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Environmental And Genetic Risk Factors For Bronchopulmonary Dysplasia In Neonates

Jessica Berger
Yale School of Medicine, jessica.berger@yale.edu

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Environmental and Genetic Risk Factors for Bronchopulmonary Dysplasia in Neonates

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by
Jessica Ashley Berger
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ENVIRONMENTAL RISK FACTORS FOR BRONCHOPULMONARY DYSPLASIA (BPD). Jessica Berger, Paulomi Mehta, Emily Bucholz, James Dziura, and Vineet Bhandari. Section of Neonatology, Department of Pediatrics, Yale University, School of Medicine, New Haven, CT.

We hypothesized that early intubation would decrease the hazard of BPD and BPD/death in premature infants regardless of need for reintubation. Specific aims included assessing rates of BPD and BPD/death in infants first extubated between day of life (DOL)1-3 versus 4-7, 8+ and impact of re-intubation. We included infants with gestational age ≤28 weeks, birth weight ≤1000g, and intubation on DOL1. Proportional hazards regression modeled time to BPD and BPD/death, adjusting for potential confounders. Of 262 infants, 101 (38.55%), 41 (15.65%) and 120 (45.80%) were extubated between DOL1-3, 4-7, and 8+, respectively. Extubation between DOL4-7 versus DOL1-3 was associated with an increased hazard of developing BPD (HR 1.7; 95%CI 1.0-2.8; p<0.05). Extubation on DOL 8+ was associated with a significantly increased hazard compared to extubation between DOL1-3 (16.9; 10.5-27.1; <0.0001) or DOL4-7 (10.0; 6.1-16.3; < 0.0001). Similar results were noted with BPD/death. Re-intubation did not affect BPD and BPD/death. Delaying extubation beyond the first 3 and 7 days was associated with an increased risk of BPD and BPD/death. Re-intubation did not impact outcomes.
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INTRODUCTION

Disease Overview

Bronchopulmonary dysplasia (BPD) is one of the most common co-morbidities of premature birth. In 1967, William H. Northway, Jr. published the earliest known description of BPD, a “chronic pulmonary syndrome” which he believed to be a consequence of therapy for neonatal respiratory distress syndrome (RDS). Northway characterized BPD by the radiographic and histopathologic changes occurring in the neonatal lungs after a period of rigorous invasive mechanical ventilation and high-concentration supplemental oxygen. In a retrospective and post-mortem (when applicable) study of 32 premature infants, Northway observed that those treated with prolonged invasive ventilation and supplemental oxygen therapy had difficulty breathing in room air when weaned from the ventilator. In those infants with evolving chronic lung disease, the lungs appeared bullous on chest x-ray. The radiographic appearance was reflected pathologically as well, with areas of bullous emphysema surrounded by areas of atelectasis. Histologically, there was also evidence of a thickened, fibrotic basement membrane and bronchial smooth muscle hypertrophy. Clinically, many of the infants also had concurrent pulmonary hypertension and cor pulmonale.

Since Northway’s initial characterization of BPD, scientists and clinicians have gathered a wealth of knowledge about the disease using animal models, retrospective clinical analysis, and randomized clinical trials. From a basic science perspective, these studies have broadened our understanding of the disease’s etiology and pathogenesis. Recent histologic investigation has offered a “new” pathologic definition of BPD. “New” BPD is characterized by decreased alveolar septation and irregular development of the
pulmonary microvasculature rather than pulmonary fibrosis \(^2,3\). In addition, “new” BPD does not always evolve from RDS \(^4\).

Clinical criteria for diagnosis have also evolved over time. Prior to 2001, BPD was diagnosed only by a need for supplemental oxygen at 36 weeks’ post-menstrual age (PMA). In 2001, a collaborative effort by the National Institute of Child Health and Human Development, the National Heart, Lung, and Blood Institute, and the Office of Rare Diseases yielded a set of new diagnostic criteria, known colloquially as the NIH Consensus Definition. The NIH Consensus Definition categorizes BPD by severity using a scale based on the number of days of supplemental oxygen use, as well as oxygen and positive pressure ventilation requirements at 36 weeks’ PMA. Any diagnosis of BPD requires a minimum of 28 days of supplemental oxygen use. Infants with mild BPD no longer require supplemental oxygen at 36 weeks’ PMA or time of discharge, whereas infants with moderate and severe disease require a fraction of inspired oxygen (Fi\(O_2\)) < 30% or Fi\(O_2\) > 30% supplemental oxygen, respectively. A diagnosis of moderate or severe disease may be made if an infant requires positive pressure ventilation at 36 weeks’ PMA even if Fi\(O_2\) requirements are not met \(^2\). Walsh et al proposed a “physiologic definition of BPD” based on Fi\(O_2\) and oxygen saturations during an oxygen reduction test at 36 weeks’ PMA in order to reduce discrepancies in diagnosis between physicians \(^5\).

**Disease Epidemiology**

Previous studies have estimated rates of BPD between 22% and 68% of pre-term neonates \(^6,7\). This variability in incidence may be attributed to the range of gestational age (GA) and birth weight (BW) of the study populations and the use of differing definitions
of BPD. Of note, the 2001 definition includes babies who would not have been diagnosed with BPD using earlier criteria. Consistently lower rates of BPD were seen across centers that used the physiologic definition of BPD\(^8\). Lower BW and/or lower GA are correlated with an increased incidence of BPD\(^6,7\), with 97% of BPD diagnoses occurring in infants weighing < 1250 g at birth\(^9\).

**Management of BPD**

Inherent to the definition of BPD is the prolonged use of positive pressure ventilation and supplemental oxygen. Conventional mechanical ventilation and high-frequency oscillatory ventilation are two modalities frequently used to support infants with BPD. Guidelines based on ventilator settings and arterial blood gases dictate when an infant may be extubated. In some severe cases, infants fail several attempts at extubation due to severity of disease or anatomical reasons, which may result in a tracheotomy for long-term respiratory support. Supplemental oxygen therapy aims to achieve an appropriate balance between hypoxia and hyperoxia, both of which may be detrimental\(^10\). To date, there remains a paucity of evidence as to what oxygen saturation range is optimal, though > 90% and < 95% are commonly-used thresholds\(^3,10\). Some infants may be discharged from the hospital with supplemental oxygen by nasal cannula for ongoing support, growth and development\(^10\).

In addition to supplemental oxygen and assisted ventilation, there are many therapies used to mitigate the effects of BPD. Post-natal steroids are often used to facilitate extubation, particularly in infants who have been invasively ventilated for long periods or who have had repeated extubation failures. In previous studies, dexamethasone worked well for this purpose. Steroid use has been associated with poor long-term...
neurocognitive outcomes. Diuretics are commonly used to reduce pulmonary edema, a frequent occurrence in infants with BPD. Data are mixed as to the appropriate classes of diuretics that should be used and their long-term efficacy, but one meta-analysis has shown a short course of a thiazide diuretic combined with spironolactone to reduce mortality and aid with lung compliance. Concurrent pulmonary hypertension may require pulmonary vasodilators such as inhaled nitric oxide or sildenafil.

**Long-Term Morbidity**

Prevention of BPD has substantial implications for reducing health care expenditures and long-term morbidity of premature infants.

*Health care costs:* Many of the comorbidities commonly linked to preterm birth are associated with increases in health care costs. High costs in the setting of BPD are primarily a consequence of prolonged respiratory support and increased length of intensive care unit stays.

*Respiratory sequelae:* Long-term respiratory sequelae of BPD may persist into childhood and even adulthood. One analysis of infants with BPD demonstrated 50% and 36% rates of re-hospitalization in the first and second years of life, respectively. Further, infants with BPD were at increased risk for pulmonary infections and reactive airway disease. Analyses of long-term respiratory outcomes suggest that subjective respiratory symptoms such as cough and wheeze continue well into adulthood. The EPICure study showed an association between extreme prematurity and reduced lung function by the age of 11. The study demonstrated an even greater reduction in lung function for children with a history of BPD.
**Neurocognitive outcomes:** Short et al. showed neurocognitive deficits on validated assessments in 8-year olds with BPD. Increased severity of BPD was correlated with poorer neurocognitive outcomes. It was also observed that Attention Deficit-Hyperactivity Disorder was more common in the BPD group than control groups. Use of post-natal steroids as part of therapy for BPD was also associated with poorer outcomes.

17,18

**Disease Etiology and Pathogenesis**

Studies done in animal models and in human subjects have shown that the etiology of BPD is multi-factorial.

**Genetic susceptibility:** Previous studies have discovered a genetic component to the etiology of BPD. A detailed discussion of these studies and techniques used to elucidate genes of interest can be found in Chapter 2.

**Antenatal Factors:** There is also evidence that environmental factors play a role in BPD pathogenesis. Intrauterine infection, or chorioamnionitis, has been associated with the development of BPD. Intra-amniotic infection drives pro-inflammatory changes including increased cytokine concentrations that may predispose infants to prematurity as well as impaired lung maturation.4 *Ureaplasma urealyticum* is a frequently-studied bacterium associated with BPD.19

**Postnatal Factors:** Though invasive ventilation and supplemental oxygen are mainstays in the early management of preterm neonates, paradoxically, prolonged exposure to these therapies is a known cause of BPD. Invasive mechanical ventilation damages lung tissue by several mechanisms. Mechanical stretch from over-inflation, atelectasis from low tidal volumes, and predisposition to infection from foreign body colonization contribute to
excess inflammation in the developing lungs. Inflammation and the subsequent tissue remodeling ultimately lead to a disruption of normal lung architecture. Prolonged exposure to hyperoxic conditions may damage lung tissue through free radical formation.

**Minimizing Invasive Ventilation**

Given the known impact of prolonged ventilation on the incidence of BPD, reduction of ventilator trauma early in post-natal life remains an important subject for inquiry. Two large trials, the Continuous Positive Airway Pressure or Intubation at Birth (COIN) Trial and the Surfactant, Positive Pressure, and Oxygenation Randomed (SUPPORT) Trial, investigated the incidence of BPD or death in infants randomized shortly after birth to either nasal continuous positive airway pressure (NCPAP) or endotracheal intubation. The investigators of the COIN Trial found that infants randomized at five minutes of life to CPAP had significantly lower rates of death or oxygen use by day of life (DOL) 28 and spent fewer days on the ventilator overall. However, there was no significant difference in the rates of BPD or death between the CPAP group and the intubation group at 36 weeks’ PMA. Nearly half (46%) of infants in the CPAP group required intubation by DOL 5. Additionally, infants initially receiving CPAP had higher rates of pneumothorax. The SUPPORT Trial randomized infants to NCPAP or intubation with surfactant administration by one hour of life. There was no significant difference in the primary outcome of BPD or death at 36 weeks’ PMA. When stratified by gestational age, post-hoc analysis did reveal a significant reduction in BPD or death at 36 weeks’ GA for infants born between 24 weeks 0 days and 25 weeks 6 days.
The CPAP group also had lower rates of death and intubation by DOL 7 and fewer days of ventilation overall.  

Many premature infants are too critically ill at birth for NCPAP to be a viable option. For those infants, reducing the number of days spent invasively ventilated is crucial to preventing or minimizing adverse outcomes. In a study of infants < 1500 g invasively ventilated up to DOL 7, DOL 14, and beyond DOL 15, Gonzaga et al. demonstrated that the hazard of developing BPD is correlated directly with prolonged invasive ventilation. Though invasive ventilation beyond week one has been associated with development of BPD, few investigators have studied the impact of ventilator use at different points during the first week of life.

One such study, by Dumpa et al., demonstrated that predominant use of non-invasive forms of ventilation (NCPAP or non-invasive intermittent positive pressure ventilation (NIPPV)) during the first week of life significantly reduced the incidence of BPD compared to endotracheal intubation. In addition, this study showed that babies extubated to NIPPV by DOL 7 were at a reduced risk of BPD or death compared to babies who remained invasively ventilated through the end of the first week of life. The sample size was not adequate, however, to study the effect of reintubation on BPD. Concerns about potential reintubation continue to play a major role in the decision to extubate despite a lack of evidence directly linking reintubation with poor long-term outcomes.
STATEMENT OF PURPOSE/ SPECIFIC AIMS

Based on the existing literature and clinical experience, we predicted that early extubation would reduce the incidence of BPD. Given that many early attempts at extubation fail, we also examined whether neonates who fail early extubation and need to be reintubated would still have a decreased incidence of BPD as compared to babies successfully extubated later in life.

To explore these hypotheses, we aim to:

(1) Identify infants at the greatest risk of developing BPD who were hospitalized at Yale-New Haven Children’s Hospital between January 2006 and December 2011.

(2) Collect retrospective clinical data about these infants, particularly with respect to mechanical ventilation and DOL of extubation.

(3) Use clinically accepted criteria to group infants by outcomes of BPD, no BPD, or death.

(4) Use appropriate statistical methods to assess for statistical differences in the hazard of BPD or death across study groups.
METHODS

Patient Population

Retrospective clinical data were collected on all infants hospitalized in the Yale-New Haven Children’s Hospital Neonatal Intensive Care Unit (NICU) between January 1, 2006 and December 31, 2012. Criteria for inclusion were all of the following: GA ≤ 28 weeks, BW ≤ 1000 g, and intubation on DOL 1. Infants who died on DOL 1, lacked any attempts at extubation, or were born with significant congenital cardiopulmonary anomalies were excluded. If a pair of twins met the inclusion criteria, one infant from the pair was randomly included to preserve the independence of study subjects for analysis and to avoid inclusion bias by birth order. Outcomes for the study included diagnosis of BPD, as defined by NIH consensus,\(^2\) and a composite outcome of BPD or death from any cause prior to 36 weeks’ PMA.

Data collection

Data were collected from the Yale NICU database and supplemented with the electronic medical record. The study was approved by Yale University’s Human Investigation Committee.

Demographic data included maternal, fetal, perinatal, and neonatal variables. Maternal data included race, prenatal care, and antenatal steroid administration. As in previous studies, antenatal steroids were only recorded if given at least 12 hours prior to delivery \(^{24}\). Fetal variables included multiple gestation, gender, GA, and BW. Perinatal variables were collected on mode of delivery, APGAR scores at 1 and 5 minutes post-partum, delivery room resuscitation details including use of supplemental oxygen (O\(_2\)), bag and mask ventilation (BMV), ETT intubation, chest compressions, and/or
epinephrine, and diagnosis of respiratory distress syndrome (RDS) documented by clinical and radiographic findings. Use of NCPAP prior to ETT intubation was also documented.

Neonatal variables included surfactant use either at delivery or at any point during hospitalization and data on common co-morbidities and treatment where applicable. These included retinopathy of prematurity (ROP), intra-ventricular hemorrhage (IVH), sepsis documented by positive blood culture, necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA) and treatment with indomethacin, ibuprofen, and/or ligation, gastrointestinal perforation, periventricular leukomalacia (PVL), and pneumothorax or pneumomediastinum documented by chest radiograph. Post-natal steroid use was also recorded.

Definitions of neonatal comorbidities used were previously outlined by our research group. ROP stage was assigned using the international classification scheme. IVH was staged using the system described by Papile. Cases of NEC satisfying stage II or greater by Bell’s criteria were included. Diagnosis of PDA was confirmed by echocardiography. PVL was identified by observation of echogenicity and cystic lesions in the periventricular white matter on cerebral ultrasound.

During the study period, there were standard guidelines in place in our NICU for delivery room (DR) management, intubation, surfactant administration, extubation, non-invasive ventilation support, re-intubation and postnatal steroid use. In brief, our approach after initial stabilization in the DR was to support neonates on NCPAP, to a maximum 6 cmH₂O, in the NICU. If requiring ≥0.35 FiO₂, infants were intubated and surfactant administered, if indicated for those with RDS (based on clinical/radiographic
criteria)\textsuperscript{30}. All infants were started on caffeine, prior to any extubation attempt, usually within the first 24h of life, and continued till they were off respiratory support. Infants were weaned on the ventilator settings and extubated to NIPPV, as per standard practice in our NICU, based our published guidelines\textsuperscript{31}. Specific instructions were in place in our NICU for NIPPV use, and specific criteria were used for re-intubation, if required\textsuperscript{31}. Use of postnatal steroids was mostly restricted to a 5-day course of dexamethasone to facilitate extubation, utilized after 4 weeks of postnatal life. As the infants improved, as per our published guidelines, they were weaned off NIPPV, to NCPAP and then nasal cannula\textsuperscript{31}. All infants underwent an oxygen-reduction test at 36 weeks PMA to assess the presence and/or severity of BPD.

Variables concerning the infant’s postnatal course were collected from medical and respiratory therapy records, such as DOL of first attempted extubation, need for reintubation, DOL of all subsequent extubation and reintubation attempts, number of days on each ventilator modality, number of days receiving supplemental O\textsubscript{2}, and length of stay in the NICU. Supplemental O\textsubscript{2} was defined as FiO\textsubscript{2} > 0.21.

Infants were divided into three study groups by DOL of first attempted extubation: DOL 1-3, DOL 4-7, and DOL 8+. These groupings were based on those previously studied by Dumpa et al\textsuperscript{24}. Extubation attempts were defined as purposeful attempts to remove the ETT. We included accidental extubation only if a decision not to replace the ETT was documented. Reintubation was defined as non-elective replacement of ETT at any time after extubation. Reintubation for elective or semi-elective surgical procedures such as ROP laser surgery, hernia repair, or bowel re-anastomosis were not included, as the majority of these cases occurred after 36 weeks’ PMA. Additionally,
elective reintubation has not been shown to affect respiratory outcomes in infants previously diagnosed with BPD\textsuperscript{32}.

**Statistical Analyses**

Baseline maternal, fetal, perinatal, and neonatal characteristics were compared across our three study groups using chi-squared tests or Fisher’s exact test, where appropriate, for categorical variables and one-way ANOVA for continuous variables. We used chi-squared tests or Fisher’s exact test to compare crude rate of BPD/death between the three groups. We used these same tests to compare crude rates of BPD/death based on DOL of extubation and need for reintubation, as well as the breakdown of BPD severity by extubation group.

We used Kaplan-Meier curves to model the unadjusted probability of survival without BPD/death between the DOL of first extubation and 36 weeks’ PMA. We set time “0” at DOL of first extubation, which varied by infant, and modeled the time course from extubation to an outcome ("BPD," “no BPD,” or “death”). For infants hospitalized at our institution through 36 weeks PMA, data collection was discontinued at 36 weeks’ PMA and outcomes were assessed at that time. An outcome of “death” was assigned at the DOL that the event occurred prior to 36 weeks’ PMA. The log-rank test was used to compare the probability between the three groups of disease-free survival as a function of time from DOL of first extubation to an outcome of BPD/death.

In a minority of cases, infants were discharged from the NICU either to home or to another institution prior to 36 weeks’ PMA (N = 31). If at time of discharge prior to 36 weeks’ PMA the infant had already received ≥ 28 days of supplemental oxygen, an outcome of BPD was assigned at the DOL equivalent to 36 weeks’ PMA (N=15). Sixteen
infants who were discharged or transferred prior to 36 weeks’ PMA had not received at least 28 days of supplemental oxygen by time of discharge. Three of these infants were discharged to home because the infant was deemed healthy enough for discharge; the likelihood of a subsequent BPD diagnosis was miniscule and these infants were designated as “no BPD.” For the 13 infants transferred to outside institutions prior to 36 weeks PMA, we used clinical judgment, including DOL of transfer, and status at time of transfer, including cumulative days of O\textsubscript{2} therapy, ventilator modality, and FiO\textsubscript{2} at time of transfer to assign the most probable outcome at 36 weeks’ PMA. Based on clinical judgment, we assumed that their clinical course at another institution would categorize eight as “no BPD” and five as “BPD.” To assess for potential bias based on transfer of infants and use of clinical judgment to designate outcomes for a minority of subjects, we also performed the proportional hazards analyses discussed below with these 13 infants omitted.

Cox proportional hazards models were used to calculate the unadjusted hazard of developing one of the primary outcomes based on DOL of extubation. Cox proportional hazards regression was used to model the hazard of developing BPD and BPD or death by adjusting for potential confounders and known contributors to BPD and/or death. Covariates were selected a priori using a combination of clinical judgment and statistical testing. They included antenatal steroid use, race, gender, multiple gestation, GA, APGAR at 5 minutes, mode of delivery, delivery room resuscitation efforts, RDS, surfactant use, ROP, IVH, NEC, sepsis, PDA, bowel perforation, PVL, pneumothorax, pneumomediastinum, and need for reintubation. Variables inherent to the definitions of the study groups or the definitions of BPD, such as days intubated, days on supplemental
O₂, or post-natal steroid use, were excluded. Given the degree of correlation between GA and BW, we compared models fit with each variable using Akaike Information Criterion (AIC) values and concluded that GA produced a better-fit model than BW, though both models produced similar estimates. To evaluate whether reintubation modified the association between DOL at extubation and development of BPD or death, we included interaction terms between extubation DOL and reintubation in our model.

Data are shown as hazard ratios (HRs) with 95% confidence intervals (CI). A p-value of 0.05 was used to determine statistical significance.

For analysis of patient demographics and rates of reintubation, we used GraphPad PRISM software, Version 6.0a (GraphPad Software Inc., San Diego, CA). For Kaplan-Meier analysis and proportional hazards modeling, we used SAS software, Version 9.3 for Windows (SAS Institute Inc., Cary, NC). For Kaplan-Meier analysis, we used the `lifetest` procedure. For unadjusted and adjusted proportional hazards modeling, we used the `phreg` procedure. To study the interaction of extubation DOL and reintubation, we used the `phreg` procedure with customized contrast statements.

**Division of Efforts**

The student was responsible for selection of study subjects, data collection and analysis of data. Statistical advice was provided by James Dziura, PhD (Yale Center for Analytical Sciences) and Emily Bucholz, MPH/PhD candidate (Yale School of Public Health). Our statistical advisors offered advice regarding which procedures to use and how to develop the initial code for the SAS software. The student then used the code provided to analyze the data and apply changes to the code when necessary.
RESULTS

Patient Categories

After applying inclusion and exclusion criteria, the final cohort included 262 infants (Supplemental Fig. 1). Of these, 101 (38.5%) were extubated between DOL 1-3, 41 (15.6%) were extubated between DOL 4-7, and 120 (45.8%) were extubated DOL 8+.

Patient Characteristics

Table 1 shows the characteristics of the sample by DOL at extubation. There were no significant differences in maternal variables. Multiple gestation and RDS were significantly more common in the late extubation group. The babies who were extubated earlier tended to be older and heavier, with higher APGAR scores at 5 minutes. Comorbidities including IVH, ROP, and PDA occurred with greater frequency in the late extubation group. Infants who were extubated later were significantly more likely to have received postnatal steroids. Additionally, infants who were extubated later had longer hospital stays.

Unadjusted rates of BPD and BPD or death increased with duration of initial intubation (Table 2A). Forty-seven (46.5%) babies extubated between DOL 1-3, 31 (75.6%) babies extubated between DOL 4-7, and 112 (93.3%) babies extubated DOL 8+ were diagnosed with BPD, respectively. Additionally, 11 infants extubated between DOL 1-3, 1 infant extubated between DOL 4-7, and 4 infants extubated DOL 8+ died prior to 36 weeks’ PMA.

Rates of reintubation did not differ significantly between the three groups (Table 2A). Unadjusted rates of BPD and BPD or death were higher among reintubated infants in the DOL 1-3 and DOL 4-7 study groups compared to non-reintubated infants (Table
2B), but this only achieved statistical significance for the infants extubated between DOL 1-3 (BPD: \( p < 0.05 \); BPD or death: \( p = 0.0004 \)). This did not hold true for infants extubated between DOL 4-7 (BPD: \( p = 0.008 \); BPD or death: \( p = 0.05 \)) or DOL 8+ (BPD: \( p = 0.4 \); BPD or death: \( p = 1.0 \)). We did not identify any significant differences in the severity of BPD by DOL of extubation (Table 3).

Unadjusted Time to BPD or Death

Kaplan-Meier survival curves for our cohort modeled the probability of BPD/death-free survival following the DOL of initial extubation (Fig. 1A-B). The BPD plot (Fig. 1A) shows a significant difference in BPD-free survival across study groups (\( p < 0.0001 \)). The plot shows that infants extubated on DOL 8+ are more likely to develop BPD in a shorter period of time following initial extubation than infants extubated DOL 1-3 or DOL 4-7. This reflects the fact that these infants are extubated later in life, far closer to 36 weeks’ PMA than infants in the other study groups, and are developing BPD at a higher rate. This difference is also seen between the curves for infants extubated DOL 1-3 and DOL 4-7. For the BPD-only analysis, the 16 infants who died prior to 36 weeks’ PMA were censored at DOL of death, represented by a hash mark on the plot. All other infants with an outcome of “no BPD” or “no BPD or death” were censored at 36 weeks’ PMA, also represented by a hash mark. The BPD or death plot (Fig. 1B) also shows significant differences in event-free survival over time (\( p < 0.0001 \)). The early downward slope in the DOL 1-3 curve reflects the 11 deaths in the DOL 1-3 group in the first weeks of life. The curve for the DOL 1-3 group subsequently plateaus, showing a greater probability of event-free survival over time than the other study groups, in particular DOL 8+. Both the BPD and BPD or death plots also reflect the GA of each
infant, as the number of days to 36 weeks’ PMA is variable based on GA of each infant in addition to DOL of extubation.

The unadjusted hazard of BPD increased with greater length of initial intubation (Table 4A). Infants who were first extubated between DOL 4-7 had an increased, though not significant, risk of developing BPD compared to babies who were first extubated between DOL 1-3 (p = 0.09). Extubation on DOL 8+ was associated with a significantly increased risk of BPD compared to extubation between DOL 1-3 (p < 0.0001) or extubation between DOL 4-7 (p < 0.0001). Similar results were observed when the composite outcome of BPD or death was modeled (Table 4B). Extubation between DOL 4-7 was associated with a non-significantly increased hazard compared to extubation between DOL 1-3 (p = 0.3). Extubation on DOL 8+ was associated with a significantly increased hazard compared to extubation between DOL 1-3 (p < 0.0001) or between DOL 4-7 (p < 0.0001).

**Adjusted Time to BPD or Death**

After adjustment for maternal and infant characteristics, differences in outcomes between extubation groups became more pronounced (Tables 4A and 4B). Extubation between DOL 4-7 was associated with an increased hazard of developing BPD compared to extubation between DOL 1-3 [HR (95% CI) 1.7 (1.0-2.8), p < 0.05], but not significantly so with BPD or death [1.3 (0.8-2.2), p=0.3]. Extubation on DOL 8+ was associated with a significantly increased hazard of BPD and BPD or death compared to extubation between DOL 1-3 [BPD: 16.9 (10.5-27.1), p<0.0001; BPD or death: 10.7 (7.0-16.5), p<0.0001] or extubation between DOL 4-7 [BPD: 10.0 (6.1-16.3), p<0.0001; BPD or death: 8.1 (5.0-13.0), p<0.0001].
To evaluate whether reintubation modified the association between time at first extubation and development of BPD or death, we examined interactions terms in our model. The predictor variable was DOL of first extubation and the modifier was reintubation. Successful first extubation between DOL 4-7 was associated with an increased hazard of BPD when compared to unsuccessful extubation between DOL 1-3 but was not statistically significant \([\text{BPD}}: 1.4 (0.5-4.0), p = 0.6; \text{BPD or death}}: 1.0 (0.3-2.9), p = 1.0\]. Successful first extubation on DOL 8+ was associated with a significantly increased hazard of BPD and BPD or death compared to unsuccessful extubation between DOL 1-3 \([\text{BPD}}: 25.0 (13.4-46.5), p < 0.0001; \text{BPD or death}}: 12.0 (6.8-21.1), p < 0.0001\] or unsuccessful extubation between DOL 4-7 \([\text{BPD}}: 14.4 (7.8-26.8), p < 0.0001; \text{BPD or death}}: 9.5 (5.2-17.3), p < 0.0001\].

As described in the methods, cox proportional hazards modeling and adjusted analyses of interactions terms were also performed after omission of the 13 infants transferred or discharged prior to 36 weeks’ PMA who did not meet criteria for BPD upon discharge. In this separate analysis, the magnitude and statistical significance of the hazard ratios were qualitatively similar.
DISCUSSION

Our retrospective analysis demonstrated that early extubation of preterm neonates is associated with a significantly reduced hazard of BPD when compared to extubation later in life. Importantly, we also show that babies who failed early extubation and needed to be reintubated remained at a lower hazard of BPD than babies who were first extubated later in life and did not need to be reintubated. Delayed extubation was also associated with increased hazard of BPD or death, though the association was not as strong as for BPD alone.

Compared to the previous literature, our study takes a novel approach by studying the interaction effect crossing the variables extubation DOL and reintubation to model the risk of BPD/death. In addition, we used a different statistical approach than previous work in this area. The use of survival analysis allowed us to model time to BPD and BPD or death and to account for censoring. This was useful, as time to BPD and BPD or death vary by extubation DOL and also by GA.

Our study builds upon previous work done in the field. We confirmed the conclusion by Dumpa et al that delayed extubation in the first week of life is associated with increased risk of BPD/death. This observation is also consistent with studies in animal models. Thomson et al have shown that preterm baboons extubated to NCPAP at DOL 5 needed more oxygen and had a greater degree of hypercapnea than baboons extubated at 24 hours of life. An equal number of animals in both groups required reintubation, but those in the delayed extubation group had more reintubation events on average and spent more cumulative days on the ventilator.
Few studies have looked at the effect of early extubation on the rate of reintubation and the rate of BPD. In a retrospective analysis, Booth et al showed success rates of 66% of extubations to NCPAP on DOL 1 and 80% on DOL 2. However, the study lacked an adequate sample size to predict success for extubations after DOL 2. Booth et al observed a statistically significant difference in rates of BPD for infants extubated by DOL 2 compared to those still on conventional ventilation. This result correlates to the reduced hazard of BPD we found in our DOL 1-3 study group, but the Booth et al study did not analyze reintubation with respect to BPD. In a randomized control trial, Danan et al selected preterm infants for extubation immediately or after 36 hours of life. There was no significant difference in reintubation rates between groups. Rates of BPD at 36 weeks’ PMA were also not significantly different between the groups. We also showed no significant difference in extubation failure based on DOL of first extubation. Both of Danan et al’s study groups would fit within our DOL 1-3 study group, which may account for the absence of difference in BPD between groups. The differences we observed for BPD across study groups may be further attributed to our use of the NIH Consensus to define BPD and our larger sample size. One unpublished study by Robbins et al showed an inverse correlation between DOL of first extubation and BPD. Using linear regression and correlation analysis, this group showed a significant correlation between DOL of extubation and need for reintubation, but found no association between need for reintubation and BPD.

Predicting extubation readiness in neonates in order to reduce extubation failures is important, but imprecise. Though investigators have studied different measures to predict success, there is no consensus as to the best method to predict successful
extubation\textsuperscript{37-41}. One method, the 3-minute spontaneous breathing trial (SBT), was shown to be safe and feasible by a prospective, non-randomized control study. However, outcomes for the SBT in days invasively ventilated, rates of extubation failure, and rates of BPD do not differ significantly from clinical judgment alone\textsuperscript{42}. Other efforts to reduce extubation failure vary in their efficacy. A trial of a new model of ultrathin-walled two-stage twin ETT did result in significantly fewer reintubations compared to a conventional ETT, yet did not significantly reduce the overall number of days ventilated or the rates of BPD\textsuperscript{43}.

Data on the long-term impact of reintubation in neonates are limited. Studies suggest that the long-term impact of reintubation varies based on the number of events and the technical skills of the clinician. Prolonged invasive ventilation itself is a risk factor for extubation failure\textsuperscript{25}, which supports the case for early extubation. We showed similar rates of extubation failure across all study groups. Though we found that a successful, delayed extubation was associated with an increased hazard of BPD or death when compared to a failed early extubation, further study is needed to confirm this observation. Early extubation combined with better methods to prevent extubation failure would likely improve outcomes for all infants. Aside from improving respiratory outcomes, other benefits of early extubation may include reduced exposure to painful procedures and smaller cumulative doses of analgesic medications\textsuperscript{44}.

One possible explanation for our observations is that early extubation reduces harmful systemic inflammation during the first 72 hours of life. In neonates $\leq$ 30 weeks’ GA, Chang et al measured the levels of pro-inflammatory cytokines interleukin (IL)-6, IL-8, and granulocyte-colony stimulating factor (G-CSF) between DOL 1-42.
Remarkably, levels of these three cytokines, which are commonly elevated during periods of active infection, were elevated between DOL 1-3 in infants in the absence of clinical, bacteriological or placental histological evidence of infection. These first few days of postnatal life may represent a critical temporal window of a propensity to an exaggerated inflammatory response in neonates exposed to intensive care. It may be that extubation during this critical period reduces the burden of inflammation and subsequent lung damage even if the infant is eventually reintubated. Most studies of pulmonary biomarkers have shown an early increase in pro-inflammatory cytokines from tracheal aspirate samples obtained from infants subsequently developing BPD. Given the major drawback in these studies of the absence of samples from non-intubated infants, further study is needed to sequentially track inflammatory markers (perhaps, in nasopharyngeal secretions) in response to invasive versus non-invasive ventilation to test the speculation.

Our study has limitations. Since our study is retrospective, our conclusions are only observational. Though much of our data were provided by the Yale NICU database, we relied upon the accuracy of the electronic medical record for extubation and reintubation DOL, as well as FiO2 levels, which were needed to assess our outcomes. As discussed in the methods, sixteen infants were discharged or transferred prior to the end of the study. While three of these infants were deemed healthy enough for discharge and were unlikely to ever develop BPD, thirteen infants were transferred to other facilities. Using clinical judgment, we assigned what we deemed to be the most likely outcome at 36 weeks’ PMA. Though these assignments could not be made unequivocally, we based them on clinical data including DOL and respiratory requirements at time of transfer.
Only five of these infants were transferred while still receiving oxygen therapy. We believe that this small number of infants would be unlikely to significantly affect our results. We also sought to ensure that including or excluding these 13 infants would not bias our study and found no differences in the resulting data with these infants included or omitted.

Our analysis of the effect of reintubation also has some limitations. For this study, we documented all necessary reintubations, with the exception of elective intubations for surgery after 36 weeks’ PMA. We did not perform separate analyses based on the reason for reintubation or based on the time to extubation failure. We recognize that an understanding of the reasons for and timing to extubation failure may provide important clinical information that can further elucidate our conclusions, and we aim to address these analyses in a subsequent manuscript. In our analysis, we investigated the impact of at least one reintubation on our outcomes. We did not study the effect of multiple reintubations as compared to a single reintubation. However, since the outcome of BPD and/or death was assessed at 36 weeks PMA, any potential impact of prolonged intubation due to multiple reintubations until that time point would have been incorporated.

We employed several strategies to strengthen our study and overcome inherent limitations. We used a large sample size to increase the power of our observations, focused our study on the subset of premature infants most vulnerable to BPD, and used the NIH Consensus definition to assign our outcomes. To minimize potential inaccuracies in our database, variables were confirmed by crosschecking with the electronic medical record. We utilized consistent data collection methods throughout.
In conclusion, delaying extubation beyond the first days of life, and certainly beyond the first week of life, is associated with an increased risk of BPD and BPD or death. We believe that we have controlled for the major confounding variables that could impact on the degree of illness in the infant – an important factor in keeping the neonate intubated. Anecdotal evidence in our NICU suggests that despite guidelines to attempt extubation when specific ventilation settings are reached, this is not always done. We have previously reported that fear of “growth failure” on non-invasive ventilation should not deter one to attempt an extubation \(^{48}\). Cooperation with trained respiratory therapists on therapist-driven weaning protocols shows promise in achieving more successful early extubations and decreasing the overall length of invasive ventilation \(^{49}\).

The high observed rate of reintubation for all infants in our study and the fact that reintubation was not associated with an increased hazard of BPD imply that potential need for reintubation should not necessarily impede early attempts at extubation. We recognize that other factors such as infection or need for surgery often affect the decision to extubate and that not all infants can be optimized for extubation in the first days of life. However, in the absence of extenuating circumstances, we recommend that extubation of infants who have been sufficiently weaned not be delayed, as our data show prolonged invasive ventilation to be associated with an increased hazard of BPD.
Table 1. Demographics of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Extubated DOL 1-3 (N = 101)</th>
<th>Extubated DOL 4-7 (N = 41)</th>
<th>Extubated DOL 8+ (N = 120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antenatal steroids, n (%)</td>
<td>91 (90.1)</td>
<td>36 (87.8)</td>
<td>109 (90.8)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>43 (42.6)</td>
<td>15 (36.6)</td>
<td>47 (39.2)</td>
</tr>
<tr>
<td>African American</td>
<td>35 (34.7)</td>
<td>18 (43.9)</td>
<td>41 (34.2)</td>
</tr>
<tr>
<td>Asian, Hispanic, and Other</td>
<td>23 (22.8)</td>
<td>8 (19.5)</td>
<td>32 (26.7)</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>49 (48.5)</td>
<td>23 (56.1)</td>
<td>59 (49.2)</td>
</tr>
<tr>
<td>Multiple gestation, n (%)</td>
<td>14 (13.9)</td>
<td>13 (31.7)</td>
<td>31 (25.8)</td>
</tr>
<tr>
<td>Caesarean delivery, n (%)</td>
<td>69 (68.3)</td>
<td>32 (78.0)</td>
<td>97 (80.8)</td>
</tr>
<tr>
<td>Gestational age (wks)*</td>
<td>26.02 ± 1.0</td>
<td>25.7 ± 1.2</td>
<td>25.3 ± 1.3</td>
</tr>
<tr>
<td>Birth weight (g)* ‡</td>
<td>809.4 ± 113.6</td>
<td>784.2 ± 127.4</td>
<td>694.5 ± 140.5</td>
</tr>
<tr>
<td>APGAR 1 minute**</td>
<td>5 (1-8)</td>
<td>4 (0.9)</td>
<td>4 (0.9)</td>
</tr>
<tr>
<td>APGAR 5 minutes**†</td>
<td>7 (2-9)</td>
<td>6 (1-9)</td>
<td>6 (0-9)</td>
</tr>
<tr>
<td>DR Oxygen, n (%)</td>
<td>100 (99.0)</td>
<td>41 (100.0)</td>
<td>116 (96.7)</td>
</tr>
<tr>
<td>DR BMV, n (%)</td>
<td>98 (97.0)</td>
<td>38 (92.7)</td>
<td>110 (91.7)</td>
</tr>
<tr>
<td>DR Intubation, n (%)</td>
<td>94 (93.1)</td>
<td>33 (80.5)</td>
<td>104 (86.7)</td>
</tr>
<tr>
<td>DR Chest Compressions, n (%)</td>
<td>13 (12.9)</td>
<td>12 (29.3)</td>
<td>22 (18.3)</td>
</tr>
<tr>
<td>DR Epinephrine, n (%)</td>
<td>4 (4.9)</td>
<td>2 (4.9)</td>
<td>10 (8.3)</td>
</tr>
<tr>
<td>DR Surfactant, n (%)</td>
<td>46 (45.5)</td>
<td>18 (43.9)</td>
<td>66 (55.0)</td>
</tr>
<tr>
<td>NCPAP prior to ETT, n (%)</td>
<td>9 (8.9)</td>
<td>8 (19.5)</td>
<td>15 (12.5)</td>
</tr>
<tr>
<td>RDS, n (%)‡</td>
<td>84 (83.2)</td>
<td>38 (92.7)</td>
<td>114 (95.0)</td>
</tr>
<tr>
<td>Anytime surfactant, n (%)</td>
<td>98 (97.0)</td>
<td>40 (97.6)</td>
<td>119 (99.2)</td>
</tr>
<tr>
<td>Postnatal steroids, n (%)</td>
<td>17 (16.8)</td>
<td>9 (22.0)</td>
<td>62 (51.7)</td>
</tr>
<tr>
<td>IVH, n (%)†</td>
<td>23 (22.8)</td>
<td>18 (43.9)</td>
<td>42 (35.0)</td>
</tr>
<tr>
<td>ROP, n (%)‡</td>
<td>54 (53.5)</td>
<td>25 (61.0)</td>
<td>101 (84.2)</td>
</tr>
<tr>
<td>NEC, n (%)</td>
<td>26 (25.7)</td>
<td>9 (22.0)</td>
<td>23 (19.2)</td>
</tr>
<tr>
<td>Bowel Perforation, n (%)</td>
<td>6 (5.9)</td>
<td>3 (7.3)</td>
<td>18 (15.0)</td>
</tr>
<tr>
<td>PDA, n (%)‡</td>
<td>19 (18.8)</td>
<td>16 (39.0)</td>
<td>66 (55.0)</td>
</tr>
<tr>
<td>Sepsis, n (%)</td>
<td>24 (23.8)</td>
<td>13 (31.7)</td>
<td>47 (39.2)</td>
</tr>
<tr>
<td>PVL, n (%)</td>
<td>2 (2.0)</td>
<td>2 (4.9)</td>
<td>8 (6.7)</td>
</tr>
<tr>
<td>PTX or PM, n (%)</td>
<td>6 (5.9)</td>
<td>4 (9.8)</td>
<td>13 (10.8)</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>Length of NBSCU Stay (d)*†</td>
<td>85.9 ± 59.0</td>
<td>105.3 ± 44.6</td>
<td>144.5 ± 92.0</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation  
** Median (range)  
† p < 0.05  
DR, delivery room; BMV, bag and mask ventilation; NCPAP, nasal continuous positive airway pressure; RDS, respiratory distress syndrome; IVH, intra-ventricular hemorrhage; ROP, retinopathy of prematurity; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; PVL, periventricular leukomalacia; PTX, pneumothorax; PM, pneumomediastinum.
### Table 2A. Unadjusted Outcomes by Extubation DOL

<table>
<thead>
<tr>
<th>Extubation Group</th>
<th>DOL 1-3 (N = 101)</th>
<th>DOL 4-7 (N = 41)</th>
<th>DOL 8+ (N = 120)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD, n (%)</td>
<td>47 (46.5)</td>
<td>31 (75.6)</td>
<td>112 (93.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BPD or Death, n (%)</td>
<td>58 (57.4)</td>
<td>32 (78.0)</td>
<td>116 (96.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Reintubation, n (%)</td>
<td>71 (70.3)</td>
<td>33 (80.5)</td>
<td>85 (70.8)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

DOL: day of life; BPD: bronchopulmonary dysplasia.

### Table 2b. Unadjusted Outcomes by Extubation DOL and Reintubation

<table>
<thead>
<tr>
<th>Extubated DOL 1-3</th>
<th>Reintubated (N = 189)</th>
<th>Not Reintubated (N = 73)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD, n (%)</td>
<td>38 (53.5)</td>
<td>9 (30.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BPD or Death, n (%)</td>
<td>49 (69.0)</td>
<td>9 (30.0)</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extubated DOL 4-7</th>
<th>Reintubated (N = 189)</th>
<th>Not Reintubated (N = 73)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD, n (%)</td>
<td>27 (81.8)</td>
<td>4 (50.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>BPD or Death, n (%)</td>
<td>28 (84.8)</td>
<td>4 (50.0)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extubated DOL 8+</th>
<th>Reintubated (N = 189)</th>
<th>Not Reintubated (N = 73)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD, n (%)</td>
<td>78 (91.8)</td>
<td>34 (97.1)</td>
<td>0.43</td>
</tr>
<tr>
<td>BPD or Death, n (%)</td>
<td>82 (96.5)</td>
<td>34 (97.1)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

DOL: day of life; BPD: bronchopulmonary dysplasia.
Table 3. Severity of BPD by Extubation DOL*

<table>
<thead>
<tr>
<th>Extubation Group</th>
<th>DOL 1-3 (N = 38)</th>
<th>DOL 4-7 (N = 26)</th>
<th>DOL 8+ (N = 107)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild BPD, n (%)</td>
<td>21 (55.3)</td>
<td>12 (46.2)</td>
<td>41 (38.3)</td>
<td>0.16</td>
</tr>
<tr>
<td>Moderate BPD, n (%)</td>
<td>4 (10.5)</td>
<td>5 (19.2)</td>
<td>14 (13.1)</td>
<td>0.60</td>
</tr>
<tr>
<td>Severe BPD, n (%)</td>
<td>13 (34.2)</td>
<td>9 (34.6)</td>
<td>52 (48.6)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* Infants with BPD of unknown severity (transferred prior to 36 weeks’ PMA) excluded from table; DOL: day of life; BPD: bronchopulmonary dysplasia; PMA: post-menstrual age.
### Table 4A. Unadjusted and Adjusted Risk of BPD by Extubation DOL

<table>
<thead>
<tr>
<th>Extubation Group</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>DOL 4-7 vs. DOL 1-3</td>
<td>1.5 (0.9-2.3)</td>
<td>0.09</td>
</tr>
<tr>
<td>DOL 8+ vs. DOL 1-3</td>
<td>7.1 (4.9-10.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DOL 8+ vs. DOL 4-7</td>
<td>4.5 (3.2-7.3)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Table 4B. Unadjusted and Adjusted Risk of BPD or Death by Extubation DOL

<table>
<thead>
<tr>
<th>Extubation Group</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>DOL 4-7 vs. DOL 1-3</td>
<td>1.2 (0.8-1.9)</td>
<td>0.34</td>
</tr>
<tr>
<td>DOL 8+ vs. DOL 1-3</td>
<td>5.3 (3.8-7.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DOL 8+ vs. DOL 4-7</td>
<td>4.3 (2.9-6.5)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Adjusted for antenatal steroid use, race, gender, multiple gestation, gestational age, APGAR at 5 minutes, mode of delivery, delivery room resuscitation efforts, respiratory distress syndrome, surfactant use, retinopathy of prematurity, intraventricular hemorrhage, necrotizing enterocolitis, sepsis, patent ductus arteriosus, bowel perforation, periventricular leukomalacia, pneumothorax, pneumomediastinum, and need for reintubation.

BPD: bronchopulmonary dysplasia; DOL: day of life; HR: hazard ratio; CI: confidence interval.
Figure Legend

**Figure 1.** Unadjusted Kaplan-Meier Survival Plot by Extubation day of life (DOL) and probability of no bronchopulmonary dysplasia (BPD) (**1A**) or no BPD or death (**1B**) in the 3 categories of infants.
Figure 1A. Unadjusted Kaplan-Meier Survival Plot by Extubation DOL (BPD)

DOL: day of life; BPD: bronchopulmonary dysplasia; PMA: post-menstrual age; * Censored subjects include infants with “no BPD” at 36 weeks’ PMA or infants who died prior to 36 weeks’ PMA.
Figure 1B. Unadjusted Kaplan-Meier Survival Plot by Extubation DOL (BPD or Death)

DOL: day of life; BPD: bronchopulmonary dysplasia; PMA: post-menstrual age; * Censored subjects include all surviving infants with “no BPD” at 36 weeks’ PMA.
Supplemental Figure Legend

Supplemental Figure 1. Flow chart showing selection of our study cohort based on inclusion/exclusion criteria.
**Supplemental Figure 1.** Flow chart of selection of our study cohort.

- **Neonates ≤ 28 weeks’ gestational age AND ≤ 1000 g**
  - **N = 490**

- **Patients Excluded (N=192)**
  - Died DOL 1
  - Not intubated DOL 1
  - Never extubated
  - Major congenital comorbidities

- **Patients Included**
  - **N = 298**

- **Missing or erroneous data (N=11)**

- **Randomized exclusion of twins (N=25)**

- **Final Cohort**
  - **N = 262**

DOL: day of life.
GENETIC RISK FACTORS FOR BRONCHOPULMONARY DYSPLASIA (BPD).
Jessica Berger and Vineet Bhandari, Section of Neonatology, Department of Pediatrics,
Yale University, School of Medicine, New Haven, CT.

BPD is a complex respiratory morbidity affecting premature infants. The etiology is multifactorial, but several studies have shown BPD to have a genetic component. Few studies have successfully identified and confirmed specific single nucleotide polymorphisms (SNPs) associated with an increased risk of BPD. The objective of this study is to collect samples for future sequencing for confirmation of specific SNPs as a replication cohort. Infants born at \( \leq 32 \) weeks’ gestational age were identified from neonates at Yale-New Haven Children’s Hospital and collaborating institutions. Genetic samples were collected by buccal swab from cases with BPD and controls. Comprehensive medical histories were collected for future genotype-phenotype correlation. 321 infants were sampled for inclusion in our study. Of these 321, 195 are BPD cases and 126 are healthy controls. Significant differences between cohorts were observed for race, gestational age, birth weight, APGAR scores, development of several common neonatal comorbidities, number of days of oxygen therapy, and number of days invasively and noninvasively ventilated. Genetic samples collected are reflective of the demographics of the neonatal population in the ICU setting. Given the racial heterogeneity of this population, the plan remains to use these samples for confirmation of specific SNPs reported by other investigators.
INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a complex respiratory process that affects premature infants. As previously described, the etiology of this disease is known to be multifactorial, with a significant component attributed to an infant’s genetic composition.

Parker et al, in a population of 108 very low birth weight identical twins, showed an adjusted odds ratio of 12.3 (p < 0.001) for developing BPD in a second twin if the other twin is diagnosed, adjusting for birth order, APGAR score, and other potential confounders. Subsequently, Bhandari and Gruen examined concordance of BPD in mono- and di-zygotic twins, showing heritability rates of up to 58.4%. Lavoie et al independently confirmed the findings of Bhandari and Gruen using similar methodology and the updated NIH Consensus Definition of BPD. This group showed a 79-82% heritability of BPD, specifically for moderate to severe BPD.

These early studies identified a genetic component to the etiology of BPD that persisted after adjustment for key demographic and clinical factors. Subsequently, investigators aimed to identify specific candidate genes that might contribute to the development of BPD in the neonate. Numerous studies explored the potential role of genes for pro-inflammatory cytokines such as interleukin (IL)-4 and tumor necrosis factor (TNF), anti-inflammatory cytokines such as IL-10, and surfactant proteins. Many of these studies found no associations between single nucleotide polymorphisms (SNPs) in these specific alleles and the development of BPD in neonates. One study by Manar et al. found a slightly significant association between a particular isoleucine isoform of the gene encoding for glutathione-S-transferase-P1 (GST-P1), which degrades damaging reactive oxygen species (ROS). This result suggested that infants afflicted with BPD
were less efficient at removing ROS from the lung, thus increasing their risk for BPD. Though the results of this and other similar studies are exciting, they are mostly limited by small sample size and poor reproducibility in larger studies. Additionally, genetic variations between races may lead to different results when exploring the same genes. Wang et al, in a study of a the Chinese Han population, found statistically significant associations between SNPs of the GST-M1 and GST-T1 alleles, both of which were not significant in Manar et al’s study. The possibility remains that racial differences, the relative infrequency of certain SNPs in the population, or a combination of multiple SNPs (polygenetic effect) will make it difficult to identify single genes contributing to the development of BPD.

Given the mixed evidence from many small candidate gene studies pointing towards multiple SNPs playing a role in BPD, there is value to interrogating the entire genome in large populations. Use of genome-wide association (GWA) studies affords investigators the ability to survey the whole genome, encompassing exons and introns, for millions of SNPs. Since many SNPs are known to have high linkage disequilibrium, “tag SNPs” may be used to represent clusters of SNPs to reduce the number of SNPs scanned in a particular assay. On a population level, GWA studies use the respective frequencies of SNPs to identify significant differences between healthy subjects and those with disease phenotypes.

The use of GWA study methodology can be applied to many study designs, including case-control, cohort, prospective clinical trial, and meta-analysis. GWA studies have many advantages, including speed, affordability, and applicability to complex diseases associated with a myriad of SNPs. There are drawbacks to this type of study,
including stringent significance thresholds requiring large sample sizes. One way to approach the need for a large sample size is to scan a large discovery population and then test SNPs with significant associations in one or more replication cohorts. Though the ideal would be to identify single gene causes for diseases of interest, the challenge of using GWA studies in complex diseases is that they may yield dozens of hits with no single SNP making a significant contribution in a large number of patients.  

To date, two large GWA studies have been conducted in neonates. Hadchouel et al used DNA pooling techniques to scan two small discovery populations, of African and white descent, respectively. The most significant polymorphism discovered in these populations, a variant of the SPOCK2 gene (p = 1.66 x 10^-7), was replicated in a separate population by genotyping and also in a population of Finnish ancestry. This study group also studied mRNA levels of SPOCK2 in rat lungs in the alveolar stage of development and found increased levels of SPOCK2 mRNA in rats exposed to hyperoxia compared to normoxic controls. Based on their findings in GWA studies and in rat models, Hadchouel et al suggest that SPOCK2 may play a role in alveolar development and in the impaired alveolarization seen in BPD cases. In another GWA study, Wang et al used a large discovery population comprised of four racial groups. Using a significance threshold of 5 x 10^-8, Wang et al were unable to find a significant SNP in the discovery or replication populations. This group also investigated SNPs previously associated with BPD in other studies, including SPOCK2, but was unable to replicate the findings of previous studies. The variability in data may be attributed to racial differences in the study populations, as well as differences in sample size.
STATEMENT OF PURPOSE/ SPECIFIC AIMS

Based on previous work studying heritability patterns of BPD in monozygotic twins, it is understood that there is a significant genetic component to the etiology of BPD. We propose to utilize a large library of DNA samples from premature infants at Yale-New Haven Children’s Hospital and other tertiary care centers to validate surveillance genes identified by other investigators that contribute to the development of BPD. Our specific aims are:

(1) To identify infants with BPD (“cases”) and without BPD (“controls”) hospitalized in the NICU at Yale-New Haven Children’s Hospital and collaborating tertiary care centers who fit inclusion criteria and to collect DNA by buccal swab.

(2) To isolate DNA from buccal swab samples using established extraction protocols.

(3) To perform high-throughput sequencing of these DNA in collaboration with the lab of Dr. Richard Lifton in order to analyze the DNA from these infants. This sequencing is to be used for validation of SNPs identified by other investigators.

(4) To perform genotype-phenotype analysis on these samples to identify genes contributing to development of BPD.

Within the time frame of this study, we have completed objectives (1) and (2).
METHODS

BPD Consortium

Prior to the initiation of this study in 2005, a BPD consortium was formed to foster sharing of genetic samples and clinical information between participating centers. Participating NICUs in this GWAS include Yale-New Haven Children’s Hospital (New Haven, CT), Magee-Womens Hospital of UPMC (Pittsburgh, PA), Coastal Carolina Neonatology (Wilmington, NC), Montefiore Medical Center (Bronx, NY), Women & Infants Hospital (Providence, RI), Stony Brook Children’s Hospital (Stony Brook, NY), Cincinnati Children’s Hospital (Cincinnati, OH), and University of Kentucky Children’s Hospital (Lexington, KY). The study was approved by Yale University’s Human Investigations Committee, as well as by an equivalent committee at all participating institutions.

Patient Population

Our study population included infants of both genders and all races born at a gestational age ≤ 32 weeks. We included infants with BPD (“cases”), based on the physiologic definition at 36 weeks’ post-menstrual age (PMA) described by Jobe et al in 2001, and infants without BPD (“controls”). Consent was obtained and samples were collected only after infants reached 36 weeks’ PMA.

Previous studies and review articles have discussed the myriad benefits of studying the genetics of particular disease processes in genetically isolated populations, including Finnish populations. Given the size and racial heterogeneity of our study population, we concluded that our study would be most powerful if a genetically isolated population was used for discovery purposes and our population was used for replication
of findings. To that end, we have been in communication with the laboratory of Dr. Mikko Hallman, a neonatologist at the University of Oulu in Oulu, Finland, to use his population of Finnish neonates for a discovery population.

**DNA Collection and Extraction**

DNA was collected via buccal swab using sterile, soft, cotton-tipped swabs inserted into the oral cavity and rubbed against the buccal mucosa 10 to 20 times. One swab was used for each cheek. Upon collection, samples were stored at -20 degrees Centigrade until extraction. Research team members at participating institutions utilized similar technique for sample collection; samples were overnight mailed on dry ice to avoid degradation of genetic material.

DNA was extracted from collected buccal swabs by proteinase-k/SDS lysis with phenol-chlorophorm extraction to degrade remaining protein material. Extracted DNA was precipitated with 70% ethanol and rehydrated with Tris-EDTA buffer. DNA was then stored at 4 degrees Centigrade prior to genotyping. We used spectrophotometry to quantify DNA concentrations in each sample.

**Clinical Data Collection**

In order to perform subsequent genotype-phenotype correlative analysis, we collected extensive demographic and clinical information about participating infants and families. At the time of initial consent, demographic information was gathered, including self-reported race. A thorough family history was obtained related to history of multiple gestations, cardiovascular disease, respiratory illness, and developmental disorders. Maternal medical history was also obtained, including questions concerning pre-natal care and pregnancy, gestational diabetes, hypertension, medications, smoking, alcohol
and drug use, miscarriages, abortions, premature births, and neonatal disease and losses if applicable. Maternal charts were consulted when necessary to confirm the diagnosis of a relevant disorder (i.e. chorioamnionitis), documentation by treating physicians, pediatricians, neonatologists, or specialists, and relevant imaging limited to X-Rays and CT scans.

Neonatal data were also collected from the electronic medical record spanning the period from birth until discharge. Delivery room data included birth weight, gestational age, multiple gestation, APGAR scores at 1 minute and 5 minutes, delivery room resuscitation modalities including oxygen therapy, bag-and-mask ventilation, and endotracheal intubation, diagnosis of respiratory distress syndrome (RDS), and surfactant use. Data from the neonate’s clinical course, including intraventricular hemorrhage, retinopathy of prematurity +/- need for surgery, patent ductus arteriosus +/- need for surgical ligation, pneumothorax or pulmonary interstitial emphysema, sepsis, and necrotizing enterocolitis, were also documented. Clinical criteria used to make these diagnoses have been described in Chapter 1 of this thesis.

The number of days on each ventilator modality and number of days of oxygen therapy were also recorded. At 36 weeks’ PMA, infants were designated as “cases” or “controls” using the NIH Consensus Definition of BPD as previously described².

Strict confidentiality regarding this patient information was maintained throughout the duration of the study.

**Genome-Wide Association Analysis**

To date, genetic and clinical data collection is ongoing and the following methods are prospective. Collaboration with the laboratory of Dr. Richard Lifton in the
Department of Genetics has been arranged to validate any surveillance genes identified in a larger discovery population. Whole genome amplification (Qiagen kit) will be used for samples that do not meet the DNA threshold for genotyping of 500 ng. For genotyping, we will use the techniques as previously described \(^6\). In brief, genome-wide genotyping will be performed using the Illumina Human 610-Quad BeadChip. Samples will be genotyped in two batches at the W.M. Keck genotyping facility of Yale University. Approximately equal number of cases and controls will be genotyped in each batch to protect against potential technical artifacts leading to differential bias in the analysis. Genotypes will be called using the automated clustering algorithm in the Illumina Genome Studio, Genotyping Module v.1.1.9. The genotyping will be performed with the Sequenom MassARRAY (MALDI-TOF) Spectrometry system at the W.M. Keck genotyping facility.

**Statistical analysis:**

**Analysis of population stratification:** We will control for potential population stratification by performing stratified Cochran-Mantel-Haenszel (CMH) analysis based on the strata defined by the cluster solution. As a confirmatory procedure, we will also adjust for two significant eigenvectors using logistic regression. For each analysis (crude, CMH, or PC-adjusted logistic), we will estimate a genomic inflation factor \(^6\).

**Genotype-phenotype correlations:** The top SNPs representative of the independent loci will be screened for associations with clinical phenotypes using age- and gender-adjusted regression models. Linear regression will be used for quantitative phenotypes and logistic regression for binary traits. The genotypes of specific SNPs will be used as
predictors and coded under an additive model. All analyses will be performed using SPSS Statistics for Windows v17.0 (SPSS, Inc., Chicago, IL).

**Division of Efforts**

Collection of samples and data for this project began in 2005 and has continued through the present date. Prior to June 2012 when this student joined the team at the Yale study site, the principal investigator, Dr. Bhandari, and other members of his research team obtained consent, collected the samples and recorded some clinical data. Haiying Meng, PhD, extracted DNA from Yale samples; these samples remain stored in the -80 degrees Centigrade freezer in the lab. At participating institutions, our collaborators obtained consent, collected the samples, and recorded clinical data on institution-specific data sheets.

Since June 2012, this student has identified appropriate subjects, obtained informed consent, collected buccal swabs, and extracted DNA using the described methods. This student produced all reagents for proteinase-k/SDS lysis and phenol-chlorophorm extraction. This student also quantified the extracted DNA using spectrophotometry. This student also compiled and organized extensive clinical data from infants previously enrolled in the study and newly enrolled infants. Additionally, this student is responsible for maintenance of Human Investigations Committee approval at the Yale site.

Once a minimum number of samples have been collected, samples will be transferred to our partners in the laboratory of Dr. Richard Lifton who will perform the genotyping and statistical analysis described above in collaboration with the W.M. Keck genotyping facility at Yale.
RESULTS

Patient Population and Characteristics

To date, 321 infants have been enrolled from Yale and partnering institutions. An additional 58 samples have been collected from first-degree relatives of study subjects, including parents, grandparents, and siblings. Of the 321 study subjects, 195 (60.7%) are “cases” and 126 (39.3%) are “controls.”

Tables 5 shows the demographics and key clinical features of the patient population sorted by BPD diagnosis. Of note, there are many significant differences between the BPD cohort and the control cohort. Infants with BPD were born at significantly lower gestational ages and birth weights and were found to have significantly lower APGAR scores at both one and five minutes in the delivery room. Additionally, racial differences were observed between the groups. The clinical courses of these groups of infants also varied, with infants with BPD also developing significantly more comorbidities, including ROP, PDA, sepsis, and pneumothorax. Infants with BPD received significantly more days of O₂ therapy, as is appropriate given the clinical definition of BPD. Those with BPD also spent more days on several ventilator modalities, including invasive ventilation (IMV, HFOV) and nasal ventilation (NIPPV, NCPAP).

DNA Quantification

Spectrophotometry was used to quantify the amount of DNA in the individual samples. Of the 206 samples extracted by this student since June 2012, 185 (89.8%) exceeded the minimum threshold of 500 ng needed for successful genotyping. These samples included swabs collected at Yale and at other institutions. Of the 21 samples that
did not meet the threshold, no single factor could be identified as the cause of low yield, though variable swabbing technique and inadequate cell collection are the most likely.
DISCUSSION

We aimed to conduct a GWA study to identify single-nucleotide polymorphisms associated with the development of BPD, a respiratory comorbidity of prematurity with known genetic causes. Within the constraints of time, we were able to collect nearly 400 samples from both neonates and their first-degree relatives. We were able to extract genetic material from these samples with a high degree of success in light of the fact that many samples had been frozen for more than one year. We also compiled a comprehensive database of clinical information about these neonates, which will be used in the future as part of genotype-phenotype correlation analysis. Given the rigid significance thresholds for this type of study, which we discussed previously, our sample size of approximately 400 is not large enough for a discovery population. To that end, we still plan on collaborating with other investigators to identify a population that is ideal for genetic discovery.

One of the leading investigators we have identified is Dr. Mikko Hallman of the Oulu University Hospital in Oulu, Finland. This study group is ideal for a number of reasons. First, Dr. Hallman has been successful in amassing a large collection of approximately 1,000 samples. Second, Dr. Hallman’s study population is derived from a genetically isolated population. As described by Arcos-Burgos and Muenke, the Finnish population is ideal for genetic studies because of the founder effect, which suggests that the population originated from a small group of individuals. It is also believed that the Finns remained geographically isolated from other European populations for nearly two millennia, leading to inbreeding and the presence of many recessive disorders in the population. Many investigators have studied the Finnish population, successfully
identifying loci for metabolic, psychiatric, and autoimmune disorders\textsuperscript{61-63}. Genetic studies in Finnish neonatal populations have also been used to show allelic variation in respiratory distress syndrome (RDS) and prematurity in general. Investigators have shown genetic variants of surfactant proteins A and C in neonates with respiratory distress syndrome\textsuperscript{64,65}. Additionally, polymorphisms in the gene coding for Toll-like receptor 4 have been associated with preterm birth in Finnish neonates\textsuperscript{66}.

One of the two previous genome-wide association studies conducted for BPD pooled DNA in the discovery population by race to eliminate confounding by race. Using one French population of African descent (N = 107) and one French population of white ancestry (N = 98), Hadchouel et al. identified a polymorphism in the gene for SPOCK2 with the most significant polymorphism achieving a p-value on the order of $10^{-7}$. This study group then used a black and white French replication population (N = 212) and a small Finnish population (N = 213) to replicate their findings. Finally, Hadchouel et al. identified elevated SPOCK2 mRNA levels in newborn rat lungs after exposure to hyperoxia\textsuperscript{57}. Though this group aimed to eliminate race as a confounder by pooling DNA into two racially distinct groups, this served to shrink an already small discovery population. As a result, they were not able to achieve a p-value on the order of $10^{-8}$, a threshold used by other studies\textsuperscript{58,60}. Quantification of SPOCK2 levels in newborn rats in the alveolar stage after exposure to hyperoxia is a useful way to confirm changes in gene expression in one animal model of BPD. However, elevation of mRNA levels does not necessary correlate with increased protein levels. Use of tracheal aspirates to measure protein levels in the cells would show a more convincing association between a polymorphism in the SPOCK2 gene and changes in phenotype.
Wang et al. also conducted a GWAS to investigate genetic variants associated with BPD in neonates. The advantage of this study was its access to a very large discovery population of 1,726 very low birth weight infants born in the state of California. Their replication population comprised 795 infants of comparable demographics and clinical characteristics. Despite access to such a large population, this study group did not identify any significant polymorphisms associated with moderate-severe BPD as compared to mild BPD or no BPD. Wang et al. note that variability in race, sample size, and other factors may have led to a negative result. Of note, several other differences exist between Wang et al.’s study and Hadchouel et al.’s study. Hadchouel et al.’s study was prospective, whereas Wang et al.’s study was retrospective and used blood samples previously collected for state-mandated newborn metabolic panels. Additionally, there was variability in the gestational ages of the populations used, both between the two studies’ discovery populations and also between Hadchouel et al.’s discovery and replication populations. Both of these studies used moderate-severe BPD as their case population, which does not reflect the current NIH consensus definition. Wang et al. also used a requirement of at least three days of positive pressure ventilation (PPV) in both cases and controls so as to control for environmental exposures, though exposure to PPV is not part of the experience for many infants without BPD.

Though our study has not yet been carried through to completion, one advantage would be the availability of a large genetically isolated population for the discovery of candidate genes to replicate in our own diverse population. With respect to our replication population, the racial demographics are grossly different from those used by
Wang et al, reflecting the differences in racial group predominance in our consortium versus that of the California group [58].

Genome-wide association studies have proven valuable in the study of many diseases with purely genetic causes or multifactorial etiologies. Though these studies have had success in diseases such as IgA nephropathy [60], success has been mixed in BPD, suggesting a complex interplay of genetic and environmental risk factors. Nevertheless, convincing evidence pointing towards the genetic contribution to BPD indicates that this is a disease area ripe for ongoing study using the most advanced technology for genetic mapping. We aim to continue this project with the objective of identifying and validating important genes in the development of such a complex and morbid disease.
Table 5. Demographics of Genome-Wide Association Study Population

<table>
<thead>
<tr>
<th></th>
<th>BPD Cases (N = 195)</th>
<th>Controls (N = 126)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antenatal steroids, n (%)</td>
<td>170 (87.2)</td>
<td>111 (88.1)</td>
<td>0.86</td>
</tr>
<tr>
<td>Race, n (%) †</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>116 (59.5)</td>
<td>54 (42.9)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Black</td>
<td>59 (30.3)</td>
<td>41 (32.5)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1 (0.5)</td>
<td>3 (2.4)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>17 (8.7)</td>
<td>19 (15.1)</td>
<td></td>
</tr>
<tr>
<td>Native American, Biracial or</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2 (1.0)</td>
<td>9 (7.1)</td>
<td></td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>102 (52.3)</td>
<td>68 (54.0)</td>
<td>0.82</td>
</tr>
<tr>
<td>Multiple gestation, n (%)</td>
<td>74 (37.9)</td>
<td>55 (43.7)</td>
<td>0.35</td>
</tr>
<tr>
<td>Caesarean delivery, n (%)</td>
<td>143 (73.3)</td>
<td>87 (69.0)</td>
<td>0.45</td>
</tr>
<tr>
<td>Gestational age (wks)* †</td>
<td>26.2 ± 1.9</td>
<td>29.5 ± 1.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Birth weight (g)* †</td>
<td>822.4 ± 243.4</td>
<td>1315 ± 333.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>APGAR 1 minute** †</td>
<td>4 (0-9)</td>
<td>7 (0-9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>APGAR 5 minutes** †</td>
<td>7 (1-9)</td>
<td>8 (0-9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>RDS, n (%) †</td>
<td>182 (93.3)</td>
<td>76 (60.3)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Anytime surfactant, n (%) †</td>
<td>184 (94.4)</td>
<td>66 (52.4)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Postnatal steroids, n (%) †</td>
<td>25 (12.8)</td>
<td>0 (0.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IVH, n (%)</td>
<td>74 (37.9)</td>
<td>42 (33.3)</td>
<td>0.41</td>
</tr>
<tr>
<td>ROP, n (%) †</td>
<td>134 (68.7)</td>
<td>15 (11.9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NEC, n (%)</td>
<td>18 (9.2)</td>
<td>10 (7.9)</td>
<td>0.84</td>
</tr>
<tr>
<td>PDA, n (%) †</td>
<td>120 (61.5)</td>
<td>20 (15.9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sepsis, n (%) †</td>
<td>90 (46.2)</td>
<td>16 (12.7)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>PVL, n (%)</td>
<td>13 (6.7)</td>
<td>3 (2.4)</td>
<td>0.12</td>
</tr>
<tr>
<td>PTX, n (%) †</td>
<td>22 (11.3)</td>
<td>6 (4.8)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Duration of invasive ventilation (d)* †</td>
<td>28.4 ± 35.2</td>
<td>2.2 ± 5.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Duration of NIPPV (d)* †</td>
<td>3.3 ± 7.5</td>
<td>1.3 ± 3.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Duration of NCPAP (d)* †</td>
<td>20.9 ± 22.9</td>
<td>4.8 ± 7.0</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Days supplemental O2 (d)* †</td>
<td>79.2 ± 47.6</td>
<td>7.3 ± 7.6</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation
** Median (range)
† p < 0.05
RDS, respiratory distress syndrome; IVH, intra-ventricular hemorrhage; ROP, retinopathy of prematurity; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; PVL, periventricular leukomalacia; PTX, pneumothorax; Invasive ventilation includes synchronized intermittent mandatory ventilation (SIMV) and high frequency oscillatory ventilation (HFOV); NIPPV, nasal intermittent positive pressure ventilation; NCPAP, nasal continuous positive airway pressure; O2, oxygen.
REFERENCES


