ORIGINAL ARTICLE

Early Pulmonary Vascular Disease in Preterm Infants at Risk for Bronchopulmonary Dysplasia

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Abstract

Rationale: Pulmonary hypertension (PH) is associated with poor outcomes among preterm infants with bronchopulmonary dysplasia (BPD), but whether early signs of pulmonary vascular disease are associated with the subsequent development of BPD or PH at 36 weeks post-menstrual age (PMA) is unknown.

Objectives: To prospectively evaluate the relationship of early echocardiogram signs of pulmonary vascular disease in preterm infants to the subsequent development of BPD and late PH (at 36 wk PMA).

Methods: Prospectively enrolled preterm infants with birthweights 500–1,250 g underwent echocardiogram evaluations at 7 days of age (early) and 36 weeks PMA (late). Clinical and echocardiographic data were analyzed to identify early risk factors for BPD and late PH.

Measurements and Main Results: A total of 277 preterm infants completed echocardiogram and BPD assessments at 36 weeks PMA. The median gestational age at birth and birthweight of the infants were 27 weeks and 909 g, respectively. Early PH was identified in 42% of infants, and 14% were diagnosed with late PH. Early PH was a risk factor for increased BPD severity (relative risk, 1.12; 95% confidence interval, 1.03–1.23) and late PH (relative risk, 2.85; 95% confidence interval, 1.28–6.33). Infants with late PH had greater duration of oxygen therapy and increased mortality in the first year of life ($P < 0.05$).

Conclusions: Early pulmonary vascular disease is associated with the development of BPD and with late PH in preterm infants. Echocardiograms at 7 days of age may be a useful tool to identify infants at high risk for BPD and PH.

Keywords: bronchopulmonary dysplasia; pulmonary vascular disease; pulmonary hypertension; echocardiography; prematurity

At a Glance Commentary

Scientific Knowledge on the Subject: Preterm infants remain at high risk for late respiratory morbidity and mortality caused by the development of bronchopulmonary dysplasia (BPD) and pulmonary hypertension (PH). Early injury to the developing lung can impair angiogenesis and alveolarization and result in simplification of distal lung airspace and the clinical manifestations of BPD and PH. However, whether early signs of pulmonary vascular disease are indicative of the subsequent development of BPD or PH at 36 weeks post-menstrual age (PMA) has not been well established.

What This Study Adds to the Field: This paper presents a longitudinal study identifying echocardiogram-derived risk factors at 7 days of age for the subsequent development of both BPD and PH. We also describe the incidence of PH at 36 weeks PMA and its relationship to BPD severity.

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Advances in perinatal medicine have dramatically increased survival of extremely premature newborns over the past few decades (1). However, preterm infants remain at high risk for late respiratory morbidity and mortality caused by the development of bronchopulmonary dysplasia (BPD), the chronic lung disease of infancy that generally occurs in preterm infants who have required respiratory support and oxygen therapy at birth (2–5). Early injury to the developing lung can impair angiogenesis and alveolarization, which results in simplification of the distal lung airspace and clinical manifestations of BPD. BPD is the most common complication of preterm birth, occurring in 68% of infants born less than 29 weeks gestation and weighing between 400 and 1,500 g as determined by the National Institutes of Health Workshop severity-based diagnostic criteria (1). BPD is characterized by persistent respiratory disease with a prolonged need for supplemental oxygen, recurrent respiratory exacerbations with frequent hospitalizations and emergency room visits (6, 7), exercise intolerance, and related respiratory problems that can extend into adulthood (8). As recently highlighted in a recent NHLBI workshop, insights into early factors that contribute to the pathogenesis of BPD are needed to develop better strategies for the prevention and treatment of at-risk premature newborns (9).

The pulmonary circulation in BPD is characterized by abnormal growth, including decreased vascular branching, an altered pattern of vascular distribution within the interstitium, and persistent precapillary arteriovenous anastomotic vessels (3, 10–14). Although the pathogenesis of BPD is complex and multifactorial (15), preclinical studies have shown that early disruption of angiogenesis in the developing lung impairs alveolarization and causes sustained abnormalities of lung structure that mimic clinical BPD (16–18). Autopsy studies examining lung tissue from infants who died with severe BPD provide further evidence that BPD is characterized by disruption of angiogenic factor expression (19, 20). Reduction of the alveolar-capillary surface area has been identified in preterm infants with BPD (21), which likely impairs gas exchange and increases the need for prolonged oxygen and ventilator therapy, worsens hypoxemia with acute respiratory infections and exercise, and may increase the risk for developing pulmonary hypertension (PH). Since the original description of BPD over 45 years ago (2), late PH has been associated with decreased survival in infants with BPD (22, 23). However, the actual incidence of PH in preterm infants with BPD is unknown and prospective studies that have systematically examined the incidence of PH in BPD are limited (24).

Therefore, to determine whether early pulmonary vascular disease contributes to the development and severity of BPD or increases the risk for late PH, we performed a prospective study designed to define the role of pulmonary vascular disease in the evolution of BPD. We hypothesized that early pulmonary vascular disease, as reflected by echocardiographic signs of PH, is associated with an increased risk for the subsequent development of BPD and its severity and late PH. To test this hypothesis, we performed a prospective study of preterm infants with echocardiographic evaluations at 7 days of age and at 36 weeks post-menstrual age (PMA) to evaluate early signs of pulmonary vascular disease, the incidence of PH at 36 weeks PMA, and its relationship to BPD status. We also sought to prospectively determine the incidence of PH in preterm infants with and without BPD and to compare the impact of different echocardiogram-derived PH criteria on the incidence of PH. Some of the results of these studies have been previously reported in the form of an abstract (25, 26).

Methods

Study Population

All data were prospectively obtained as part of an observational research study that included subjects who were enrolled between July 2006 and March 2012 at hospitals affiliated with two academic institutions (the University of Colorado School of Medicine, Anschutz Campus and Indiana University School of Medicine). The protocol was approved by the institutional review boards at each of the participating sites, and written informed consent was received from the parents or guardians of all participants.

Criteria for enrollment included gestational age less than 34 weeks and birthweight between 500 and 1,250 g. Exclusion criteria included clinical evidence of congenital heart disease (except patent ductus arteriosus [PDA], patent foramen ovale or atrial septal defect <1 cm, or ventricular septal defect <2 mm if known before enrollment); lethal congenital abnormality; and futile cases (anticipated death before hospital discharge). Subjects were required to be enrolled within 7 days of age. Infants who died (n = 20), withdrew (n = 2), or were transferred or discharged (n = 17) without undergoing echocardiogram and/or BPD assessment at 36 weeks PMA were excluded from analysis (*see* Table E1 in the online supplement).

Birthweight *z* scores were calculated using data from Oken and colleagues (27). BPD status and severity was assessed at 36 weeks PMA using a modification of the National Institutes of Health workshop definition (3) with application of the oxygen reduction test as described by Walsh and coworkers (28). Additional detail on the method for assigning BPD status is provided in the online supplement. Morbidities occurring during the birth hospitalization and length of oxygen use and mortality in the first year of life were systematically collected.

Echocardiogram Screening

Research echocardiograms were standardized at both sites before initiation of the study and were performed at 7 days of age and at 36 weeks PMA (*see* online supplement). All echocardiograms were interpreted by a single cardiologist who remained masked to subjects' clinical status to provide consistent evaluation of findings. To more rigorously define PH in this study population, evaluations of each echocardiogram included assessments of three categories of criteria for making the diagnosis of PH (*see* Table E2). The primary criteria for PH were met by any of the following findings: an estimated right ventricular systolic pressure (RVSP) greater than 40 mm Hg, RVSP/systemic systolic blood pressure greater than 0.5, any cardiac shunt with bidirectional or right-to-left flow, or any degree of ventricular septal wall flattening. Because of uncertainty regarding echocardiogram-derived definitions of PH and subjectivity of septal wall flattening (29), we evaluated two other definitions for PH: included only moderate or severe septal flattening (alternate PH criteria-1) and excluded septal flattening from the criteria altogether

(alternate PH criteria-2; *see* online supplement for details). Echocardiograms at 7 days of age were used to assess "early PH," and at 36 weeks PMA to assess "late PH."

All research echocardiograms were also separately interpreted by each institution's clinical cardiologists to provide real-time results to the attending physicians. However, these clinical interpretations were not used for research data analyses. Management decisions based on the clinical echocardiogram results and the timing of additional clinical echocardiograms were at the discretion of the attending neonatologist.

Statistical Analysis

All data were prospectively collected and managed using REDCap (Research Electronic Data Capture) (30) database hosted at the University of Colorado Denver Development and Informatics Service Center. Chi-square tests were used to assess the association between categorical variables. Distributions of continuous variables were assessed using the Shapiro-Wilk test. All outcome variables with nonnormal distributions were analyzed in simple comparisons using Wilcoxon rank sum tests or Kruskal-Wallis one-way analysis of variance for tests with more than two independent groups.

A generalized logistic regression model was developed to identify risk factors for PH at 36 weeks PMA and an ordinal logistic regression model was developed to identify risk factors for BPD at 36 weeks PMA using stepwise variable selection (*see* online supplement). Multiple linear regression analyses were used to assess the relationship between durations of oxygen use, PH, and BPD severity. Days of oxygen use were log transformed to correct for a nonnormal distribution. *P* values of less than 0.05 were accepted to indicate statistical significance. All statistics were computed using SAS v 9.4 (Cary, NC).

Results

During the study period, 277 of the enrolled infants survived and received a "late" echocardiogram at 36 weeks PMA (Figure 1). An "early" echocardiogram at 7 days of age was performed in all but 3 of the 277 infants. Characteristics of the study population are listed in Table 1. For the entire cohort, the median (interquartile

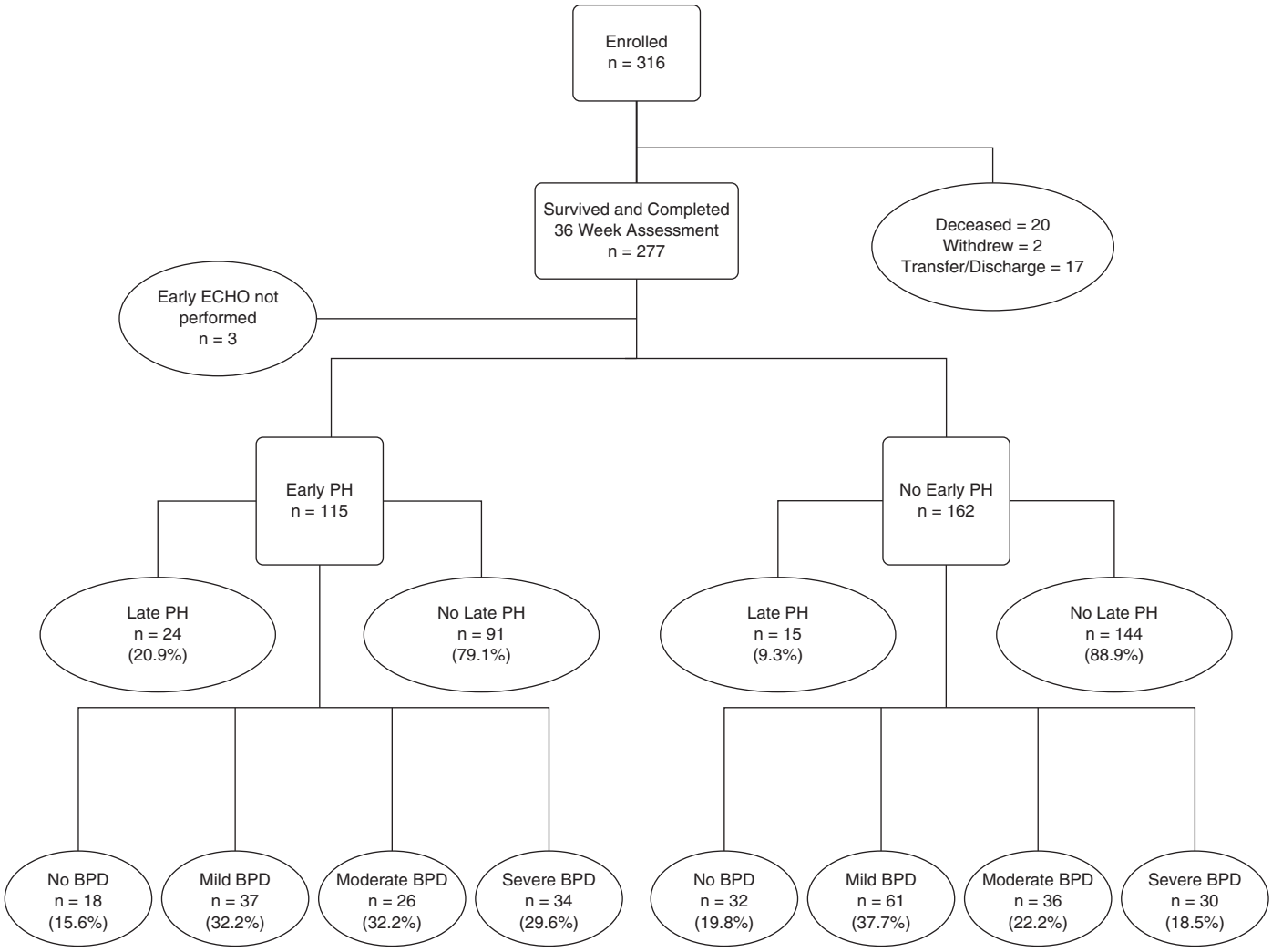

Figure 1. Enrollment and follow-up of study participants. BPD = bronchopulmonary dysplasia; ECHO = echocardiogram; PH = pulmonary hypertension.

Table 1. Subject Characteristics

	All [*n (%) or Median (IQR)*] (*N = 277*)	PH [*n (%) or Median (IQR)*] (*N = 39*)	No PH [*n (%) or Median (IQR)*] (*N = 238*)	*P* Value
Birthweight (g)	909 (750 to 1,075)	810 (695 to 1,000)	922 (765 to 1,080)	0.03
Birthweight *z* score	−0.27 (−0.82 to 0.29)			
Birthweight strata (g)				
500–749 (n = 67)	660 (600 to 695)	670 (615 to 695)	657 (600 to 690)	0.94
750–999 (n = 116)	870 (810 to 940)	832 (790 to 880)	882 (810 to 944)	0.06
1,000–1,250 (n = 94)	1,129 (1,070 to 1,190)	1,115 (1,020 to 1,150)	1,133 (1,073 to 1,195)	0.31
Gestational age	27 (25 to 28)	26 (25 to 28)	27 (25 to 28)	0.31
Sex (male)	135 (48.7)	19 (48.7)	116 (48.7)	0.99
Race				0.15
American Indian or Alaska Native	0 (0)	0 (0)	0 (0)	
Asian	1 (0.36)	0 (0)	1 (0.42)	
Black or African American	50 (18.1)	4 (10.3)	46 (19.3)	
Hawaiian or Pacific Islander	0 (0)	0 (0)	0 (0)	
White	224 (80.9)	34 (87.2)	190 (79.8)	
Other	1 (0.36)	1 (2.6)	0 (0)	
Unknown	1 (0.36)	0 (0)	1 (0.42)	
Ethnicity				0.09
Hispanic or Latino	69 (25)	14 (35.9%)	55 (23.2%)	
Not Hispanic or Latino	207 (75)	25 (64.1%)	182 (76.8%)	
Small for gestational age	29 (10.5)	9 (23.1)	38 (16)	0.27
Maternal smoking	38 (13.7)	5 (12.8)	33 (13.9)	0.86
Antenatal corticosteroids	217 (78.3)	30 (76.9)	187 (78.6)	0.82
Multiple gestation	70 (25.3)	16 (41)	54 (22.3)	0.01
Cesarean section	210 (75.8)	34 (87.2)	176 (74)	0.07
Maternal complications				
Preexisting diabetes	10 (3.6)	0	10 (4.2)	0.37
Gestational diabetes	17 (6.1)	1 (2.6)	16 (6.7)	0.48
Preexisting hypertension	31 (11.2)	1 (2.6)	30 (12.6)	0.09
Prolonged rupture of membranes	48 (17.3)	7 (18)	41 (17.2)	0.95
Chorioamnionitis	56 (20.2)	5 (12.8)	51 (21.4)	0.21
Preeclampsia	74 (26.7)	12 (30.8)	62 (26.1)	0.55
Antepartum hemorrhage	39 (14.1)	7 (18)	32 (13.5)	0.45
PDA at 7-d echocardiogram	95 (40.9)	17 (47.2)	78 (39.8)	0.41
PDA medical treatment	118 (42.6)	17 (43.6)	101 (42.4)	0.24
PDA surgical ligation	44 (15.9)	9 (23.1)	35 (14.7)	0.34
IVH (grade 3 or 4)	14 (5.1)	1 (2.6)	13 (5.5)	0.7
Pneumonia	35 (12.6)	5 (12.8)	30 (12.6)	0.97
Necrotizing enterocolitis	45 (16.3)	7 (18)	38 (16)	0.76
Sepsis	59 (21.3)	10 (25.6)	49 (20.6)	0.48
Threshold retinopathy	35 (12.6)	8 (20.5)	27 (11.3)	0.11
Days of CPAP	12 (6 to 25)	15 (7 to 30)	12 (5 to 24)	0.14
Required CPAP at 36 wk PMA	15 (5.4)	6 (15.4)	9 (3.4)	0.003
Days of MV	16 (5 to 41)	29 (7 to 52.5)	14 (4 to 36)	0.02
Required MV at 36 wk PMA	20 (7.2)	7 (18)	13 (5.5)	0.005
Length of stay (NICU)	90 (74 to 114)	90 (80 to 135)	90 (72 to 114)	0.23
Discharged on oxygen	166 (59.9)	30 (76.9)	136 (57.1)	0.01
Mortality	7 (2.5)	4 (10.3)	3 (1.3)	0.009
Total oxygen days (NICU)	79 (46 to 104)	87 (62 to 117)	75 (44 to 103)	0.027

Definition of abbreviations: CPAP = continuous positive airway pressure; IQR = interquartile range; IVH = intraventricular hemorrhage; MV = mechanical ventilation; NICU = neonatal intensive care unit; PDA = patent ductus arteriosus; PH = pulmonary hypertension; PMA = post-menstrual age.

range) gestational age at birth and birthweight of the infants were 27 weeks (25–28) and 909 g (750–1,075), respectively. Two hundred twenty-seven (82%) infants developed BPD, which included mild (n = 100; 36%), moderate (n = 62; 22%), and severe (n = 65; 23%) disease.

At the time of the 7-day echocardiogram, 108 (39%) neonates were treated with mechanical ventilation support, 111 (40%) with continuous positive airway pressure, 15 (5%) with high-flow nasal cannula (Vapotherm, Exeter, NH), 30 (11%) with nasal cannula oxygen, and eight (3%) were in room air. At 7 days of age, 115 (42%) infants met the primary criteria for early PH (Figure 2A), 97 of whom met criteria based on septal wall flattening (Table 2). The incidence of septal flattening was highest in those infants who subsequently developed severe BPD (50%), although this was not statistically significant ($P = 0.06$). Fifty-five (47.8%) infants with early PH required mechanical ventilation at 7 days of age, whereas 53 (33.3%) infants without early PH required mechanical ventilation at 7 days ($P = 0.015$). The presence of a PDA at 7 days of age was significantly associated with the development of more severe

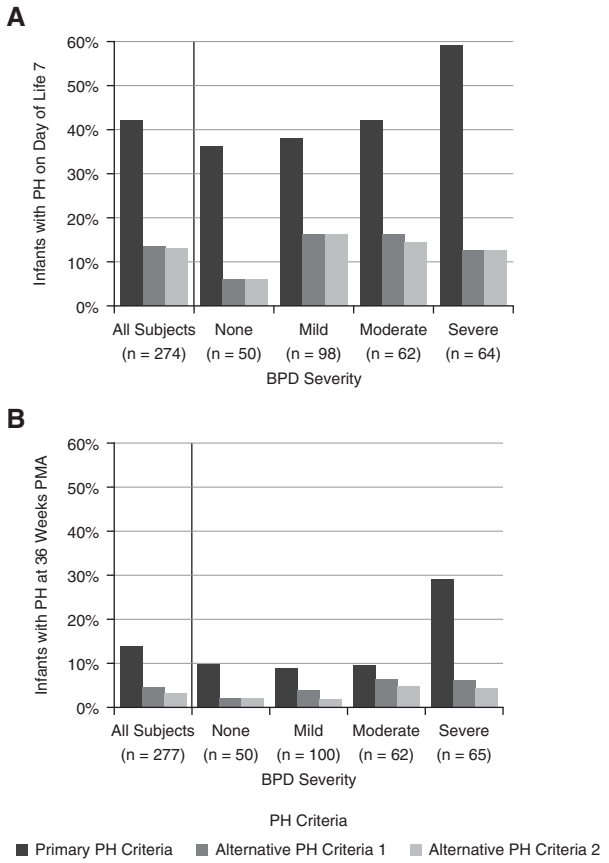

Figure 2. The proportion of subjects with pulmonary hypertension (PH) according to the primary criteria (detailed in the text) at 7 days of age displayed by bronchopulmonary dysplasia (BPD) status (*A*), and at 36 weeks post-menstrual age (PMA) (*B*). There was a significantly higher proportion of infants with PH in the severe BPD group at 36 weeks PMA compared with the other BPD status groups ($P < 0.001$).

BPD at 36 weeks PMA ($P = 0.003$). Echocardiogram evidence of PDA at 7 days was associated with early PH ($P = 0.002$) but was not associated with late PH ($P = 0.41$). Thirty-nine (14%) infants met criteria for late PH by the primary criteria (Table 2). Of the infants meeting early PH criteria at 7 days, 24 (21%) also met criteria at 36 weeks PMA. Although the proportion of infants with PH in the no BPD, mild BPD, and moderate BPD groups was relatively similar (9–10%), 29% of severe BPD infants met criteria for PH ($P < 0.001$) (Figure 2B). At 36 weeks PMA, 78% of all infants still had evidence of a patent foramen ovale/atrial septal defect, whereas 12% had a persistent PDA, but there were no significant differences in the proportion of infants with these lesions between the BPD severity groups.

The incidence of PH using alternative PH criteria and the association with BPD status for both echocardiogram evaluations are presented in Figure 2. Application of these criteria reduced the incidence of PH at 7 days of age and 36 weeks PMA. Very few patients (8% and 6% at 7 d and 36 wk PMA, respectively) had tricuspid regurgitant velocity that were deemed acceptable to reliably estimate RVSP (Table 2), thus the criteria involving RVSP could not be applied to many infants.

The sensitivity and specificity of the primary criteria applied at 7 days of age to predict the presence of PH at 36 weeks was 62% and 61%, respectively, and the positive and negative predictive values were 21% and 91%, respectively. These performance measures were much lower for both of the alternate criteria (*see* Table E3).

We further investigated clinical risk factors for the development of BPD and found that the following were significantly associated with the diagnosis of BPD: decreasing gestational age at birth (relative risk [RR], 1.12; 95% confidence interval [CI], 1.01–1.24), decreasing birthweight *z* score (RR, 1.16; 95% CI, 1.08–1.25), ventricular septal flattening (RR, 1.12; 95% CI, 1.03–1.23), multiple gestation (RR, 1.12; 95% CI, 1.01–1.24), and the Colorado center (RR, 0.89; 95% CI, 0.82–0.98) (Figure 3A; *see* Table E4). No clinical risk factors for PH at 36 weeks PMA were identified, but the 7-day echocardiographic risk factors of septal wall flattening (RR, 2.85; 95% CI, 1.28–6.33) and right ventricle dilation (RR, 9.4; 95% CI, 1.58–55.93) were associated with late PH (Figure 3B; *see* Table E4).

The characteristics of the infants with and without PH at 36 weeks PMA using the primary criteria are shown in Table 1. Patients with PH had lower birthweights ($P = 0.03$) but there were no differences in gestational age at birth between groups. Infants with late PH were more likely to be from a multiple gestation birth than infants without late PH ($P = 0.01$). Infants with PH were more likely to have received positive pressure ventilation support at 36 weeks PMA, had increased mechanical ventilation days and oxygen days, were more likely to be discharged on supplemental oxygen, and had increased mortality ($P < 0.05$).

Pharmacologic therapy for late PH was relatively uncommon in this cohort. Five infants were treated with both inhaled nitric oxide and sildenafil, and one infant was treated with sildenafil alone. Three of these infants had echocardiograms at 36 weeks PMA that were negative for signs of PH, but had subsequent clinical echocardiogram evaluations that had signs of PH later in their course. Seven infants died, including four with evidence of late PH. Of these, PH with progressive respiratory failure was the cause of the death for three, whereas the fourth subject died of sepsis. Three of these infants died in the neonatal intensive care unit (NICU), and one died in hospice care after discharge. Of the three infants who died without evidence of late PH, one died of progressive respiratory failure in the NICU, one suffered hemorrhagic stroke after discharge home, and the other died of acute aspiration after discharge home.

Evaluating the relationship between PH as determined by the primary criteria and duration of oxygen use, we found

Table 2. Echocardiogram Data

	BPD Severity					
	All [*n (%) or Median (IQR)*]	**None [*n (%) or Median (IQR)*]**	**Mild [*n (%) or Median (IQR)*]**	**Moderate [*n (%) or Median (IQR)*]**	**Severe [*n (%) or Median (IQR)*]**	***P* Value***
At 7 Days of Age	N = 274	N = 50	N = 98	N = 62	N = 64	
PDA	94 (34.3)	8 (16)	30 (30.6)	26 (41.9)	30 (46.9)	0.003
PFO/ASD	221 (80.7)	42 (84)	74 (75.5)	52 (83.9)	53 (82.8)	0.45
VSD	8 (2.9)	2 (4)	1 (1)	2 (3.2)	3 (4.7)	0.51
TRJV present	30 (8)	4 (8)	12 (12.2)	8 (12.9)	6 (9.4)	0.82
RVSP > 40 mm Hg	2 (6.7)	0	1 (8.3)	0	1 (16.7)	0.78
RVSP/sBP > 0.5	15 (50)	1 (25)	7 (58.3)	4 (50)	3 (50)	0.76
Septal wall flattening	97 (35.4)	15 (30)	28 (28.6)	22 (35.5)	32 (50)	0.06
RA enlargement	1 (0.36)	0	0	0	1 (1.6)	0.63
RV hypertrophy	5 (1.8)	1 (2)	0	2 (3.2)	2 (3.1)	0.17
RV dilation	6 (2.2)	0	2 (2)	0	4 (6.3)	0.09
Primary PH criteria	115 (42)	18 (36)	37 (37.8)	26 (41.9)	34 (53.1)	0.19
Alternative PH criteria 1	37 (13.5)	3 (6)	16 (16.3)	10 (16.1)	8 (12.5)	0.31
Alternative PH criteria 2	36 (13.1)	3 (6)	16 (16.3)	9 (14.5)	8 (12.5)	0.35
At 36 Weeks PMA	N = 277	N = 50	N = 100	N = 62	N = 65	
Gestational age at birth (range)	27 (25–28)	29 (28–30)	26.5 (26–28)	26 (25–27)	26 (24–27)	<0.0001
PDA	34 (12.3)	4 (8)	11 (11)	13 (21)	6 (9.2)	0.15
PFO/ASD	215 (77.6)	41 (82)	78 (78)	48 (77.4)	48 (73.9)	0.78
VSD	7 (2.5)	2 (4)	2 (2)	2 (3.2)	1 (1.5)	0.77
TRJV present	18 (6.5)	2 (4)	4 (4)	5 (8.1)	7 (10.8)	0.3
RVSP > 40 mm Hg	2 (11.1)	0	1 (25)	0	1 (14.3)	0.77
RVSP/sBP > 0.5	7 (38.9)	1 (50)	1 (25)	2 (40)	3 (42.9)	1
Septal wall flattening	33 (11.9)	5 (10)	8 (8)	3 (4.8)	17 (26.2)	0.002
RA enlargement	15 (5.4)	2 (4)	4 (4)	2 (3.2)	7 (10.8)	0.28
RV hypertrophy	9 (3.3)	0	1 (1)	0	8 (12.3)	<0.0001
RV dilation	25 (9)	2 (4)	5 (5)	4 (6.5)	14 (21.5)	0.004
Primary PH criteria	39 (14.1)	5 (10)	9 (9)	6 (9.7)	19 (29.2)	0.001
Alternative PH criteria 1	13 (4.7)	1 (2)	4 (4)	4 (6.5)	4 (6.2)	0.66
Alternative PH criteria 2	9 (3.3)	1 (2)	2 (2)	3 (4.8)	3 (4.6)	0.65

Definition of abbreviations: ASD = atrial septal defect; BPD = bronchopulmonary dysplasia; IQR = interquartile range; PDA = patent ductus arteriosus; PFO = patent foramen ovale; PH = pulmonary hypertension; PMA = post-menstrual age; RA = right atrial; RSVP = right ventricular systolic pressure; RV = right ventricular; sBP = systolic blood pressure; TRJV = tricuspid regurgitant velocity; VSD = ventricular septal defect.
**P* values represent results of Kruskal-Wallis one-way analysis of variance for tests.

that infants with PH at 36 weeks PMA had significantly increased duration of supplemental oxygen use compared with infants without PH (RR, 1.35; 95% CI, 1.17–1.55) (Figure 4; *see* Table E4). Gestational age at birth, BPD severity, and center were also independently associated with duration of oxygen use (*see* Table E5).

Discussion

This prospective study sought to determine the incidence of PH in preterm infants in the first week after birth and at 36 weeks PMA, and the relationship of early signs of pulmonary vascular disease with both BPD and late PH. Using a broad echocardiogram-based definition of PH, we found an overall incidence of PH of 42% at 7 days of age, and 14% at 36 weeks PMA, with the highest incidence of late PH (29%) occurring in infants with severe BPD. Significant numbers of preterm infants with none, mild, and moderate BPD (9–10% in each group) also had evidence of late PH. Applying alternate, stricter criteria for PH dramatically reduced the incidence at both time points. Early signs of pulmonary vascular disease at 7 days of age (ventricular septal wall flattening and right ventricle dilation) were associated with higher risk of late PH. Risk factors for BPD in this cohort also included lower gestational age at birth, lower birthweight *z* score, ventricular septal wall flattening at 7 days of age, multiple gestation pregnancy, and the center of admission. Late PH was also associated with prolonged use of positive pressure ventilation and supplemental oxygen therapy. These results suggest that even subtle signs early pulmonary vascular disease in the first week after birth, as evidenced by echocardiographic markers of PH, are risk factors for both BPD and late PH, and that late PH is associated with increased duration respiratory support.

Although PH has long been associated with established BPD in preterm infants, recognition of the importance of early disruption of pulmonary vascular growth in the pathogenesis of BPD and late respiratory sequelae has been growing (11, 16, 17, 19, 21). This prospective study suggests that even mild signs of PH at 7 days of age, independent of gestational age at birth, birthweight, and other maternal and NICU comorbidities, are associated with BPD severity and late PH. Although infants with severe BPD have the highest risk for late PH, it is striking that nearly 10% of infants

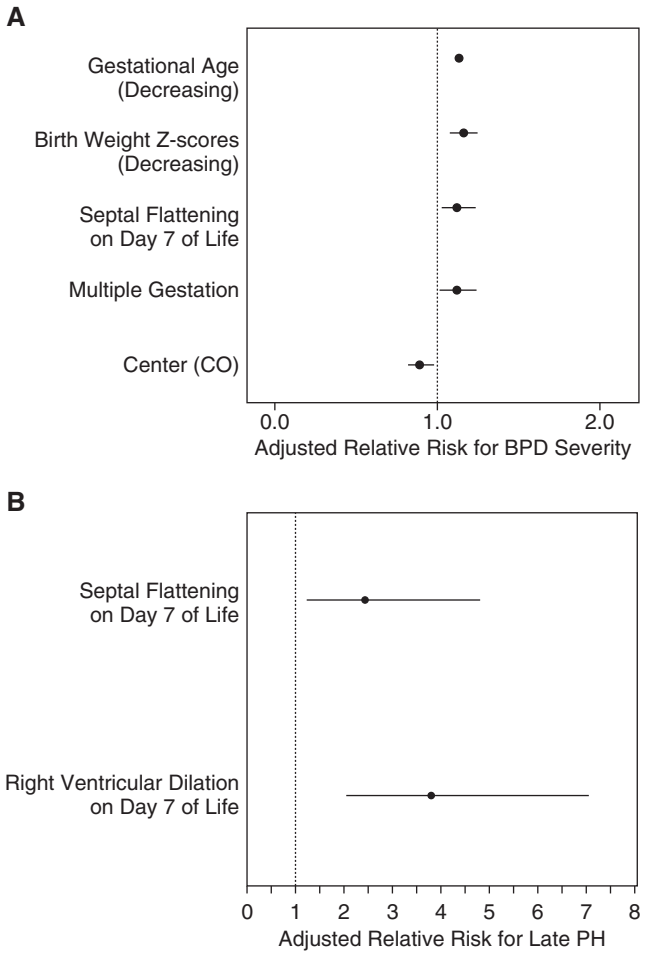

Figure 3. Adjusted relative risk for the development of bronchopulmonary dysplasia (BPD) (*A*) after adjusting for maternal, birth, and early infant factors (*see* METHODS). Gestational age at birth, birthweight *z* score, ventricular septal flattening, multiple gestation, and center were significantly associated with BPD. (*B*) Echocardiographic risk factors at postnatal day 7 (septal wall flattening and right ventricular dilation) were significantly associated with pulmonary hypertension (PH) at 36 weeks post-menstrual age.

with none, mild, and moderate BPD were also found to have late PH, and that echocardiogram findings of PH in these subjects were also strongly associated with duration of respiratory therapy.

We had previously studied a small group of infants with severe BPD and known or highly suspected PH to determine the reliability of echocardiogram measures of PH in this population (29). We found that echocardiogram measurements (including septal flattening) were fairly sensitive for diagnosing PH compared with cardiac catheterization. However, we reported that the echocardiogram was not completely reliable for determining the severity of PH as reflected by cardiac catheterization. In the present study we attempted to identify sensitive measures of PH as a screening tool for early pulmonary vascular disease and as a marker for BPD. Further studies need to determine whether this screening method can identify high-risk infants who would benefit from early intervention.

Overall, these findings support the hypothesis that early pulmonary vascular disease in preterm infants contributes to increased susceptibility for BPD and late respiratory morbidity. These data may further provide useful prognostic information for parents and caregivers, and suggest that routine echocardiographic screening may identify preterm infants who are at increased risk of late cardiopulmonary morbidities. In addition, early identification of preterm infants at greater risk for respiratory morbidities may allow the opportunity to better study novel interventions for disease prevention (9).

This is a first study of PH in preterm infants using blinded interpretation of echocardiograms with strict *a priori* definitions of PH. We found that the tricuspid regurgitant velocity was rarely of sufficient quality to adequately estimate RVSP (present in 8.7% of echocardiograms). Thus, the usefulness of the more rigorous alternate PH criteria using objective echocardiogram findings is of limited value in this population, and use of subjective findings of PH, such as ventricular septal flattening, is likely required to identify infants with PH. Although the use of a single masked cardiologist for interpretation of echocardiograms enhances the consistency of reporting PH for this study cohort, it is unclear whether these interpretations are generalizable to clinical practice because assessment of interventricular septal flattening may be variable.

As may be expected with delayed cardiopulmonary transition from *in utero* life after preterm birth, ventricular septal wall flattening was very common on postnatal day 7 (42%), but 62% of infants with evidence of PH at 36 weeks also had evidence of PH at 7 days. Conversely, absence of PH at 7 days was associated with a very low incidence of late PH (<10%) and 91% negative predictive value. The presence of right ventricular dilation at 7 days of age, which was not included in the criteria for PH, was also a risk factor for late PH, which reinforces the possibility that evidence of elevated pulmonary artery pressure at 7 days of age is associated with risk for late PH. We also found that septal flattening at 7 days of age was strongly associated with the development of BPD. These findings suggest that sustained evidence of elevated right ventricular pressure through the first week after birth may reflect early pulmonary vascular injury that increases risk for BPD. Several studies have reported an association between PDA and BPD (31–33). In this cohort, evidence of PDA at 7 days of age was associated with early PH and BPD severity, but not late PH in univariate analyses. The presence of increased left-to-right flow through a PDA could impact assessments of PH by echocardiogram, but also could lead to

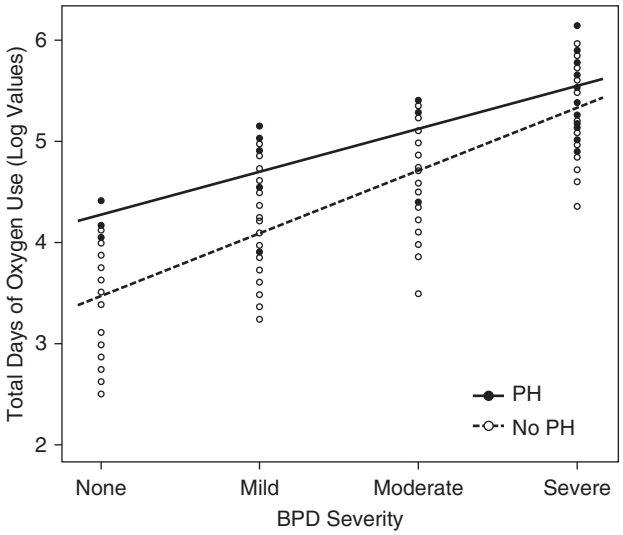

Figure 4. Infants with late pulmonary hypertension (PH) were treated with oxygen longer (from birth) than infants without late PH after adjusting for gestational age at birth, center, and bronchopulmonary dysplasia (BPD) status.

pulmonary vascular remodeling and pulmonary vascular disease. Although PDA at 7 days or treatment for PDA were not directly associated with BPD or late PH in our risk models, its impact could be encompassed by the association of early PH with BPD and late PH. Further studies are required to isolate the impact of PDA on the risk for PH in this population.

Previous studies of preterm infants with BPD have suggested that the diagnosis of PH was associated with increased mortality risk (34–36). Despite advances in the management of preterm infants over the recent decades, there is continued evidence that PH is still associated with poor outcomes (23, 37, 38). PH was often thought to be a rare complication only impacting infants with most severe respiratory disease. However, recent data indicate that angiogenesis and alveolarization are intricately related, and even mild perinatal lung injury after preterm birth disrupts the normal sequence of lung development, increasing risk for pulmonary vascular disease, including PH (10, 37, 39).

Past studies have shown that PH is commonly found in preterm infants with BPD (24, 40), with incidence rates of 25% in BPD infants and 18% in extremely low-birthweight infants, respectively. Our prospective study includes larger birthweight infants than the study reported by Bhat and colleagues (24) but reveals similar incidence rates of PH (16% for extremely low-birthweight infants). In the previous study, median age of diagnosis was 112 days (range, 93–122 d) with 65% of BPD infants who were ultimately diagnosed with PH having a "normal" echocardiogram between 4 and 6 weeks of age, suggesting that PH can be a late finding (24). PH was diagnosed as late as 232 days of age in the study by An and coworkers (40). A recent study by Mirza and coworkers described a lower incidence of PH (8% at 10–14 d of age and 4% at 36 wk PMA), but the criteria for PH diagnosis in that study was more similar to our alternate criteria-1 (41), which achieved similar rates of PH in our cohort (Figure 2). Higher death rates in preterm infants with BPD and PH compared with those without PH have been reported as well (24, 40, 42). Factors associated with late PH in these studies include lower birthweight and longer periods of respiratory support. PDA (40), infection (40), oligohydramnios (42), and small for gestational age (24, 43) have also been associated with PH; however, with the exception of low birthweight z score, we did not identify these findings as strong risk factors for BPD or PH in the present study.

This study has several potential limitations, including the lack of confirmation and quantification of PH by right heart catheterizations, which is the gold standard for diagnosis, because performing these invasive evaluations was not practical in this population. Furthermore, clinical decision-making was independent of the research team, but decisions for duration of respiratory support, including oxygen therapy, may have been influenced by clinical interpretations of the research echocardiogram results, which may have confounded our results. We identified the center of care to be a risk factor for the development of PH. Although the centers were at different altitudes, differences in the care provided at each of the hospitals were not controlled, limiting the ability to conclusively determine whether altitude was indeed the factor accounting for this difference. Additionally, this study population may not represent a consecutive birth cohort because enrollment in this study required informed consent and not all eligible patients participated.

In conclusion, we found that echocardiographic evidence of PH was present in a significant proportion of preterm infants at 36 weeks PMA and was strongly associated with the diagnosis of BPD and duration of oxygen use during infancy. Although PH was highly prevalent in infants with severe BPD, significant proportions of infants with none, mild, and moderate BPD also had evidence of PH, suggesting that PH is not solely related to the severity of lung disease. Early signs of PH at 7 days of age were common and were associated with increased risk for both BPD and late PH. Conversely, absence of PH at 7 days of age was associated with a very low risk for late PH. Overall, these findings support the hypothesis that early pulmonary vascular disease precedes the development of BPD and are strongly associated with late PH and respiratory morbidities. We speculate that early echocardiograms may be useful for the early identification of preterm infants at high risk for BPD and late PH for future preventive and therapeutic studies. ■

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