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Predictors of pulmonary hypertension in very low birth weight infants in the neonatal intensive care unit

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Predictors of pulmonary hypertension in very low birth weight infants in the neonatal intensive care unit

By

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M.D., University of Alabama Birmingham, 1999

Advisor: Anne Fitzpatrick, PhD MSCR

An abstract of

A thesis submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Master of Science

in Clinical Research

2016

ABSTRACT

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By Shilpa Vyas-Read

Approximately 8-23% of premature infants develop pulmonary hypertension (PH), and up to 50% of infants with PH die by 3 years. As a result, professional societies recommend PH screening in premature infants. However, little evidence is available to guide which patients warrant screening, and this void may result in a delay of therapeutic intervention or follow-up.

The two Aims of this proposal were: 1) Determine clinical factors associated with PH 2) Develop a predictive model for PH>30 days of life.

Infants who had the following billing codes: < 32 weeks, birth weight < 1500 grams (BW), neonatal unit, and echocardiograph had records abstracted from a data warehouse. For Aim 1, echocardiographic PH at any point, and for Aim 2, echocardiographic PH >30 days, were the primary outcomes. . For Aim 2, early predictor variables on the outcome of PH> 30 days were evaluated and receiver-operating curves (ROC) were generated to determine cut-points for sensitivity analyses. . Odds ratios and 95% confidence intervals are expressed as (OR, CI) below.

559 infants were included in the overall study, and 92 (16.5%) had PH. For Aim 1, Black race (1.79, 1.02-3.14), atrial septal defect (ASD, 2.72, 1.45-5.11) and patent ductus arteriosus (2.04, 1.19-3.49) increased the odds of PH in multivariable analyses, whereas caffeine decreased the odds (0.49, 0.29 -0.84). 321 patients were included in Aim 2, and a model of birth (birth weight, Apgar 1 minute, Black Race) and early neonatal variables (caffeine, ASD), with positive-pressure ventilation controlled was determined. The ROC showed an area of 0.766, corresponding to a validated sensitivity 80%, specificity 44%, positive predictive value 21%, and a negative predictive value 92%.

Through this proposal, we have identified new factors that contribute to PH, such as race and caffeine. Further, we have developed a model that utilizes early neonatal factors to begin to predict PH. Future steps will include external validation of the model, and implementation of a formalized screening protocol in the neonatal intensive care unit.

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INTRODUCTION

Premature birth, with its multitude of insults, results in an arrest of the normal growth and expansion of the lung capillary network, a pathology that manifests clinically as pulmonary hypertension (PH) (1-3). The incidence of PH in very low birth weight infants, which ranges between 8 and 23%, approximates that of other serious neonatal morbidities, such as necrotizing enterocolitis and periventricular leukomalacia (4-7). The exact mortality attributable to PH is unclear, but retrospective studies suggest that 50% of infants with PH may die by 2-3 years (8,9). This potential for neonatal morbidity and mortality has led professional societies such as the American Thoracic Society to recommend the implementation of routine echocardiographic screening for premature infants (10). However, few predictors for PH have been identified and guidelines for care are vague (11-14). Given the lack of understanding of which patients to screen, and when to begin screening, neonatologists are faced with the possibility of delaying detection of PH, or delaying the initiation of effective therapies for its treatment.

The completion of this study will allow neonatologists insight into which very low birth weight infants to screen for PH in the neonatal intensive care unit. The study had 2 Aims: 1) to determine clinical factors associated with the development of PH at any time during the neonatal hospital course, and 2) to determine early variables that could be used to develop a predictive model for the development of PH after 30 days of life. To complete these Aims, we utilized a comprehensive clinical data warehouse through the Children's Hospital of Atlanta to identify 559 very low birth weight infants at risk for PH. Of these, 92 infants had PH at any time by our study definition, and 50 had PH > 30 days of life. Predictive factors for the development of PH after 30 days included lower birth weight, lower Apgar scores at one minute, Black race, and a significant atrial septal

defect. Caffeine therapy decreased the odds of PH overall and after 30 days of life. Our predictive model performed modestly with a sensitivity of 84%, a specificity of 46%, a positive predictive value of 21%, and a negative predictive value of 94%.

Though the completion of this proposal, we have identified new predictors for PH in premature infants that have previously not been described, such as Black race and caffeine therapy. We have additionally taken a first step toward the development of a predictive model that can guide which patients to screen in the neonatal intensive care unit. Further steps will revolve around external validation of the model, and implementation of a screening protocol for PH in local neonatal intensive care units.

BACKGROUND

Depending on the gestational age of the infant, preterm infants may be at the end of the cannilicular or at the beginning of the saccular stage of lung development at the time of their birth. During the cannilicular stage, peripheral airways are branching rapidly, and along with the airspace growth, the capillary network is expanding as well (1). As newly formed capillaries come in contact with overlying epithelium, type 2 alveolar epithelial cells differentiate into the type 1 cells that line the mature alveolus. With the saccular stage of lung development, which begins after 24 weeks gestation, the capillary network continues to grow in length and diameter, and new blood vessels expand to provide for the rapidly developing terminal airspace. Premature delivery, with its exposure to high oxygen concentrations, mechanical ventilators, infections, and nutritional deprivation, abruptly disrupts this complex choreography of airspace and vascular growth and leads to an impaired lung formation and function that can persist beyond the neonatal years (15). Indeed, infants who are born prematurely and have a subsequent lung disease termed bronchopulmonary dysplasia have been shown to have disruptions in their alveolar and capillary growth, which leads to increased resistance in and remodeling of their pulmonary blood vessels. (2,3). Fetal factors, such as maternal preeclampsia, *in utero* infection, or intrauterine growth restriction, coupled with premature birth and postnatal injury are hypothesized to lead to pulmonary vascular disease, that manifests clinically as pulmonary hypertension (PH) (16).

Retrospective studies of premature infants have estimated the incidence of PH in very low birth weight (< 1500 grams) infants to be between 25-43% (8,9,14). Concerns that selection bias and small sample size may have resulted in less accurate PH rates in these studies have led several investigators to interrogate the incidence prospectively. In one study of extremely low birth weight (<1000 grams) infants, the overall incidence of PH was 17.9% by the time of death or discharge from the neonatal intensive care unit (4).

Analyses of Danish registries have suggested that the incidence of PH in infants less than 1500 grams was as high as 23%, if they also had bronchopulmonary dysplasia, a lung disease of prematurity (12). In other studies, 8% of infants who were born at less than 28 weeks had evidence of PH at 36 weeks corrected gestational age, and the incidence of the diagnosis differed depending on the timing of echocardiographic studies (5). Taken together, these studies suggest an incidence of PH in very low birth weight, premature infants of somewhere between 8 and 43%. Even at the lower range, this incidence approximates that of other severe neonatal morbidities including necrotizing enterocolitis, periventricular leukomalacia, and severe retinopathy of prematurity (6). Additionally, the outcome of infants with PH may be guarded when they are compared with their premature counterparts. Although the majority of PH improves over the first year of life potentially due to postnatal lung growth, the odds of long-term mortality related to PH has been suggested to be as high as 4.6 in infants who have BPD and require prolonged ventilation (8,14). Indeed, survival rates for one subset of extremely premature patients were approximately 50% by 2-3 years following diagnosis (9).

Given the potential for neonatal and post-neonatal mortality, professional societies' such as the American Heart Association and the American Thoracic Society recommend screening premature infants for the development of PH and implementation of therapies to treat pulmonary vascular disease in PH (10). Unfortunately, precisely *which* infants to screen with echocardiography and *when* to screen infants and *how long* to screen an infant is still problematic for clinicians. The most widely studied potential predictor for the development of PH is the diagnosis of bronchopulmonary dysplasia (BPD), a premature lung disease that shares pathophysiologic etiology with PH, in very low birth weight, premature infants. Of infants who required positive-pressure ventilation at 36 weeks corrected gestational age, indicating that they have the most severe form of BPD, 29 to 53 % also had a diagnosis of PH (5,13,16). Intrauterine growth

restriction, or birth weight for gestational age less than the 10th percentile, has also been associated with the development of PH, suggesting that fetal vascular programming may play a role in the development of hypertension (14,17,18). Few other potential predictors have been identified other than these, postnatal infection, and possibly illicit drugs (11,12). The optimal timing of the initiation of screening has also not been identified. In one investigation, early echocardiographic screening at around 4 weeks of age identified only one-third of all the infants with PH, whereas other investigations have shown that early PH at less than one week of age was a risk factor for both BPD and later PH (4,7). Lack of information about predictive strategies and optimal timing of screening have resulted in screening around 36 weeks in most neonatal intensive care units, a time when respiratory morbidity is more apparent and infants may be nearing discharge to home (9,19,20). Aside from potentially delaying PH therapies, this approach may be problematic because the low sensitivity of echocardiographs for the detection of PH may result in infants going home with "normal" echocardiograms with no formal surveillance for the development of disease (21). Indeed, the median age of PH development is not during the neonatal period but at approximately 4 months of age (22), suggesting some infants may leave the neonatal unit and ultimately develop PH.

Bioinformatics and the electronic medical health record are being used in research in order to identify patients and capture clinical variables in a comprehensive manner (23,24). We utilized data in the Epic electronic health record system to identify patients who were very low birth weight, premature, and at risk for pulmonary hypertension within our Children's Healthcare of Atlanta system. In the first Aim of this study, we sought to define clinical variables that are associated with the development of PH at any time during the hospital course. In the second Aim of this study, we determined birth and early hospital variables that are predictive of the development of PH after 30 days of life, and constructed a predictive model for PH using these factors.

Completion of these Aims will enhance our understanding of which infants should be screened for the development of PH during the neonatal period.

Research goal

The main goal of this study was to determine clinical factors that are associated with the development of PH at any time prior to death or discharge from the neonatal intensive care unit. We additionally sought to define birth and early hospital risk factors and to use those risk factors for the development of a predictive model for the development of PH after 30 days of life.

METHODS

Study design

The study population was a retrospective cohort of patients who had been seen at the Children's Healthcare of Atlanta, Egleston or Scottish Rite campus from January 2010 to January 2015. For the first Aim, the primary outcome was pulmonary hypertension (PH), as defined by echocardiographic parameters (see definition below under Pulmonary hypertension as the outcome variable), at any point prior to death or discharge for the neonatal intensive care unit. For the second Aim, the primary outcome was PH after 30 days of life.

Characteristics of the study population

Inclusion/Exclusion criteria

Infants who were less than 32 weeks gestational age at birth, had a birth weight of less than 1500 grams, were in the neonatal intensive care unit, and had an echocardiographic procedure were included in the initial study population (n=586). Patients were excluded from the study if they had multiple anomalies or aneuploidy (n=8), congenital heart disease other than atrial septal defect (ASD), ventricular septal defect (VSD), or patent ductus arteriosus (PDA) (n=11), congenital lung disease (n=2), or missing medical record information (n=4). A total of 25 patients were excluded, and 561 very low birth weight, premature infants were eligible for inclusion. Of these, 2 infants had missing outcome data for PH, and 559 infants were included in the overall study cohort for the determination of PH in Aim 1. For Aim 2, 321 infants had a diagnosis of PH or their last echocardiographic procedure after 30 days of life.

Measurements

Data collection

Initial patient identification from the electronic health record data warehouse was performed by the Department of Biomedical Informatics at Emory University (P.

Shankar) and the Business Information Technology Department at Children's Healthcare of Atlanta using ICD-9 codes for gestational age < 32 weeks, birthweight < 1500 grams, neonatal intensive care unit, and echocardiographic procedure. Study patients were identified using Qlikview-based Population Discovery data mart and verified using the electronic health record-based Clarity Database in use at Children's Healthcare of Atlanta by investigators and analysts who were blinded to the study design. Variables that were discrete searchable fields were extracted from the clinical data warehouse for the study population (such as caffeine use, birthweight, gestational age, positive blood cultures, and surgery). Maternal and birth-related variables that could not be searched through discrete fields were abstracted manually from the infant's medical record (S. Vyas-Read). The respiratory support modality was abstracted from the clinical respiratory flowsheet in the infant's electronic health record and the level of support at 28 days and 36 weeks post-conceptual age was determined by calculating the number of weeks from the date of birth to each time point of assessment (P. Shankar, C. Travers). Echocardiographic variables, such as blood pressure, atrial septal defect, ventricular septal defect, patent ductus arteriosus, tricuspid regurgitation jet velocity, right ventricular hypertrophy/dilation, left ventricular hypertrophy, septal flattening, and directionality of shunting were abstracted from the clinical report for each echocardiogram in the infant's medical record (J. Stremming). Mortality and the time to death were manually determined by subtracting the date of death from the birth date (S. Vyas-Read).

Pulmonary hypertension as the outcome variable

For the first Aim, the primary outcome variable was pulmonary hypertension on any echocardiograph prior to death or discharge from the neonatal intensive care unit. Pulmonary hypertension was defined as in 3 possible ways based on prior literature: 1) a moderate-to-large patent ductus arteriosus with bidirectional or right-to-left shunting;

2) a tricuspid regurgitation jet velocity of $\geq$ 32 mm Hg with septal flattening, right ventricular hypertrophy, or right ventricular dilation; or 3) a tricuspid regurgitation jet velocity of $\geq$ 45 mmHg (7,11,17). Echocardiographs were ordered at the discretion of the attending neonatologist and performed by pediatric cardiologists within the Sibley Heart Center. Echocardiographic variables were collected from standard reports in clinical use at our center during the study period. Although it is a competing risk, death was not included as an outcome in this study because the cause of mortality in very low birth weight infants is usually multi-factorial and could not be determined from the medical record. However, the proportions of infants who died in the PH and No PH groups were not significantly different.

For the second Aim, the day of echocardiogram was defined as the day of life that an infant received a study that first showed PH. If an infant did not have PH on any echocardiogram after 30 days of life, the day of life of the last echocardiographic study was recorded. Infants who received echocardiograms at > 30 days and developed PH were then compared to infants who received echocardiograms at >30 days and did not develop PH during the hospital course (Median duration of follow-up from hospital admission to death or discharge from the neonatal intensive care unit is shown in Tables 1 and 6).

Predictors and co-variates

Demographic variables

Maternal and birth characteristics were abstracted from the infant electronic health record. Variables such as “maternal drug use” are indicative of the use of tobacco and/or alcohol, while “illicit drug use” refers to the use of marijuana, amphetamines, or other illegal substances. For multivariable analyses, Black race was compared with a composite of White, Hispanic, Asian and Other races. Death was defined as mortality from any cause during the hospital course. Positive bacterial and fungal cultures for were

determined from captured laboratory data in the clinical data warehouse. A positive blood culture included any culture that was deemed "positive" for bacterial or fungal growth by the microbiology laboratory at Children's Healthcare of Atlanta (with the exception of *Streptococcus viridans)*. Respiratory support at 36 weeks was defined as the respiratory modality at 36 weeks $\pm$ 5 days.

Echocardiographic variables

The echocardiographic characteristics of infants with pulmonary hypertension were ascertained at the time of the echocardiogram that first demonstrated evidence of pulmonary hypertension (Table 2). The timing of PH diagnosis was determined by subtracting the date of birth for each infant from the date of the echocardiogram showing PH. Directionality of the shunt through an ASD, ventricular septal defect, or patent ductus arteriosus (PDA) was determined by the pediatric cardiologist at the time of the echocardiogram as 1) left-to-right or none 2) bidirectional or 3) right-to-left. The tricuspid regurgitation jet velocity (TRJV) was graded as 1) normal, < 32 mmHg 2) mild, 32-44 mmHg 3) moderate 45-60 mmHg and 4) severe $\geq$ 60 mmHg at that time. Systolic and diastolic blood pressure was recorded as a cuff pressure unless the infant had an existing arterial line. Septal flattening was defined subjectively as none, any, or severe subjectively by the pediatric cardiologist performing the echocardiogram. Right ventricular dilatation, hypertrophy, and dysfunction were defined as either present or absent. Atrial septal defects were categorized as 1) none or patent foramen ovale (PFO) or 2) patent foramen ovale versus atrial septal defect (PFO vs. ASD) or atrial septal defect (ASD). PDA was defined as 1) none or small or ligated versus 2) moderate-to-large on the first study echocardiogram. Ventricular septal defects were defined as 1) intact, tiny, and small or 2) moderate-to-large or multiple.

Missing data

No outcome data for the diagnosis of PH was missing. For Aim 1, co-variates with missing observations are listed: intrauterine growth restriction, n= 548; caffeine therapy, n=555; ASD, n=538; race, n= 546. For the predictive model in Aim 2, the predictors with missing observations are listed: Apgar 1 minute, n=311; Race, n=314; ASD, n=311. For variables not listed, no data were missing. Since the percentage of missingness in both models was maximally 4%, no imputation for missing data was performed prior to analyses.

Descriptive statistics

The distribution of continuous variables was evaluated using skewness and kurtosis measurements, by comparing the mean and median value for each variable, and by examining histograms of each variable. For variables that were normally distributed, the mean and standard deviation was reported, and two-sample t-tests were performed between outcome groups. For variables with a skewed, non-normal distribution, the median and interquartile range was reported and a Wilcoxon rank sum test was used to compare groups. For categorical variables, frequency tables were constructed and the proportion of infants in each cell evaluated. Chi-square tests of proportion were used to compare outcome groups unless the cell frequency was ≤ 5, in which case the Fisher's exact test was used.

Univariable analyses

For Aim 1, variables that were statistically significant in the descriptive analyses were then evaluated to determine their impact on the outcome of pulmonary hypertension at any time during the neonatal course (Table 5). Positive pressure ventilation at 36 weeks was not evaluated as a predictor since its diagnosis is often concomitant with the diagnosis of pulmonary hypertension. The number of echocardiograms and the length of study were greater in patients with pulmonary hypertension, reflective of their overall acuity, and these variables were not included

evaluated as covariates. The remaining significant variables were evaluated by univariable logistic regression to determine odds ratios, and 95% confidence intervals.

For Aim 2, birth characteristics and early hospital variables that were present before 30 days of life and could be used as predictors for the later development of PH (after 30 days of life) were evaluated by univariable logistic regression (Table 6). Caffeine therapy was assumed to be initiated prior to 30 days of life given a consensus within our group to initiate caffeine therapy as soon after birth as possible for infants < 32 weeks gestational age at birth. Atrial septal defect was defined at the first echocardiogram (Table 4).

Multivariable analyses

A multivariable model was constructed by the manual addition of each significant variable to the intercept and birthweight variable. The -2Log likelihood values were determined, and the value each additional variable was determined using a likelihood ratio test with $p \geq 0.05$ as a stopping rule. For the first Aim, multivariable logistic regression was performed with the following variables: birthweight (per 100 gram change), growth restriction (yes vs. no), race (Black vs. all other races), caffeine (y vs. n), atrial septal defect (none or PFO vs. PFO vs. ASD or ASD), and patent ductus arteriosus (none or small vs. moderate or large). For the second Aim, multivariable logistic regression was performed with the following predictor variables: birthweight, Apgar 1 minute, race, caffeine, ASD, and positive-pressure ventilation at 28 days. Although maternal illicit drug use was positively associated with PH>30 days, it was not included in the model because of concerns that maternal recollection and reporting may hinder the validity of the data for this factor. Additionally, positive-pressure ventilation was controlled because of prior reports that oxygen use at 30 days affects the risk for PH, although we did not find this to be true in our data (25). Additionally, interaction was assessed between biologically plausible variables including birthweight*race,

birthweight*caffeine, birthweight*ASD, ethnicity*caffeine, ethnicity*ASD, and caffeine*ASD. None of the interaction terms met the $p< 0.05$ level of statistical significance for inclusion in the final model. Odds ratios and 95% confidence intervals were constructed.

The final multivariable logistic models are shown below:

AIM 1 :

Logit [Pr (PH= 1)] = β0 + β1birthweight + β2Growthrestriction (Yes vs. No) + β3Race (Black vs. Other) + β4Caffeine (Yes vs. No) + β5ASD (ASD or PFO/ASD vs. none or PFO) + β6PDA (moderate/large vs. none/small).

AIM 2:

Logit [Pr (PH > 30days = 1)] = β0 + β1birthweight + β2Apgar1minute + β3Race (Black vs. Other) + β4Caffeine (Yes vs. No) + β5ASD (ASD or PFO/ASD vs. none or PFO) + β6Positive-pressure ventilation (HFNC/CPAP/Vent vs. Room Air or nasal cannula).

Predictive Model Generation

A receiver-operating curve was generated using the final selected model in Aim 2 and the area under the curve was evaluated. Calibration plots of observed versus predicted proportions were performed, and the Hosmer and Lemeshow goodness of fit test was examined. In order to increase the detection of pulmonary hypertension among infants at risk, recognizing that the false positive rate would consequently be also increased, we chose a predicted probability of 8% as a cutpoint for sensitivity analyses. A frequency table comparing model prediction to disease was generated and the sensitivity, specificity, positive and negative predictive value was calculated from the counts in the frequency table. Because of the relatively small sample size, it was not possible to divide

the data into a training and validation dataset. Therefore, cross-one-out validation procedures were performed and model diagnostics for the validated model were repeated.

All statistical procedures were performed using SAS 9.4 statistical software and the level of significance for comparisons was a p-value < 0.05.

RESULTS

Patient selection

An initial search of the electronic health record (Clarity) database from January 2010 to January 2015 yielded 586 infants that met the inclusion criteria of having a gestational age of less than 32 weeks at birth, having a birthweight less than 1500 grams at birth, being admitted to the neonatal intensive care unit, and having an echocardiographic procedure (Figure 1). Of these, 25 infants were excluded from the study population due to multiple anomalies or aneuploidy (8 infants), congenital heart disease other than PDA, ASD or VSD (11 infants), congenital lung disease (2 infants), or missing medical information (4 infants). Two patients were excluded due to missing outcome data for the 561 patients who met inclusion criteria. 559 infants were included in the study cohort. 238 (43%) infants had PH diagnosed on an echocardiogram or had their last echocardiographic follow-up at less then or equal to 30 days of life, and 321 (57%) infants had PH or their last echocardiographic follow-up after 30 days of life. Of the infants who were evaluated at < 30 days, 42/238 (18%) had PH, and of the infants who were evaluated at > 30 days, 50/321 (16%) developed PH. Overall, 99 patients (18%) died during the follow-up period, and 21 (4%) infants had an echocardiogram with PH at the time of their death. In the entire study cohort, 92 infants developed PH during the study period (16%) and 467 (84%) did not.

Because the neonatal intensive care units that were studied are referral-care units and the study population is dynamic, we then described the study population at < 30 days, 31-60 days, 61-90 days, and over 90 days to determine if death or length-of-follow-up differed significantly for patients that were referred early in their neonatal course versus later. The number of studies for each time interval ranged between 2 and 3 per patient, and the median duration of follow-up for each time period was between 240 – 250 days (Day of discharge minus day of admission; Table 1). The day of life of the

echocardiogram that first showed PH or was the last study for the infant were similar between PH and No PH groups in each time interval. The majority of death (58/559 or 10%) occurred within the ≤ 30 days of life, and 41/559 infants (7.3%) died after 30 days of life. The day of death was later for the PH group for the first 2 time intervals, but infants who were in the >90 days interval and had PH died at a median of 181 days versus 273 days for the No PH group.

Echocardiographic characteristics of infants with PH

The echocardiographic characteristics of infants with PH were evaluated to determine the severity of their disease at the time of diagnosis. The median day of life when pulmonary hypertension was diagnosed was 48, and 30% of infants with an atrial septal defect (or patent foramen ovale) had bidirectional or right-to-left shunting noted on echocardiograph (Table 2). Similarly, 32% of infants who had shunting noted through a patent ductus arteriosus had bidirectional or right-to-left shunting. The tricuspid regurgitation jet velocity was nearly equally distributed between the normal, mildly elevated, moderately elevated, and severely elevated range, and 26% of infants had tricuspid regurgitation in the moderate-to-severe range. Some septal flattening was noted in 68% of infants with PH, and 10% in our study had severe septal flattening. Right ventricular hypertrophy or dilation was diagnosed in 40-42% of infants, and 20% had right ventricular dysfunction. Of the infants with PH, 17% were on sildenafil and 2% were being treated with bosentan.

Neonatal and hospital characteristics

Birth characteristics, such as Apgar scores, gestational age, mode of delivery, gender, placental abruption, maternal betamethasone administration, race, multiple gestation, and legal and illicit drug use did not differ between patients who had pulmonary hypertension and those who did not (Table 3). Infants who had pulmonary hypertension had a lower weight at birth (0.75 ± 0.24 vs. 0.82 ± 0.25 kg, p=0.01) and

were more like to have intrauterine growth restriction (14% vs. 7%, p=0.01), than infants without pulmonary hypertension. The incidence of common neonatal co-morbidities, such as intraventricular hemorrhage, necrotizing enterocolitis, and retinopathy of prematurity were similar between groups (Table 4). The median number of echocardiographs was 5 (range, 3-8) for infants with pulmonary hypertension, and 2 (range, 1-3) for infants without pulmonary hypertension (p <0.01). However, the day of life on which the diagnostic echocardiograph was performed did not differ between groups (median, day of life 26). The median length of hospital stay was significantly longer for infants with pulmonary hypertension (70, range 7-136) than for those without pulmonary hypertension (47, range 4-106; p=0.03).

Interestingly, significantly fewer infants with PH had received caffeine, than infants who did not have PH (55% vs. 69%, p=0.02). To determine if respiratory acuity was different between the outcome groups, we evaluated the need for respiratory support at both 28 days and at a corrected gestational age of approximately 36 weeks. While "any respiratory support" was defined by a requirement for any modality from nasal cannula to mechanical ventilation, "positive pressure ventilation" included high-flow nasal cannula, continuous positive airway pressure, and mechanical ventilation only. The need for any respiratory support or positive pressure ventilation did not differ at 28 days between the PH and No PH groups. Similarly, any respiratory support also did not differ between outcome groups at 36 weeks corrected gestational age. However, a higher proportion of infants with pulmonary hypertension required positive pressure ventilation at 36 weeks corrected (45% vs. 31%, p= <0.01), when compared with those who did not have PH. The proportion of infants with positive blood cultures did not differ between the 2 outcome groups. On their initial echocardiography, infants with pulmonary hypertension had more clinically significant atrial septal defects (patent foramen ovale/ASD or ASD; 25% vs. 11%, p =<0.01) and more moderate-to-large sized

patent ductus arteriosus (52% vs. 34%, p = <0.01) than those without pulmonary hypertension. The percentage of infants with ventricular septal defects did not differ between groups.

Associations between neonatal characteristics and echocardiographic PH

We then performed univariable logistic regression for significant c0-variates on the outcome of pulmonary hypertension at any point during the neonatal course (Table 5). For every 100-gram increase in birth weight, the odds of pulmonary hypertension decreased by 0.87 (95% CI 0.78 - 0.96). To determine which birth weight categories were at higher risk of PH, the cohort was divided into infants with birth weights < 500 grams, 501-750 grams, 751-1000 grams, and > 1000 grams. The odds of PH were highest for the smallest infants, whose birth weights were < 500 grams at birth (3.14, 95% CI 1.16 - 8.48, relative to infants whose birth weights were >1000 grams). The diagnosis of intrauterine growth restriction and Black race increased the odds of PH by over 1.8-fold (Intrauterine growth restriction 2.32, 95% CI 1.16 - 4.62; Black race 1.78, 95% CI 1.10 - 2.89). Caffeine administration during the hospital stay decreased the odds of pulmonary hypertension (0.58, 0.37-0.92). In contrast, the presence of a patent foramen ovale/ASD or ASD increased the odds of pulmonary hypertension of 6.6-6.7 fold, when compared with infants with no ASD or patent foramen ovale. Additionally, the diagnosis of a moderate or large patent ductus arteriosus on initial echocardiography increased the odds of pulmonary hypertension 2.21-fold (95% CI 1.37 - 3.58), when compared with studies that showed either no or a small patent ductus arteriosus. Initial echocardiography was performed for the PH group at a median of 14 days of life, and for the No PH group at 26 days of life, and this difference was not significantly different (p=0.36; Table 4).

In a multivariable model, birthweight and intrauterine growth restriction trended towards being associated with PH, but they did not meet statistical significance when race, caffeine, ASD and PDA were controlled (Table 5). With other factors held constant,

race, ASD and PDA increased the odds of PH [OR 1.79; 95% CI (1.02 – 3.14); 2.72 (1.45-5.11); 2.04 (1.19-3.49); respectively], and caffeine therapy decreased the odds of PH [0.49 (0. 0.29 – 0.84)].

Birth and early hospital variables predictive of PH after 30 days of life

To identify variables that may be predictive of PH later in the neonatal course, we evaluated factors that are present at birth or within the first 30 days of life using descriptive statistics and univariable logistic regression (Table 6). Infants who developed PH > 30 days had lower 1 and 5 minute Apgar scores, a lower birthweight, and a lower gestational age than those who did not develop PH, suggesting they were potentially sicker from birth (Table 6). Intrauterine growth restriction proportions were not different between groups, although numbers were small. Race continued to be associated with an increased odds of PH after 30 days (OR 2.56, 95% CI 1.23 – 5.34), and maternal illicit drug use was also associated with an increased odds of the outcome (OR 2.76, 95% CI 1.12 – 6.80). Similar to the larger cohort, fewer infants with PH > 30 days received caffeine compared with No PH, although the association was not statistically significant (49% vs 63%, p= 0.06). The presence of a PFO/ASD or ASD, or a moderate/large PDA on initial echocardiography increased the odds of PH after 30 days by 2.48 (95% CI 1.17 – 5.27) and 2.53 (1.28 – 5.01), respectively. However, since bidirectional or right-to-left shunting was used a definition of PH, and PDA size may potentially influence the degree of shunting, we did not include PDA in the final predictive model.

Development of a predictive model for pulmonary hypertension

To determine a predictive model, each co-variate that was both clinically and statistically significant from descriptive analyses (Table 6) was manually added to a logistic regression model and models were compared using likelihood ratio tests. In multivariable analyses, increasing birthweight, increasing Apgar score at 1 minute, and

caffeine therapy was associated with a reduction in the odds of PH after 30 days when other factors were held constant (Table 7). Race was strongly associated with PH after 30 days, and the odds were 3.81 (95% CI 1.55 – 9.36) for Black infants compared with White/Hispanic/Asian infants. When birthweight, Apgar at 1 minute, race, caffeine, and positive-pressure ventilation were controlled, the presence of a PFO vs. ASD or an ASD on initial echocardiography increased the odds of PH after 30 days 2.67-fold, similar to the association found in univariable analyses.

The contribution to the area under the curve of a receiver operating curve of each significant predictor was evaluated, and a final model was determined which included the following co-variates: birthweight, Apgar 1 minute, race, caffeine, ASD and positive-pressure ventilation at 28 days. To determine the proportion of PH after 30 days of life predicted by the selected model, and to compare it with the observed proportion of pulmonary hypertension, a calibration plot was performed (Figure 2, panel A). The Hosmer and Lemeshow goodness of fit test had a p-value of 0.36, which confirmed the model not to be a poor-fit for the data. A receiver operating curve of sensitivity versus 1-specificity was then constructed for the selected model, and the area under the curve for the model was 0.766 (shown in Figure 2, panel C). A binary classification system was then developed in which a predicted probability of over 8% for pulmonary hypertension was designed as "yes" for the model, and $\leq$ 8% was designated "no." The sensitivity, specificity, positive predictive value and negative predictive value of the model were then determined manually from a frequency table (Table 8). The selected model had a sensitivity of 84%, a specificity of 46%, a positive predictive value of 22%, and a negative predictive value of 94% at the given cut-point.

Validation of the predictive model

Given the small number of infants with the outcome of interest, the dataset could not be split into a training and validation dataset, and cross one out validation was used

to validate each predicted probability against the remainder of the data. Using this method, a receiver-operating curve was constructed for the validated data set and it had an area under the curve of 0.714 (see Table 9). A predicted probability of > 8% was designated as a "yes" outcome for the validated model, and $\leq$ 8% predicted probability was designated as a "no." Sensitivity, specificity, positive and negative predictive values for the validated model were determined from the defined frequency table and are listed in Table 9.

DISCUSSION

In this study, we have shown the association between common birth and hospital characteristics and the development of pulmonary hypertension in the neonatal period. Due to the comprehensive nature of the electronic health record and clinical data warehouse, we were able to identify new associations between demographic variables, such as birthweight and Black race, clinical variables, such as Apgar score at 1 minute and caffeine use, and echocardiographic variables, such as atrial septal defect, and the development of PH after 30 days of life in a referral neonatal intensive care unit. By identifying infant characteristics, at birth or within the first postnatal month, that contribute to PH, these findings may lead to the development of echocardiographic protocols that target screening of populations at the highest risk of disease, rather than relying on respiratory support requirements as a sole guide. The ability to predict PH after 30 days using our model was modest overall, (sensitivity 84%, specificity 46%, positive predictive value 22%, and negative predictive value 94%) as we intentionally set cut-points for predictive probabilities in sensitivity analyses low, at the risk of increasing false-positive rates, in order to avoid missing infants who may develop PH later in their course. Negative prediction for the model was stronger, as 94% of infants who test negative would not develop PH after 30 days of life.

Clinical centers have begun utilizing the clinical data warehouse in order to identify patients for treatment, to implement provider reminders for care, and to connect patient populations across centers (23,24,26). We were able to identify a research cohort using common neonatal billing codes, such as very low birth weight, less than 32 week gestational age, neonatal intensive care unit, and echocardiographic procedure. The overall incidence of PH was 16.5 % in our study population, which is in keeping with other prospective investigations of the disease (4,5,12). Further, the proportions of infants with common neonatal morbidities, such as intraventricular hemorrhage and

bronchopulmonary dysplasia (respiratory support at 28 days), were similar to those in a multi-center, national cohort (6). Our population had a slightly higher rate of necrotizing enterocolitis and retinopathy of prematurity than other cohorts, likely due to the fact that the neonatal intensive care units studied were referral-based, and infants were often transferred in to the center for surgical management of these diseases.

Pulmonary hypertension based on echocardiographic parameters is difficult to define due to difficulty in obtaining a measurable tricuspid regurgitation jet velocity, and poor sensitivity for the detection of PH (21). However, echocardiography is widely used in neonatal intensive care units because ethical considerations prohibit the use of cardiac catheterization as a screening tool (27). In our study, infants were defined as having PH if they had a patent ductus arteriosus shunt directionality of bidirectional or right-to-left (indicating systemic or supra-systemic pulmonary artery pressure), or a mildly elevated tricuspid regurgitation jet velocity with septal flattening, or right ventricular hypertrophy or dilation, or a tricuspid regurgitation jet velocity of over 45 mmHg. Although a discrete number for the tricuspid regurgitation jet velocity may be difficult to obtain, 99% of our infants with PH had jets that could be categorized into normal, mild, moderate or severe. In other investigations, a measurable tricuspid jet velocity in combination with septal flattening, right ventricular hypertrophy, or right ventricular dilation had a positive predictive value of 89-100%. Given these values, our patients who were classified as having PH echocardiographically would also be likely to have PH if they underwent catheterization (21). However, if infants did not have echocardiographic evidence of PH in our study, it is still possible that they may truly have had or will develop PH. Future investigations that include long-term outpatient follow up would allow us to determine the magnitude of this misclassification. Further, the definitions of PH applied in our study are comparable to those used in other investigations (4,8,13). The optimal echocardiographic parameters for infants at risk of PH is not known, but

algorithms to detect PH in a systematic manner are now being developed based on available literature (27).

Our findings were in keeping with other investigators with respect to the association between intrauterine growth restriction and pulmonary hypertension at any point during the neonatal course. In a single center, retrospective study, infants with BPD, who had a birth weight for gestational-age ratio percentile of less than 25%, had a 3.9-fold increase in the odds of PH at 36 weeks gestation. When this model was further controlled for gestational age, multiple gestation, gender and race, the odds of PH for growth-restricted infants were increased to 5.9 (17). Our population differed slightly from that in the prior study, in that we did not restrict our cohort only to infants with BPD, and the overall proportion of infants with intrauterine growth restriction was only 8% (compared with 30%). However, in our univariable analyses, intrauterine growth restriction doubled the odds of developing PH during the neonatal period. When birth weight and race were simultaneously controlled, intrauterine growth restriction trended towards an increased odds of overall PH, but the association was not statistically significant. The discrepant association between growth-restriction and PH between our study and that of Check et al. may be explained in part by the larger proportion of Black infants in our PH population (71% vs. 18%), and the lower incidence of PH in our population (16% vs. 28%). Further, we did not grade the severity of intrauterine growth restriction on Fenton curves in our population, but rather relied on obstetrical diagnosis of intrauterine growth restriction in the infant birth record. Regardless, the relationship between maternal vascular diseases, fetal growth, and future cardiovascular risk is strengthening (28). In one study, infants who were growth-restricted had alterations in echocardiographic findings and aortic media-intima thickness that were present prenatally and persisted for 6 months postnatally (29). Additionally, the offspring of mothers who had preeclampsia during pregnancy had lower weight at birth, and had an

exaggerated response to hypoxic pulmonary hypertension during adolescence (30). These studies and others point to a nearly certain role for the *in utero* environment and fetal epigenetic programming on future cardiovascular risk in infants, a relationship that needs to be further characterized in premature infants.

In our multivariable model, the presence of a PFO/ASD or an ASD increased the odds of any PH or PH after 30 days of life substantially, even when birth weight, race, and caffeine use were controlled. Supporting these findings, other investigators have also found an association between the presence of an ASD and the development of PH in infants with BPD, a diagnosis that conferred an increased risk of mortality (31). Animal models of chronic left-to-right shunting have increased pulmonary vascular resistance and arteriolar medial thickness through alterations in angiotensin, endothelin, and endogenous nitric oxide signaling, implying biologic plausibility to the association between ASD and PH (32,33). Epidemiologically, ASDs affect 8-10% of congenital heart defects, and the majority of them close spontaneously in the first year of life (34). For those with persistent defects, small studies have suggested that infants with BPD may have improved respiratory outcome following transcatheter closure of left-to-right shunts at approximately 6 months of age (35,36). Unfortunately, for infants in whom chronic left-to-right shunting through an ASD persists beyond the early infancy or potentiates respiratory morbidity, the optimal timing of intervention to prevent or treat PH is unclear.

Interestingly, caffeine therapy strongly decreased the odds of any PH and PH after 30 days of life in our study. Caffeine is a methylxanthine that has been widely used in neonatal intensive care units due to its safety profile and effectiveness in the prevention of apnea of prematurity (37). The Caffeine Therapy for Apnea trial and four separate observational studies have now shown that caffeine is useful not only as a treatment for apnea, but also has a protective agent against the development of BPD in

premature infants (38-43). In spite of the therapeutic effects of caffeine on lung mechanics and BPD, we were unable to find any literature that has investigated the role of caffeine in pulmonary hypertension. It is possible that BPD mediates the protective effect of caffeine on PH. However, in our multivariable model in which the need for positive pressure ventilation at 28 days was controlled, caffeine administration was still associated with lower odds of PH after 30 days of life (0.40; 95% CI 0.20 – 0.82). The mechanism of caffeine action is thought to be through the release of intracellular calcium, the inhibition of phosphodiesterases, and possibly antagonism of adenosine at its receptor (44). Further, caffeine must undergo *N*-demethylation in the liver to be converted to its active compound. In adults, the acute cardiovascular effects of caffeine include changes in arterial stiffness, blood vessel resistance, and aortic pressure, and these effects differ based on whether a patient has baseline hypertension (45,46). Many studies have additionally shown that the timing of caffeine is important in the efficacy of its protective effect on BPD (39,40). In this study, we only captured if caffeine was given during the hospital course but the timing of initiation and the length of therapy was not abstracted. Future investigations should now be performed that determine the most favorable dose, timing, and length of caffeine therapy to prevent PH in neonatal infants.

In our study cohort, infants with Black race had a much higher odds of PH compared with infants of other races. In other PH studies, the proportion of infants with Black race has ranged from 3% to 42%, whereas 58% of our overall study population was Black, potentially allowing us a greater power to determine subtle differences in outcome (5,7,17,47). Differences in health based on race and other individual-specific factors are becoming more appreciated in scientific investigations, but reports are still relatively rare in the pediatric literature (48-50) . In univariable analyses, we found that the odds of PH were 1.8 higher for Black infants and this relationship was sustained when significant neonatal and hospital factors were controlled (Odds ratio 1.79, 95% CI 1.02-

3.14). Infants who were Black who survived at least 30 days had a 3.8-fold increase in the odds of PH compared with infants who were White, Hispanic, or Asian races. Whether this association is due to decreased mortality for Black infants compared with other races, or is the result of biologic differences between races should be interrogated further.

One limitation of this study relates to the temporal sequence of predictors relative to the diagnosis of PH. For Aim 1, we acknowledge that a few variables, such as caffeine therapy, positive-pressure ventilation, and patent ductus arteriosus size, may not have occurred prior to or may have occurred concomitant with the diagnosis of PH at any time during the hospital course. For this reason, a causal association between these factors and PH is ambiguous and should not be assumed. For Aim 2, we attempted to address temporality by including only factors that were present prior to the first 30 days of life in the predictive model. Our institution tends to implement caffeine therapy early (usually within the first week of life), so caffeine was included in the predictive model. However, this was an assumption since we did not have the timing of caffeine initiation for this project. Additionally, due to the observational, retrospective nature of this study, we did not have a formalized echocardiography schedule for patients but we utilized studies that were performed at the discretion of the clinician at the bedside. As such, there may have been indications to obtain an echocardiogram that were more prevalent in the PH group, introducing some ascertainment bias. However, the PH and non-PH groups were similar with regard to common neonatal and hospital morbidities, and respiratory support at 28 days, suggesting that there were not drastic differences in patient acuity between groups. We additionally acknowledge that very low birth weight, premature infants who are sick have a higher risk of death than other neonates, which precludes the development of PH. We found death rates and the timing of death (approximately 33 weeks corrected gestational age) in the PH and non-PH groups in the

entire study cohort to be comparable. Since the 42.8% of premature infants die within the first month of life, we further analyzed only infants who survived beyond 30 days (51). Infants who had echocardiograms after 30 days of life who developed PH were smaller and of younger gestational age than their No PH counterparts. However, whether this is true because only the smallest, youngest premature infants who survive can be evaluated for PH, or whether the association is causal is difficult to determine from this retrospective analysis. Additionally, we utilized cross one out validation techniques to attempt to assess the validity of our model, but the generalizability of the model would certainly be enhanced by external validation.

The strengths of this study are that we utilized a novel procedure for patient identification from an existing clinical data warehouse. As such, we were able to capture hospital variables more comprehensively than if we had manually abstracted data, and were able to identify new variables associated with PH. Additionally, we included objective clinical variables in our predictive model that would be readily available early in the neonatal hospital course, and steered away from variables that have traditionally been associated with PH but are present later in the neonatal course. We focused on utilizing these variables to predict PH after 30 days of life, so that screening and possible therapy may be implemented earlier in the hospital course. Once the model has been validated in an independent population, it would be possible to utilize this type of model with traditional echocardiography to determine the need for screening using a Bayesian approach. For example, if the pre-test probability of PH after 30 days for a very low birth weight infant in our unit is 16% (50/321), then the pre-test odds are 0.19. The likelihood ratio positive for our model is 1.43, so the post-test odds are 0.27. If we combine this post-test odds with the likelihood ratio positive for an echocardiogram with $\geq$ 1 abnormality (LR + 1.43), then the post-test odds for the model and a positive echocardiogram are now 0.39 and the post-test probability is 28% (21). Based on the

higher probability of the infant developing PH, we may choose to put him/her in a different level of surveillance for disease. In contrast, if the screening test were negative then the pre-test odds of 0.19 multiplied by the likelihood ratio negative of 0.45 would result in post-test odds of 0.09. If the infant also had a negative echocardiogram (with a LR – of 0.12), then the post-test probability would be 1% and we might feel less committed to serial echocardiograms.

In summary, in this study, we have confirmed clinical variables that were known to be associated with the development of PH in premature infants, such as intrauterine growth restriction, birth weight, and atrial septal defect, and we have identified new factors that predict PH, such as Black race and therapy with caffeine. Our predictive model is unique in that it utilizes only readily available clinical variables, rather than solely relying on echocardiographic or respiratory characteristics, to establish risk for PH. Validation of this model and implementation of a formalized screening program will enhance our understanding of which very low birth weight infants should be screened and when clinicians should begin PH screening in the neonatal intensive care unit.

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TABLES/FIGURES

Figure 1. Flowchart of study patient selection

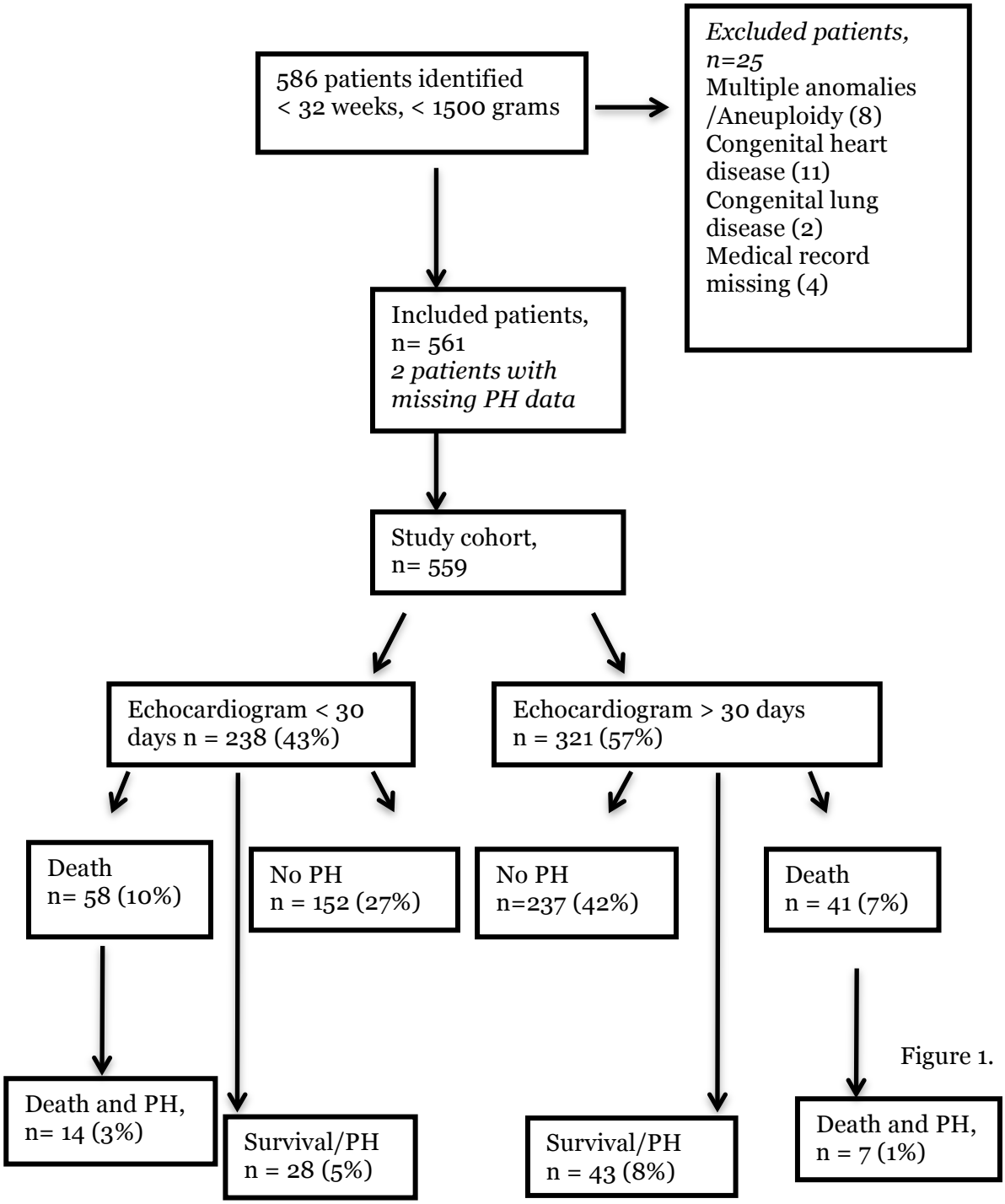


Figure 1.

Figure 1. Flowchart of study patient selection. An electronic health record database query was performed for < 32 weeks gestation at birth, < 1500 grams birthweight, neonatal intensive care unit, and echocardiographic procedure. 586 infants were identified, and 25 of these met exclusion criteria. 561 infants were eligible for inclusion in the study, and 559 were included (2 had missing data). Infants were then divided into patients with echocardiograms less than or greater than 30 days. Of the patients with echocardiograms less than 30 days of life, 58 died, 28 survived and developed pulmonary hypertension (PH), and 152 survived and did not develop PH. Of the infants with echocardiograms > 30 days of life, 41 died, 43 survived and developed PH, and 237 survived and did not develop PH. Overall, 92 infants met the criteria for pulmonary hypertension, 467 did not.

Table 1. Descriptive Characteristics of the study cohort throughout the study period

	≤30 days	31 – 60 days	61 – 90 days	> 90 days
Number of echos (n = 1013)	437	480	483	727
Number of echos per patient Median (IQR)	3 (1-4)	2 (1-4)	2 (1-4)	3 (1-5)
Number of patients (n)	238	100	52	169
Day of life of admission Median (IQR)	16 (9 – 23)	41 (36 – 49)	74 (67 – 81)	141 (114 – 195)
Day of life of death or discharge Median (IQR)	261 (60 – 271)	288 (282 – 297)	319 (314 – 330)	382 (346 –435)
PH diagnoses n, %	42, 18%	7, 7%	5, 10%	38, 22%
Day of life of echo—No PH (days) Median (IQR)	18 (10-24)	40 (35-48)	74 (66-81)	155 (114-209)
Day of life of echo—PH (days) Median (IQR)	12 (4-15)	43 (39-50)	73 (72-77)	135 (114-170)
Death n, %	58, 24%	18, 18%	7, 13%	16, 9%
Death, PH n, %	14, 6%	3, 3%	0, 0%	4, 2%
Day of death, No PH Median (IQR)	20 (14-49)	51 (38-63)	91 (72-188)	273 (208-670)
Day of death, PH Median (IQR)	25 (13-63)	151 (46-1022)	_____	181 (156-197)

Table 1. Descriptive Characteristics of the study cohort throughout the study period. 1013 echocardiograms were performed on 559 infants during the study period. The timing of the echocardiogram (echo) was defined as the infant's day of life that the study first showed pulmonary hypertension (PH) or the infant's day of life at the time of the last study. The infants were divided into 4 groups based on the timing of the echo: ≤30 days, 31-60 days, 61-90 days, and > 90 days. Descriptive characteristics for each study group are listed in columns, and percentages represent the proportion of patients with

death or PH, relative to the number of patients receiving echocardiograms in that time interval.

Table 2. Echocardiographic characteristics of infants with pulmonary hypertension

Echocardiographic parameter	**Infants with PH, n=92**
Timing of diagnosis (days) Median (IQR)	48 (13-122)
Atrial shunt direction (n, %)	
None or left-to-right	53, 58%
Bidirectional or right-to-left	28, 30%
Patent ductus arteriosus shunt direction (n, %)	
None or left-to-right	20, 21%
Bidirectional	18, 19%
Right-to-left	12, 13%
Tricuspid regurgitation jet velocity (n, %)	
Normal, < 32 mmHg	36, 38%
Mild, 32-44 mmHg	33, 35%
Moderate, 45-60 mmHg	19, 20%
Severe, > 60 mmHg	6, 6%
Systolic blood pressure (mmHg) Mean ± SD	69.9 ± 20.2
Diastolic blood pressure	40.3 ± 14.2
Septal flattening (n, %)	
None	1, 1%
Any	63, 68%
Severe	9, 10%
Right ventricular dilation (n, %)	
None	51, 55%
Any	39, 42%
Right ventricular hypertrophy (n, %)	
None	48, 52%
Any	37, 40%
Right ventricular dysfunction (n, %)	
None	73, 79%
Any	18, 20%
Sildenafil (n, %)	16, 17%
Bosentan	2, 2%

Table 2. Echocardiographic characteristics of infants with pulmonary hypertension. Echocardiographic and clinical variables were extracted from the infant medical record at the time of pulmonary hypertension diagnosis (PH). Echocardiograms were performed by clinical pediatric cardiologists and quantitative variables (tricuspid regurgitation jet velocity) and qualitative variables (shunt directions, septal flattening,

degree of right ventricular dysfunction/dilation/hypertrophy) were measured. Sildenafil and bosentan use was extracted from the clinical data warehouse for infants with PH. The variables for which data is missing are: atrial shunt direction, n= 81; PDA shunt direction, n= 50; septal flattening, n=73; right ventricular dilatation, n=90; right ventricular hypertrophy, n=85; right ventricular dysfunction, n=91.

Table 3. Birth characteristics of very low birth weight infants with and without pulmonary hypertension at any point during the hospital course

Birth characteristics	Overall N=559	PH N=92	No PH N= 467	p-value
Apgar 1 min. Mean ± SD	4.05 ± 2.60	3.66 ± 2.64	4.13 ± 2.59	0.11
Apgar 5 min. Mean ± SD	6.49 ± 2.27	6.29 ± 2.24	6.53 ± 2.27	0.35
Birthweight (kg) Mean ± SD	0.82 ± 0.25	0.75 ± 0.24	0.83 ± 0.24	0.01*
Gestational age (wks) Mean ± SD	26.13 ± 2.25	25.95 ± 2.30	26.17 ± 2.24	0.39
Mode of Delivery (n, %)				0.74
Vaginal	181, 32%	29, 32%	152, 33%	
C/S	372, 67%	62, 67%	310, 66%	
Gender, M vs. F (n, %)				0.90
Male	323, 58%	53, 58%	270, 58%	
Female	232, 42%	39, 42%	193, 41%	
Intrauterine growth restriction (n, %)	45, 8%	13, 14%	32, 7%	0.01*
Placental abruption (n, %)	60, 11%	11, 12%	49, 10%	0.64
Chorioamnionitis (n, %)	53, 10%	8, 9%	46, 10%	0.55
Maternal betamethasone (n, %)				0.30
0-1 doses	182, 33%	33, 36%	149, 32%	
2 or more doses	296, 53%	43, 47%	253, 54%	
Race (n, %)				0.17
White	158, 28%	20, 22%	138, 30%	
Black	326, 58%	65, 71%	261, 56%	
Asian	20, 4%	2, 2%	18, 4%	
Hispanic	35, 6%	5, 5%	30, 6%	
Other	7, 1%	0, 0%	7, 2%	
Illicit drug use (n, %)	43, 8%	11, 12%	32, 7%	0.07
Multiples (n, %)	102, 18%	17, 18%	85, 18%	0.94
Maternal drug use	45, 8%	10, 11%	35, 7%	0.24

Table 3. Birth characteristics of very low birth weight infants with and without pulmonary hypertension at any point during the hospital course. Pulmonary hypertension (PH) was defined as a tricuspid regurgitation jet velocity > 45 mmHg alone, > 32 mmHg with right ventricular hypertrophy or septal flattening, or a right-to-left shunt through a patent ductus arteriosus on any echocardiogram. Gestational age, Apgar scores, intrauterine growth restriction, placental abruption, multiple gestation, and chorioamnionitis were determined by the obstetrical or neonatal assessment as recorded in the infant medical record. Maternal drug use was defined by the use of alcohol or tobacco, and illicit drug use was defined as the use of any other substance (THC, methamphetamines, etc.) during pregnancy as reported by the mother. 559 infants were evaluated by two-sample t-test or Wilcoxon rank sum test for continuous variables, or Chi-square/Fisher's exact test for categorical variables. *$p<0.05$ for comparison groups. Variables for which data is missing are listed below: Apgar 1 minute, n=545; Apgar 5 minutes, n=547; Mode of delivery, n=552; Gender, n=555; Intrauterine growth restriction and maternal chorioamnionitis, n=548; Placental abruption, n=549; Maternal betamethasone, n=478; Race, n=546; Illicit drugs, n=493; Multiple gestation, n=554; Maternal drug use, n=492.

Table 4. Hospital characteristics of infants with and without pulmonary hypertension at any point during the hospital course

Hospital characteristics	Overall N=559	PH N=92	No PH N= 467	p-value
Intraventricular hemorrhage (n, %)	122, 22%	21, 23%	101, 22%	0.74
Necrotizing enterocolitis (n, %)	128, 23%	20, 22%	108, 23%	0.84
Retinopathy of prematurity (n, %)	258, 46%	50, 54%	208, 45%	0.06
Number of echocardiograms Median (IQR)	2 (1-4)	5 (3-8)	2 (1-3)	<0.01*
Echocardiogram timing, day of life Median (IQR)	26 (13-55)	26 (11-78)	26 (14-51)	0.85
Length of stay, days Median (IQR)	49 (5-110)	70 (7-136)	47 (4-106)	0.03*
Caffeine (n, %)	373, 67 %	51, 55 %	322, 69%	0.02*
Any respiratory support, 28 days (n, %)	240, 43%	38, 41%	202, 43%	0.73
Positive pressure ventilation, 28 days (n, %)	231, 41%	37, 40%	194, 42%	0.81
Any respiratory support, 36 wks (n, %)	226, 40%	44, 48%	182, 39%	0.27
Positive pressure ventilation, 36 wks* (n, %)	185, 33%	41, 45%	144, 31%	<0.01*
Positive culture, Any (n, %)	93, 17%	17, 18%	76, 16%	0.60
Blood	66, 12%	11, 12%	55, 12%	0.96
Day of life, 1st echo Median (IQR)	26 (12 – 51)	14 (8 – 85)	26 (13 – 50)	0.36
Atrial septal defect (n, %)				<0.01*
None/PFO	464, 83%	64, 70%	400, 86%	
PFO/ASD	74, 13%	23, 25%	51, 11%	
Ventricular septal defect				0.90
None/Small	501, 90%	84, 91%	417, 89%	
Mod/Large	14, 3%	3, 3%	11, 2%	
Patent Ductus Arteriosus Size				<0.01*
None/Small	283, 51%	34, 37%	249, 53%	
Mod/Large	207, 37%	48, 52%	159, 34%	

Table 4. Hospital characteristics of infants with and without pulmonary hypertension at any point during the hospital course. Pulmonary hypertension was defined as a tricuspid regurgitation jet velocity > 45 mmHg alone, > 32 mmHg with right ventricular hypertrophy or septal flattening, or a right-to-left shunt through a patent ductus arteriosus on any echocardiograph. Intraventricular hemorrhage, necrotizing enterocolitis, retinopathy of prematurity, and length of stay variables were abstracted from the clinical data warehouse. Any respiratory support refers to the use of low-flow nasal cannula, high-flow nasal cannula, continuous positive airway pressure, or ventilation, and positive-pressure ventilation includes any of these modalities other than low-flow nasal cannula. Respiratory support was ascertained at 28 days of life, and at 36 weeks $\pm$ 5 days corrected gestational age. Positive culture includes blood cultures that were positive for bacterial or fungal growth. Atrial septal defects were categorized into none/patent foramen ovale (PFO) and PFO vs. atrial septal defect (ASD) and ASD. Patent ductus arteriosus was divided into none/small or moderate/large based on the size at the first echocardiogram. 559 infants were evaluated by two-sample t-test or Wilcoxon rank sum test for continuous variables, or Chi-square/Fisher's exact test for categorical variables. *$p<0.05$ for comparison groups. Variables for which data are missing are listed below: Intraventricular hemorrhage and necrotizing enterocolitis, n=555; Number of echocardiographs, n=543; Day of life echocardiograph, n= 557; Visit length of stay, n=549; Caffeine, n=555; Respiratory support at 36 weeks, n=557; Positive pressure ventilation at 36 weeks, n=260; Atrial septal defect, n=538; Ventricular septal defect, n=515; Patent ductus arteriosus size at first echo, n= 490.

Table 5. The effect of neonatal and hospital characteristics on pulmonary hypertension at any point during the hospital course

Neonatal characteristics	**Unit**	**Odds ratio (95% CI)**	**p-value**	**Adj Odds ratio (95% CI)**	**Adj p-value**
Birthweight	per 100 gms	0.87 (0.78 - 0.96)	0.01*	0.94 (0.83 – 1.06)	0.29
Birthweight categories (kg)	<0.500	3.14 (1.16 - 8.48)	0.03*	_____	_____
	0.501-0.750	1.70 (0.91 - 3.20)	0.55	_____	_____
	0.750-1.000	1.00 (0.49 - 2.04)	0.06	_____	_____
	1.000-1.500	Ref	Ref	_____	_____
Intrauterine growth restriction	Y vs. N	2.32 (1.16 - 4.62)	0.02*	1.86 (0.83 – 4.17)	0.13
Race	Black vs. Other	1.78 (1.10 - 2.89)	0.02*	1.79 (1.02 – 3.14)	0.04*
Hospital characteristics					
Caffeine	Y vs. N	0.58 (0.37 - 0.92)	0.02*	0.49 (0.29 – 0.84)	0.01*
Atrial septal defect	ASD/PFO vs. None/PFO	2.73 (1.55 - 4.81)	0.03*	2.72 (1.45 – 5.11)	<0.01*
	ASD	6.61 (1.98 - 22.06)	0.02*	_____	_____
	PFO vs. ASD	6.70 (1.90 - 23.58)	0.03*	_____	_____
	PFO	2.59 (0.91 - 7.40)	0.27	_____	_____
	None	Ref	Ref	Ref	Ref
Patent ductus arteriosus	Mod/Large Vs. None/Small	2.21 (1.37 – 3.58)	<0.01*	2.04 (1.19– 3.49)	0.01*

Table 5. The effect of neonatal and hospital characteristics of very low birth weight infants on pulmonary hypertension any point during the hospital course. Analyses were performed on significant co-variates from Tables 3 and 4. The comparisons were as follows: Black vs. other races; atrial septal defect (ASD), PFO/ASD or ASD versus

PFO/none on first echocardiograph; patent ductus arteriosus (PDA), moderate or large versus none/small at the first echocardiograph; all others, yes vs. no. Univariable logistic regression was performed to determine the odds of PH and 95% confidence intervals (Odds ratio and p-value column). A multivariable logistic regression model with birthweight, growth restriction, race, caffeine, ASD, and PDA was performed and odds of PH and 95% confidence intervals were obtained (Adj odds ratio and adj p-value column), *indicates $p<0.05$.

Table 6. Evaluation of birth and early hospital characteristics for the outcome of PH after 30 days of life

Birth / Early Hospital Characteristics	PH > 30 days n=50	No PH >30 days n= 271	Odds ratios (95% CI)	p-value
Day of life of admission Median (IQR)	122 (96 – 163)	88 (48 – 146)	1.00 (0.99-1.00)	<0.01*
Day of life of death or discharge Median (IQR)	204 (149 – 376)	326 (290 – 386)	0.994 (0.992-0.997)	<0.01*
Apgar 1 min. Mean ± SD	3.06 ± 0.98	4.08 ± 2.55	0.84 (0.73 – 0.96)	0.01*
Apgar 5 min. Mean ± SD	5.77 ± 1.97	6.56 ± 2.26	0.86 (0.75 – 0.98)	0.02*
Birthweight (kg) Mean ± SD	0.70 ± 0.19	0.82 ± 0.24	0.08 (0.02 – 0.39)	<0.01*
Gestational age (wks) Mean ± SD	25.08 ± 1.78	26.16 ± 2.26	0.78 (0.66 – 0.91)	<0.01*
Mode of Delivery (n, %)				
C/S	30, 63%	180, 67%	0.82 (0.44 – 1.56)	0.55
Vaginal	18, 38%	89, 33%	ref	
Gender (n, %)				
Male	30, 60%	161, 60%	0.99 (0.54 – 1.85)	0.99
Female	20, 40%	20, 40%	ref	
Intrauterine growth restriction (n, %)	4, 9%	24, 9%	0.96 (0.32 – 2.91)	0.94
Placental abruption (n, %)	7, 15%	28, 11%	1.49 (0.61 – 3.64)	0.38
Chorioamnionitis (n, %)	5, 11%	20, 8%	1.50 (0.53 – 4.22)	0.44
Maternal betamethasone (n, %)				
2 or more doses	23, 53%	149, 63%	0.67 (0.35 – 1.29)	0.23
0-1 doses	20, 47%	87, 37%	ref	
Race (n, %)				
Black	40, 80%	161, 61%	2.56 (1.23 – 5.34)	0.01*
White/Asian/Hispanic	10, 20%	103, 39%	ref	

Illicit drug use (n, %)	8, 19%	19, 8%	2.76 (1.12 – 6.80)	0.02*
Multiples (n, %)	5, 10%	47, 17%	0.54 (0.20 – 1.43)	0.21
Maternal drug use	6, 14%	20, 8%	1.80 (0.68 – 4.78)	0.23
Caffeine (n, %)	24, 49%	170, 63%	0.56 (0.30 – 1.03)	0.06
Any respiratory support, 28 days (n, %)	13, 26%	90, 34%	0.70 (0.35 – 1.38)	0.30
Positive pressure ventilation, 28 days (n,%)	13, 26%	87, 32%	0.74 (0.38 – 1.47)	0.39
Atrial septal defect (n, %)				
PFO/ASD	12, 26%	33, 12%	2.48 (1.17 – 5.27)	0.02*
None/PFO	34, 74%	232, 88%	ref	
Ventricular septal defect				
Mod/Large	0, 0%	6, 2%	0.75 (0.09 – 6.19)	0.78
None/Small	46, 100%	244, 98%	ref	
Patent Ductus Arteriosus Size				
Mod/Large	25, 61%	87, 38%	2.53 (1.28 – 5.01)	<0.01*
None/Small	16, 39%	141, 62%		

Table 6. Evaluation of birth and early hospital characteristics for the outcome of PH after 30 days of life. Descriptive analyses were performed for birth and hospital characteristics on the outcome of PH after 30 days of life. Shown are selected characteristics that were clinically and statistically significant, and were present early enough in the neonatal course to precede the development of the outcome. Gestational age, Apgar scores, intrauterine growth restriction, placental abruption, multiple gestation, and chorioamnionitis were determined by the obstetrical or neonatal assessment as recorded in the infant medical record. Maternal drug use was defined by the use of alcohol or tobacco, and illicit drug use was defined as the use of any other

substance (THC, methamphetamines, etc.) during pregnancy as reported by the mother. Caffeine therapy was abstracted from the clinical data warehouse and was presumed to be initiated < 30 days of life. Positive-pressure ventilation includes the need for high-flow nasal cannula, continuous positive airway pressure, or mechanical ventilation at 28 days of life. Atrial septal defect (ASD) and patent ductus arteriosus (PDA) variables reflect their status on the first echocardiogram. 321 infants were evaluated by two-sample t-test or Wilcoxon rank sum test for continuous variables, or Chi-square/Fisher's exact test for categorical variables, and *$p<0.05$ for comparison groups. Univariable logistic regression was performed to determine odds ratios and 95% confidence intervals.

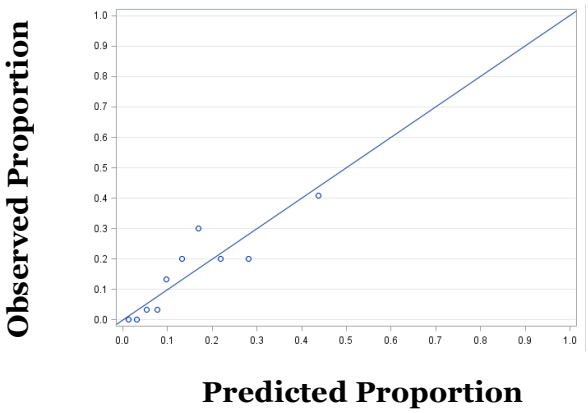
Table 7. Multivariable logistic regression of birth and early hospital characteristics on the development of PH > 30 days of life

Variable	Unit	Odds ratio	95% confidence Interval	p-value
Birthweight	per 100 grams increase	0.80	0.67 – 0.96	0.02*
Apgar 1 minute	per 1 increase	0.81	0.69 – 0.96	0.01*
Race	Black vs. Other	3.81	1.55 – 9.36	0.01*
Caffeine	Yes vs. No	0.40	0.20 – 0.82	<0.01*
Atrial septal defect	PFO/ASD & ASD vs. None/PFO	2.67	1.10 – 6.47	0.03*
Positive pressure ventilation at 28 days	Yes vs. No	1.09	0.50 – 2.40	0.83

Table 7. Multivariable logistic regression of birth and early hospital characteristics on the development of PH > 30 days of life. Odds ratios and 95% confidence intervals for each co-variate, controlling for the others, were determined using multivariable logistic regression. The number of observations for each co-variate is as follows: birthweight, n=321; Apgar 1 minute, n= 311; Race, n= 314; atrial septal defect, n= 311; positive-pressure ventilation, n=321. *$p<0.05$ for comparison groups.

Figure 2. Model diagnostics for the selected model

A. Calibration plot



B. Hosmer and Lemeshow Goodness of Fit

p-value = 0.36

C. Receiver operating curve

Area under curve 0.766

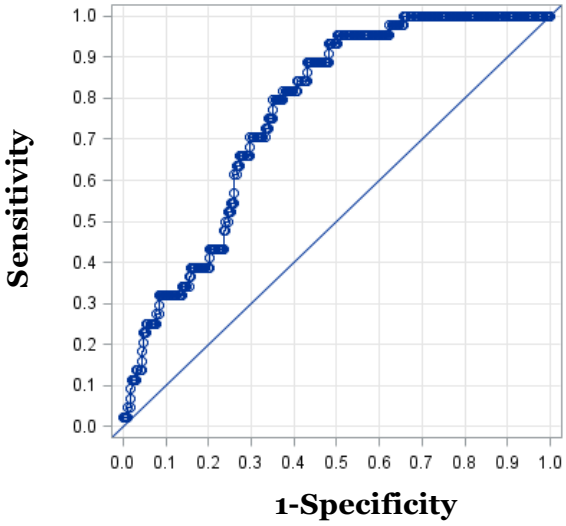


Figure 2. Model diagnostics for the selected model (specified in Table 7). A predictive model for pulmonary hypertension (PH) after 30 days of life that maximized the area under the receiver-operating curve (ROC) was determined using multivariable logistic regression. Panel A shows a calibration plot of observed proportions of the outcome of PH > 30 days (Y-axis) compared with those predicted by the model (X-axis). Panel B shows the p-value of the Hosmer and Lemeshow goodness of fit test for the selected model. Panel C shows a ROC of the sensitivity of the model (Y-axis) compared with 1-specificity (X-axis). Total n = 297, PH n = 44.

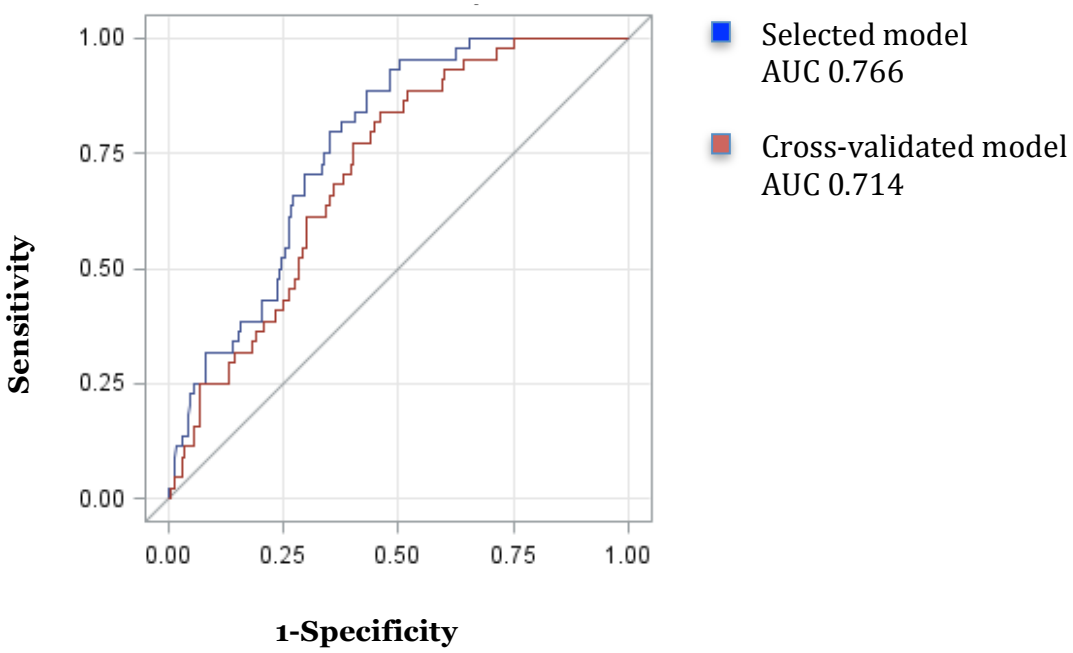
Table 8. Model performance for disease prediction

	PH>30 days = No	PH>30 days = Yes	Total
Model Prediction			
No	124	8	132
Yes	147	42	189
Total	271	50	321

Performance Measures	Counts	Percentage
Sensitivity	42/50	84%
Specificity	124/271	46%
Positive Predictive Value	42/189	22%
Negative Predictive Value	124/132	94%

Table 8. Model performance for disease prediction. Predicted probabilities were coded into dichotomous variables as follows: Predicted probability > 8% = yes, $\leq$ 8% = no. Model prediction versus disease outcome is shown in the frequency table (top). Sensitivity, specificity, positive and negative predictive values were calculated from frequency table counts manually.

Table 9. Cross-Validated Model Performance



Performance Measures	Counts	Percentage
Sensitivity	40/50	80%
Specificity	119/271	44%
Positive Predictive Value	40/192	21%
Negative Predictive Value	119/129	92%

Table 9. Cross-Validated Model Performance. Cross-one out validation was performed in SAS and a receiver-operating curve was determined for the validated model (Panel A, red line) and compared to the predictive model (Panel B, blue line). Predicted probabilities for the validated model were coded into dichotomous variables as follows: Predicted probability > 8 % = yes, $\leq$ 8% = no. Model prediction versus disease outcome is shown in the frequency table (Panel B). Sensitivity, specificity, positive and negative predictive values were calculated from counts in the frequency table.