperoxia (**Figure 7** and **Table 2**). Mechanisms linking preeclampsia to accelerated alveolarization have not been completely described, but recent data suggests that accelerated placental aging, a feature of preeclampsia (37, 38), is associated with the certain vascular phenotypes of BPD (39), and that reversal of accelerated lung aging characterized by cell senescence and apoptosis could attenuate hyperoxia-induced lung injury (40). Britt and colleagues reported reduced levels of monocyte chemoattractant protein (MCP-1) in bronchoalveolar lavage fluid at p14 in hyperoxia-exposed mice, suggesting a possible mechanism by which hyperoxia is associated with decreased human CD45+ cells in lungs at p14 in our MISTRG model (41). Our findings also suggest that the hypoxic insult of preeclampsia alone may be a significant driver of *increased* migration of monocytes to the lung. These findings are consistent with previous *in vitro* observations of hypoxia-induced directed migration of human monocytes (42). While the effects of subsequent hyperoxia following hypoxia on monocyte migration are not well-understood, double-hit models of monocyte replenishment at p7 in mice support our findings that hypoxia-hyperoxia leads to decreases in circulating lung monocytes (24).

As gestational immaturity is an independent risk factor for BPD, it is not surprising that we saw differences in monocyte function among the 3 gestational age subgroups. Delayed, rather than accelerated alveolarization appeared to be associated with

adoptive transfer of monocytes from very preterm births. In our humanized mouse model, the pattern of alveolar simplification with lower gestational age was modified by hyperoxia such that the differences between the gestational age subgroups were lost (**Table 2**). However, the decrease in CD45+ cell count with hyperoxia was preserved (**Figure 7**), suggesting that monocytes engraft differently with hyperoxia when certain pathologies (extreme prematurity, intrauterine inflammation or placental vascular dysfunction) are also present. It cannot be determined from our study whether the decrease in CD45+ cells contribute to delayed alveolarization, and further studies of the function of these circulating lung cells are needed. In a separate study of cord blood monocytes derived from this same patient cohort, bulk RNAseq revealed distinct gene expression profiles mediated by inflammatory versus vascular processes (7). These findings, coupled with single cell RNAseq studies conducted by Evren et al, that identified distinct developmental pathways from circulating MNCs to lung macrophages (3) support a pivotal role of circulating fetal-derived monocytes in early lung development and subsequent BPD.

Certain findings of cord blood cellular count and plasma contribute to our understanding of monocyte interactions and their microenvironment. It is important to note that across all types of patient samples in our cohort, classical monocytes were the most predominant at birth, and that differences in proportion of monocyte subsets may play a role. Consistent with Evren et al, it is likely that the classical subpopulation represents the predominant source of human CD45+ cells present in circulation at p14, which are possible precursors to the CD206+ alveolar macrophages present after 3 weeks (**Figure 9**). Our humanized mice findings coupled with recent data from the larger cohort support the emerging role of atypical, non-classical monocytes in vascular processes by which preeclampsia is associated with distinct endotypes of BPD (43, 44).

In multiplex analysis of corresponding cord blood plasma samples obtained at the time of monocyte isolation at birth, we found that MCP-1, MIP-1β and IL-8–chemokines known to be induced by monocytes in various disease states and are key regulators of migration and infiltration of monocytes and macrophages (45–47)—were differentially associated with several parameters of alveolarization, microvessel density, and circulating human CD45+ cells in the lungs. These chemokines, in addition to G-CSF, are known to play an interactive role in neutrophil recruitment and function (48, 49). Yet it remains unclear

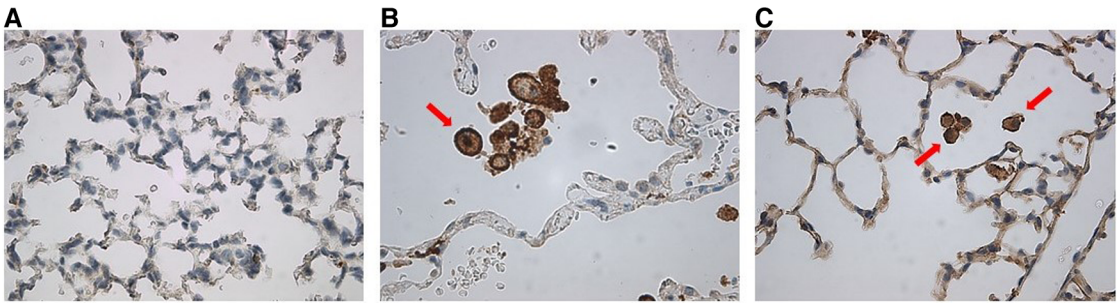

FIGURE 9
Anti-human CD206 stained MISTRG mouse lung slides showing (**A**) absence of alveolar macrophages at p14 as compared with (**B**) positive human control. (**C**) At p28, there was emergence of positively-stained cells with prominent nuclei similar to human controls, as indicated by red arrows.

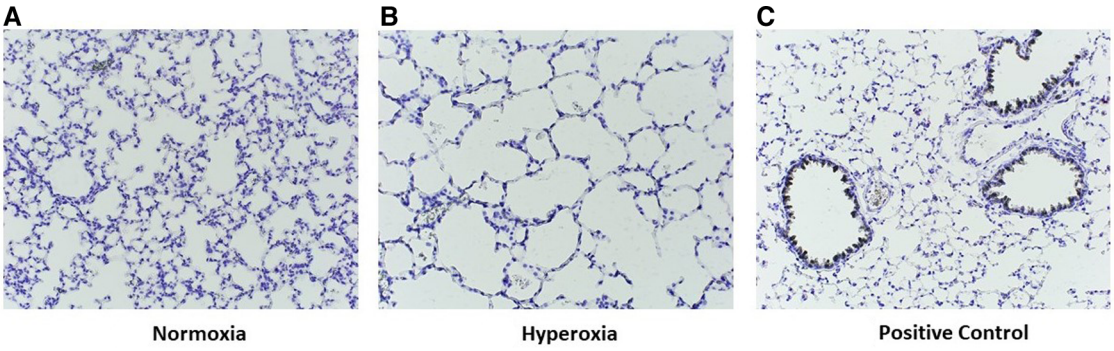

FIGURE 10
Representative images of p14 lungs with F4/80 immunostaining. Lungs harvested at p14 were stained with F4/80 to identify murine lung macrophages. Note the absence of positive staining in both normoxia-exposed (**A**) and hyperoxia-exposed (**B**) lungs as compared with positive control lung from an adult wildtype non-humanized mouse lung tissue (C57BL/6J, compliments of Dr. Eniko Sajti) (**C**). For all slides, biotin labelled secondary antibody used, with counterstain incubation time 30 s Antibody dilution for both primary and secondary was 1:200. Streptavidin-HRP dilution 1:1500. Images taken at 20×.

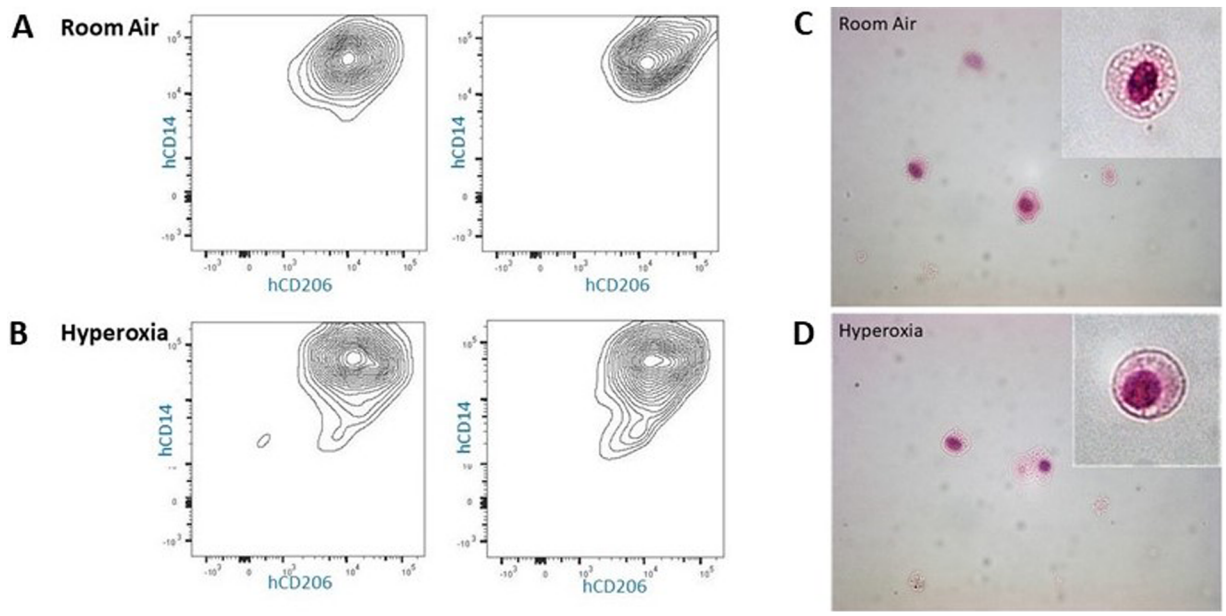

FIGURE 11
Cytospin images (40×) of hCD14$^+$/hCD206$^+$ lung cells isolated from p56 MISTRG lungs. (Panel A,B) Flow cytometry gating by HLA-DR$^+$/mouseCD45$^-$ followed by FACS for isolation of human CD14$^+$/human CD206$^+$ cells from lungs exposed to room air or hyperoxia ×14 days followed by recovery in room air until 8 weeks of age. (Panel C,D) Cell surface markers and morphology (inlay) confirm the presence of human alveolar macrophages at p56 in MISTRG mice exposed to room air and early hyperoxia.

whether and which proteins present in plasma represent expression from circulating monocytes and whether human monocyte expression is similar after transplantation into MISTRG mice. In the RT-qPCR studies (Figure 12) human gene expression, but not mouse gene expression, correlates with presence of human cells in the lungs. These findings suggest, albeit preliminarily, that monocytes after birth continue to contribute to their microenvironment and may have paracrine effects on the lungs (perhaps from the liver) in the absence of human lung macrophages.

There were several limitations in this study. Firstly, a much larger sample of patients is needed to more completely understand the diversity of monocytes and the influence of perinatal risk factors. At the onset of this study, we had

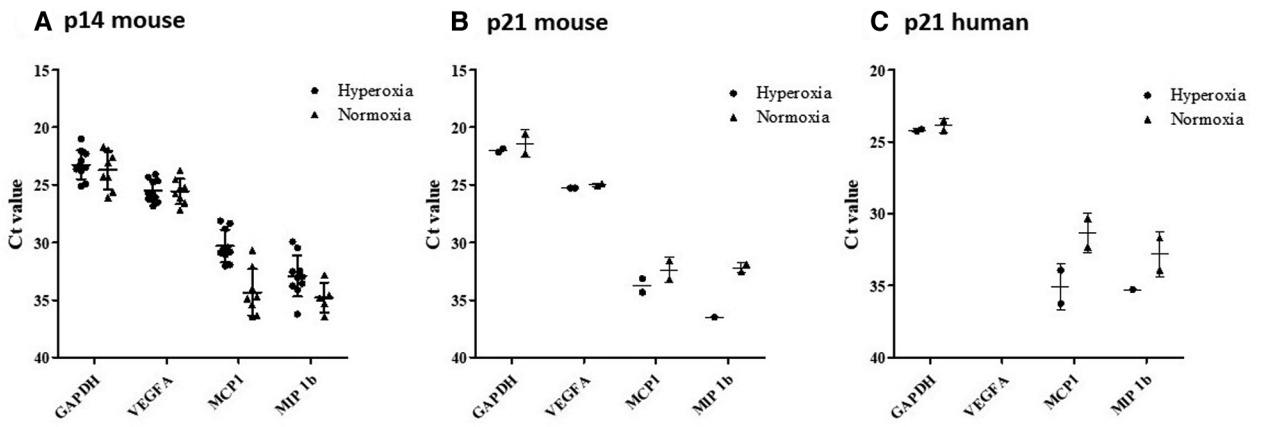

FIGURE 12
RT-qPCR results from MISTRG lungs at p14 showing gene expression changes with hyperoxia. According to plotted Ct values (*y*-axis) VEGF-A for normoxia and hyperoxia were not remarkably different. Whereas, MCP-1 and MIP-1B lower Ct values were observed in hyperoxia as compared to normoxia, indicating relatively higher levels of RNA (Panel A). Relative to p14, there was down-regulation (as noted by higher Ct value) of mouse MCP-1 and MIP-1B but not mouse VEGF-A after recovery in normoxia at p21 (Panel B). There was emergence of human MCP-1 and MIP-1B expression at p21, which was not seen at p14 (Panel C).

originally hypothesized that monocytes would readily engraft in the MISTRG mice and influence lung development via monocyte-derived alveolar macrophages. Emerging literature has since shown that these alveolar macrophages do not appear until much later in development, beyond the perinatal period, which is supported by our findings. But the finding that monocytes may accelerate early microvessel development before the appearance of macrophages generates new hypothesized mechanisms of perinatal lung vascular development influenced by innate immune mechanisms. Further investigations of how vascular changes arise in the lungs despite the lack of transplanted immune cells in early development are needed, as these studies may redirect our focus towards other cell types or perhaps paracrine effects on the lungs from distant or circulating monocytes. As the MISTRG model has been used in various studies of adult lung disease, there is a steep learning curve for leveraging the unique aspects of this model for more mechanistic interrogation of BPD—a developmental lung disease that is multifactorial and highly complex.

As our cord blood monocyte archive expands, we hope to interrogate more samples from patients who develop BPD. A significant challenge in using human infant-specific cord blood monocytes is that the process requires cryopreservation of the monocytes, sometimes for longer periods than traditionally done, to allow well-matched patient samples to be adoptively transferred in sequential studies with well-coordinated timed matings. Variations in litter size and patient-specific factors led to variations in the number of pups injected for each exposure group. There are also limitations in the volume of blood and cells that can be collected from extremely preterm infants, requiring pooled analysis of patients according to their birth characteristics. While our preliminary studies showed excellent cell viability and function in gene expression studies using the methods described in this study, the workflows to optimize viability and improve cell recovery are critically important for the success of each experiment, and to study more litters of MISTRG mice simultaneously to minimize batch effects. Lastly, another important limitation of this study is that we have yet to study the long-term effects of monocyte injection in larger samples, in particular the sustained effects of early microvessel development after recovery in normoxia. If sufficient human lung macrophage populations are successfully established using the above processes, limitations of alveolar proteinosis in adult mice may be overcome so that these further timepoints can be investigated. Methods for following the course and trafficking of immune cells can be employed in real-time using recently developed immunophenotyping technologies. In addition, studying other organ systems and larger scale analysis of the lungs, such as RNAseq and proteomics, will be highly informative.

In summary, we conducted this study to test the hypothesis that fetal monocytes influence early lung development in a novel humanized mouse model of hyperoxia-induced lung injury. We found that fetal monocytes directly influence pulmonary vascular development, despite the absence of alveolar macrophages in the neonatal period of MISTRG mice. Furthermore, the vascular effects induced by monocytes are inhibited by hyperoxia, but may be independent of pulmonary hypertension. The above study describes a novel *in vivo* experimental model of BPD by which human monocytes and their associated clinical and perinatal characteristics can be investigated for effects of neonatal hyperoxia on early lung development. Elucidation of the mechanisms by which fetal monocytes, programmed by *in utero* processes such as vascular dysfunction, influence lung injury and repair is essential for developing more targeted, patient-specific approaches in the management of multifactorial BPD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Northwestern University. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Northwestern University.

Author contributions

RB, JN, AS, LB and EL contributed to the design and execution of experimental methods, as well as acquisition of data for the work. SS and AM contributed their expertise in study design conception, data interpretation and analysis. KM is responsible for conceptual design of the study, oversight of experiments, data analysis and interpretation, and drafting of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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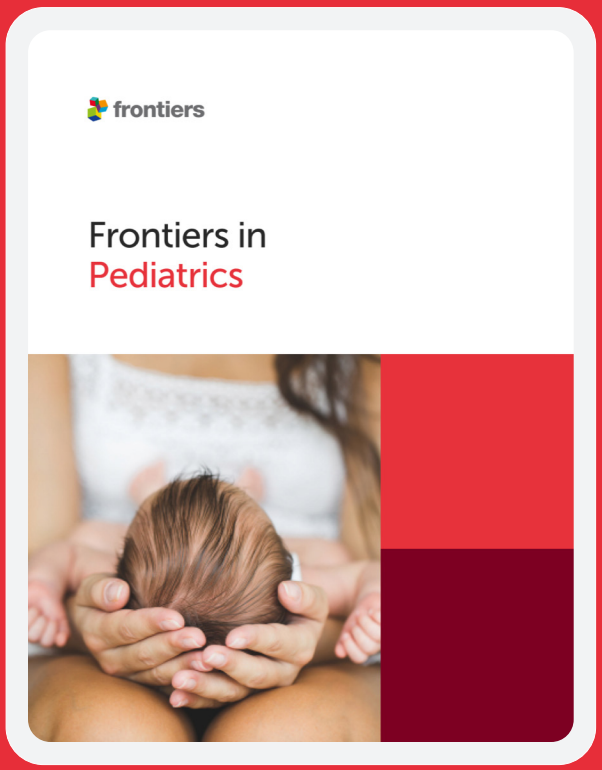

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