Late Oxygen Exposure and Retinopathy of Prematurity: A Propensity Score Analysis

A thesis submitted by

Jennifer W. Lee, MD

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Abstract:

Objective: It is unclear whether late oxygen exposure after 31-32 weeks postmenstrual age (PMA) is associated with retinopathy of prematurity (ROP). The study aim is to explore the association of late oxygen exposure with development of ROP in preterm infants.

Patients and Methods: Clinically important variables for preterm infants weighing <1500g at birth were recorded as part of the Vermont Oxford Network (VON) registry. Data were prospectively collected for 1003 premature infants born between 23 and 28 weeks gestation. Exposure to oxygen at 36 weeks PMA, during phase II of ROP pathogenesis, was recorded. Multivariable logistic regression models were created to examine the effects on the risk of severe ROP. A propensity score for late oxygen exposure was used to control for potential confounding variables.

Results: Infants who received late oxygen had lower birth weights and gestational ages. They were more likely to be male, receive oxygen at day of life 28, have a patent ductus arteriosis (PDA), a PDA ligation, respiratory distress syndrome (RDS), pneumothorax, and late bacterial sepsis compared to those who did not receive late oxygen. After adjustment by propensity score stratified into quintiles for late oxygen therapy, infants who were still receiving oxygen at 36 weeks PMA were twice as likely as those who did not receive late oxygen to develop severe ROP [OR 2.00 (95%CI 1.29-3.09); p=0.002].

Conclusion: Late oxygen administered at 36 weeks PMA during phase II of ROP pathogenesis, might increase the risk of developing ROP in preterm infants. More research is needed to confirm this association and to further elucidate the mechanisms.

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Introduction:

Retinopathy of prematurity (ROP) is a disorder of the developing retina that predominantly affects premature infants. It is a leading cause of impaired vision and blindness throughout the world [1]. While normal retinal vasculature development occurs in utero and is completed between 36-40 weeks gestational age (GA)[2], the abnormal vessel growth of ROP begins to develop at the time of premature birth. Exposure to oxygen has been shown to be a critical factor in the development of ROP [3].

ROP develops in two sequential phases [4]. The initial vaso-obliterative phase is triggered by hyperoxia in the immediate postnatal period (since the arterial PO_2 of a fetus *in utero* is 25 torr, even room air is supraphysiologic for the preterm infant). This results in cessation of normal retinal vessel growth and regression of existing vessels, predominantly via down regulation of vascular endothelial growth factor (VEGF) by relatively high concentrations of oxygen [3]. The subsequent phase occurs when areas of the developing retina grow and thicken, with internal cells becoming relatively hypoxic (approximately 31-32 weeks PMA). This stimulates the release of VEGF and incites proliferative vascularization leading to the development of severe ROP and the possibility of retinal detachment.

Many studies have shown that early oxygen exposure in the first few weeks of life increases the risk of ROP [5-9]. However, few studies have explored whether exposure to oxygen later in gestation during phase II of ROP development, can alter this risk[10]. A recent meta-analysis of relevant ROP studies suggested a 46% reduction in risk of severe ROP by high oxygen saturation (>94-99%) at a PMA of $\geq$ 32 weeks, indicating possible benefit of oxygen during the second phase of ROP[11].

By using observational data collected prospectively as part of the Vermont Oxford Network (VON) and propensity score (PS) analyses, we aimed to determine if the use of late oxygen, oxygen at 36 weeks PMA can alter the risk of severe ROP in premature infants. We hypothesized that infants born at 28 weeks gestation or less who are still receiving supplemental oxygen therapy at

36 weeks PMA are less likely to develop severe ROP when compared with similar neonates who do not receive late oxygen.

Patients and Methods:

This is a prospective cohort study of premature infants born at 23-28 completed weeks gestation, who survived to 36 weeks gestation, using data obtained from the VON registry. The VON registry maintains a database which includes data about the care and clinical outcomes of high-risk neonates. It provides this information to participating Neonatal Intensive Care Units (NICUs) for use in quality improvement, peer review, and long term outcomes. The database includes over 900 nurseries throughout the world, three of which were included in this study.

Infants were born and/or admitted to 1 of 3 large level III NICUs in Massachusetts during the years 2002-2010. Centers included Tufts Medical Center, Baystate Medical Center, and University of Massachusetts Medical Center. Infants who died in the delivery room, died within 12 hours of birth, had a major congenital anomaly, died before 36 weeks PMA, were discharged home before 36 weeks, or who were transferred before 36 weeks were excluded. The final sample size for this study was 1003 infants. (Figure 1)

Pertinent clinical characteristics obtained from the VON registry were extracted. Brief definitions of selected variables are provided below. For detailed data definitions by VON, see www.vtoxford.org/tools/2010ManualofOperationswithindex14.pdf. Using this registry ensured consistency in data definitions/entries among the three participating hospitals.

Pregnancy/Birth characteristics included the presence of any obstetrical prenatal care (yes/no) prior to the admission during which birth occurred, administration of antenatal steroids (yes/no) during the pregnancy at any time before delivery, mode of delivery (cesarean section vs vaginal delivery), multiple gestation (yes/no) if two or more live fetuses were documented at any time

during pregnancy, and delivery room oxygen (yes/no) if the infant received any supplemental oxygen in the delivery room.

Infant characteristics included sex (male vs female) and gestational age, which was recorded as the best estimate of gestational age in weeks based on last menstrual period, obstetrical parameters, and prenatal ultrasound. If not available in the maternal chart, the neonatologist assessment based on physical exam was utilized. Birth weight was entered in grams and was the weight from the labor and delivery record or weight on admission to the NICU. Surfactant administration (yes/no) was recorded as any exogenous surfactant given at any time after birth. The presence of respiratory distress syndrome (RDS) (yes/no) was defined as a PaO2 <50mmHg in room air, central cyanosis, or an oxygen requirement within the first 24 hours of life combined with radiographic findings consistent with RDS. Pneumothorax (yes/no) was recorded based on radiographic evidence of extrapleural air or the need for thoracentesis. A need for ventilator therapy (yes/no) was noted if the infant received intermittent positive pressure ventilation via an endotracheal tube (ETT) with a conventional ventilator, or high frequency ventilation (yes/no). Early bacterial sepsis (yes/no) was recorded when a bacterial pathogen was recovered from a blood and/or cerebrospinal fluid (CSF) culture obtained on day 1, 2, or 3 of life. Late bacterial sepsis was recorded when a bacterial pathogen was recovered from a blood/CSF culture obtained after day of life 3. Fungal infection (yes/no) was noted when a fungus was recovered from a blood culture obtained via a central or peripheral blood sample after day of life 3. Necrotizing enterocolitis (NEC) (yes/no) was diagnosed by having one clinical sign and one radiographic sign consistent with NEC and surgery for NEC (yes/no) included laparotomy, resection, or drain placement. A patent ductus arteriosus (PDA) (yes/no) was recorded when clinical signs consistent with a PDA were noted or as seen on echocardiography and a PDA ligation (yes/no) was recorded if surgical ligation was attempted either in the NICU or the OR. Early oxygen exposure on day of life 28 (yes/no) was noted if the infant received any supplemental oxygen on that day. Intraventricular hemorrhage was recorded as the worst grade (0-4) noted

on cranial imaging. In these analyses, only severe IVH (grade 3 and 4) was used, as grades 1 and 2 are generally not associated with adverse outcomes.

ROP exams were completed as per current AAP guidelines[12]. Infants were excluded if they did not have an eye exam recorded. ROP was documented as the worst stage (0-5) noted on eye exams, using the International Classification of Retinopathy of Prematurity criteria[13]. Stage 3, 4, and 5 are classically considered severe ROP, and this is how severe ROP was defined in this study. If infants required ROP Surgery (retinal cryosurgery or laser surgery) this was also noted.

A random subset of patients was selected for a limited chart review. This audit was performed to obtain the timing of ROP exams in relation to oxygen therapy at 36 weeks PMA. The gestational age corresponding to the exam for which the worst stage of ROP was noted was determined to ensure that the infant was receiving late oxygen before severe ROP developed. Ten patients from even years of birth (2002-2010) were selected from the VON registry at Tufts to have the charts reviewed (see appendix). Oxygen exposure was noted as present or absent in the delivery room, at 28 days of life, and at 36 weeks PMA. Early oxygen was defined as oxygen administered during phase I of ROP development up to day of life 28. Late oxygen was defined as oxygen therapy at 36 weeks PMA (yes/no).

The study was approved by the institutional review boards at all 3 centers prior to conducting the study.

Statistical Analysis:

Data from the 3 centers were combined to create the final database and the consistency of the results was examined by study center. The database was first examined for completeness and entry accuracy and found to be complete. Sample characteristics were then obtained. Continuous variables (gestational age, birth weight, and apgar scores) were examined with 2 sample t tests and mean values with standard deviations were calculated for the outcome of severe ROP. Categorical variables were also analyzed with chi square tests and

percents were calculated. Birth year was analyzed as a categorical variable (2002-2010) by chi square analysis to account for possible changes over time.

The incidence of ROP by ROP stage was determined for the entire study sample. Next, variables likely related to ROP were examined by univariable logistic regression with the outcome of severe ROP (greater than or equal to stage 3). After univariable analyses, a multivariable model for severe ROP was created. Clinically important variables included in the model were gestational age, birth weight, sex, respiratory distress syndrome, early sepsis, patent ductus arteriosus, oxygen at 28 days, necrotizing enterocolitis, antenatal steroids, prenatal care, and oxygen at 36 weeks PMA. Stepwise logistic regression was used and entry criteria were standard, at 0.05 significance level.

Logistic regression based propensity scores were then generated for the probability of receiving late oxygen therapy at 36 weeks PMA and used in subsequent analyses as a covariate, as a propensity score is the probability of receiving treatment conditional on observed baseline covariates. In this way, a propensity score can reduce the number of covariates in the model, and also reduce the bias of confounding variables[14]. A list of variables was selected to create the propensity score model for receiving late oxygen at 36 weeks PMA (see appendix). These variables included pregnancy, birth, and infant characteristics. Twenty-three were selected in total as potentially significant clinical variables. These variables were included in a model and then subsequently clinically important interactions were added to create the final propensity score model. Stepwise logistic regression was again used, however, the entry and stay criteria were increased to a 0.3 significance level. This was utilized to be sure no potentially useful variables were excluded in the propensity model. This model is also useful by providing predictors for oxygen therapy at 36 weeks – one definition of bronchopulmonary dysplasia. Histograms were created that show the propensity score distribution for both oxygen use at 36 weeks PMA and no oxygen exposure at 36 weeks PMA, and the overlap that exists – important for stratification analysis (figure 2).

For the propensity score analyses, the propensity score was used as a continuous covariate in logistic regression, as well as stratified into quintiles based on propensity to be treated with late oxygen. The primary analysis chosen was stratification by propensity score. By utilizing this technique, all the data are used and the results are more generalizable, as no subjects were excluded from analysis. The propensity scores to receive late oxygen were stratified into quintiles. Stratification on the propensity score helps to balance covariates that are used to estimate the propensity score, and using 5 strata or quintiles removes over 90% of the bias in each of the covariates[15]. To further analyze the quintiles that were created, minimum, mean, and maximum values for each quintile were compared (see appendix). A logistic regression model for severe ROP using only the propensity score quintiles and oxygen exposure at 36 weeks PMA was then created and compared to a similar logistic regression model using propensity score quintiles, oxygen at 36 weeks PMA, as well as other clinically significant variables. In this final model, we forced in the primary variable (late oxygen use) as well as the propensity score quintiles and stepped in the other variables, including hospital center. The continuous propensity score in an additional logistic regression model was used to further validate our findings in our primary analysis.

We also examined the outcome of ROP surgery given late oxygen use and propensity scores and found similar results (see appendix).

Results:

Of the 568 infants who were excluded due to lack of data for eye exam and/or oxygen at 36 weeks, 182 infants died, 104 were discharged to home and 284 were transferred. Of the infants who died, the mean gestational age was 24.6 weeks (median 24) and the mean length of stay was 11.8 days (median 6.5days). Those that were transferred or discharged had higher mean gestational ages at birth (26.7 weeks and 26.8 weeks respectively) as well as longer lengths of stay (35.1 days and 59.9 days). Comparing available data for those infants who were excluded to those that had data at 36 weeks, those who

were excluded were more significantly likely than those who were included to be born by vaginal delivery (42% vs 34%), more likely to have severe IVH (19% vs 10%), less likely to have PDA (37% vs 47%), less likely to have PDA ligation (6% vs 14%), and less likely to be treated with a ventilator (85% vs 91%) or high frequency ventilator (43% vs 50%).

A total of 1003 infants were ultimately included in the final cohort. Severe ROP ($\geq$ stage 3) was detected in 190 infants (18.9%) and 157 infants (15.7%) underwent ROP surgery (see appendix). In those infants who developed severe ROP, mean gestational age was lower (24.9 $\pm$ 1.2 wks vs 26.3 $\pm$ 1.4wks; $p<0.0001$), as was birth weight (702 $\pm$ 125g vs 911 $\pm$ 223g; $p<0.0001$). Almost 80% of infants who developed severe ROP had oxygen use at 36 weeks PMA, while 46% of those without severe ROP had oxygen therapy. Other clinical characteristics significantly more present in the severe ROP group included lower apgar scores at 1 and 5 minutes, oxygen on day of life 28, severe IVH, PDA, PDA ligation, NEC, NEC surgery, late bacterial sepsis, and late fungal sepsis (table 1).

A random subset of the sample including 10 patients was examined by a chart review to further explore the timing of the ROP exams in relation to treatment with oxygen at 36 weeks PMA. This chart review included only 10 infants given significant time constraints. In this small subset of infants born on even years during the study period, 5 received late oxygen and 5 did not. Nine of the 10 infants had their worst eye exam after 36 weeks PMA (see appendix). This subset was chosen at random and is likely representative of the sample as a whole. 90% of the infants had the worst eye exam noted after 36 weeks PMA, after receiving oxygen (or not) during phase II of ROP development. Given this potential misclassification, 100 simulations were performed to test the results if 10% of the data were misclassified. The OR for oxygen at 36 weeks ranged from 1.19-2.13, with 95% of the ORs in the interval 1.31-1.91. This simulation is reassuring in that even with a potential 10% misclassification, the results remain fairly consistent.

In univariable logistic regression utilizing the outcome of severe ROP (≥ stage 3), both increasing gestational age weeks [OR 0.50, (95%CI 0.44-0.56), p<0.0001] and birth weight grams [OR 0.99, (95%CI 0.993-0.995), p<0.0001] were associated with a reduced risk of ROP. The presence of a PDA, RDS, NEC, oxygen at day of life 28, severe IVH, late bacterial sepsis, fungal sepsis, high frequency ventilation, and oxygen at 36 weeks significantly increased the risk of severe ROP (see appendix).

In multivariable logistic regression for severe ROP while controlling for other factors, gestational age, birth weight, PDA, and late oxygen at 36 weeks PMA were significantly associated with severe ROP. Once again, increasing gestational age [OR 0.74 (95%CI 0.63-0.86); p=0.0002] and birth weight [OR 0.99 (95%CI 0.995-0.997); p<0.0001] were associated with a reduced risk of ROP, while PDA [OR 1.73 (95%CI 1.2-2.5); p=0.0036] and late oxygen use at 36 weeks PMA [OR 2.21 (95%CI 1.46-3.35); p=0.0002] were associated with an increased risk of severe ROP (see appendix).

The propensity score model was created using the characteristics described. This logistic regression models the probability of receiving late oxygen given selected covariates (table 2). The c statistic for the PS model was 0.797 suggesting that the model differentiates subjects with and without late oxygen use, but there is still some overlap between the treated and untreated groups. This indicates that the propensity score is useful, in that there is not perfect discrimination or non-overlap with the logistic regression model for late oxygen use.

In univariable analysis, the OR for severe ROP given oxygen therapy at 36 weeks PMA was 4.50. This decreased to 2.21 after adjustment in multivariable analysis for severe ROP. For the primary analysis, stratification into quintiles by propensity to receive late oxygen was performed and used in logistic regression with late oxygen use. The OR for late oxygen treatment was 2.03 (95%CI 1.34-3.07); p=0.0009. Next, multivariable logistic regression including propensity score quintiles and late oxygen use, as well as adjustment for other clinically important variables including hospital center, was performed yielding

similar results (OR 2.00; 95%CI 1.29-3.09; p=0.0019) (tables 3 and 4). There was no center effect detected or interaction between center and late oxygen use. The c statistic increased from 0.767 to 0.823 and -2LL decreased from 823.65 to 757.47 (p<0.001) with the adjustment for other clinically important variables. Odds ratios by PS quintile were also calculated. For the most extreme PS quintiles, 1st and 5th quintile, the ORs were less than 1, however in the middle 3 quintiles with the most overlap between oxygen exposure at 36 weeks, the OR were > 2.5. In addition, to further explore the propensity score, it was also used as a continuous variable in the logistic regression model, along with late oxygen use, also showing similar results with an OR 1.98 (95%CI 1.30-3.01).

Discussion:

ROP is a cause of morbidity in premature infants that can result in visual impairment and blindness. Most research to date has focused on the pathogenesis of phase I ROP and its possible prevention via vigilant early oxygen control and avoidance of hyperoxia[16,17]. Although important and successful in decreasing the incidence of ROP, phase II of ROP pathogenesis can also be targeted to decrease the severity of ROP. In our study, we have attempted to further explore the theory that supplemental oxygen during phase II of ROP might reduce the ROP severity by decreasing the confounding factors that are present in the ELBW population via propensity scores in this prospectively collected cohort. In our adjustment for confounding factors by the use of propensity scores, late oxygen use remained significantly associated with the development of severe ROP – in contrast to our proposed hypothesis that late oxygen would be a protective factor.

The development of ROP occurs in 2 phases. In phase I, exposure to hyperoxia suppresses vascular endothelial growth factor (VEGF) expression, resulting in cessation of normal vessel growth and regression of existing vessels[3]. This occurs from the time of preterm birth to approximately 30-32 weeks PMA. Phase II of ROP begins when the avascular portion of the retina undergoes significant growth and local hypoxia develops. This hypoxia

stimulates VEGF release and in the presence of elevated insulin-like growth factor 1 (IGF-1), proliferative neovascularization occurs which can lead to retinal detachment and blindness [2,4].

Given this, we hypothesized that exposure to late oxygen during phase II of ROP development would be associated with a reduced risk for severe ROP, as suggested by a recent meta-analysis including 10 cohort and randomized studies for a total of 3088 infants. If supplemental oxygen therapy could reduce local retinal hypoxia, than it might also reduce the aberrant neovascularization and retinal detachment. After adjusting for possible confounding factors, an increased risk of severe ROP was noted. We speculate that this might be due to the increased production of reactive oxygen species and oxidative stress that is damaging in many complications of prematurity, including ROP. In addition to the increased risk of severe ROP with late oxygen use, the presence of a patent ductus arteriosus also remained significantly associated with severe ROP in the final PS adjusted model. As expected, as gestational age and birth weight increased, the risk of severe ROP decreased.

Other studies have shown inconclusive results in oxygen therapy during phase II of ROP development[10,11,18]. Oxygen protocols in the NICU are very carefully regulated in the first few weeks of life in premature infants, keeping tight control of oxygen saturations between 86-92% via early and aggressive oxygen adjustments. It is unknown as to when these parameters should be adjusted and a higher oxygen saturation goal should be adopted, and therefore, increased oxygen administered.

Our data suggest that exposure to oxygen at 36 weeks PMA is associated with an increased risk of severe ROP, contrary to our original hypothesis. A recent meta-analysis demonstrated that high oxygen saturation (94-99%) at 32 weeks PMA or greater was associated with a reduced risk of severe ROP, as suggested in our hypothesis. It is unclear as to how much oxygen was administered to these infants to achieve these goal saturations during phase II of ROP development. It could be that the infants with less severe illness had higher oxygen saturations and less ROP.

Association of illness severity, as measured by a cumulative SNAP score, has been studied with respect to progression of prethreshold ROP to threshold ROP[19]. A higher cumulative SNAP score during phase I of ROP development was associated with an increased risk of progressing to severe ROP. It was also noted that in the first 28 days, infants progressing to severe ROP were more likely than those who did not develop severe ROP to be on supplemental oxygen and mechanical ventilation. The study did not evaluate effects of oxygen at 36 weeks PMA, or during phase II of ROP development. Despite this, it still remains unclear as to the optimal oxygen saturation targets in growing premature infants, as some studies have suggested an increased risk of death when lower oxygen saturation targets are continued through 36 weeks PMA[20].

Many studies examining neonatal outcomes and morbidities are observational studies, including those targeting ROP; however, observational studies are prone to bias and confounding. For example, infants who receive late oxygen might be more critically ill compared to those who do not receive late oxygen, and might be more likely to receive respiratory support longer, be maintained at higher oxygen saturations, and be less likely to be weaned off oxygen as rapidly as other infants. In this study, we have attempted to reduce this potential confounding by using propensity scores to balance the treatment based on selected covariates. With propensity score adjustment, we have demonstrated that infants who receive oxygen during the initial part of phase II ROP development at 36 weeks PMA are twice as likely to develop severe ROP compared to those who do not receive late oxygen.

In creating our propensity score model, we have also created a predictive model for the likelihood of receiving oxygen at 36 weeks PMA, one definition of bronchopulmonary dysplasia (BPD). Significant factors included a decreased risk of late oxygen use with higher birth weights, severe IVH, and surgery for NEC. This decreased risk of late oxygen use in infants requiring surgery for NEC is surprising given that these infants are often critically ill. It is possible that in the infants who receive surgery, the intense inflammatory state that occurs with NEC is reduced. Therefore, compared to infants who do not receive surgery the

duration of inflammation is shorter. The PS model was also significant for an increased risk of late oxygen use in males, early oxygen at day of life 28, high frequency ventilation, late bacterial sepsis, and PDA ligation. This model may be useful in predicting infants at risk for BPD, as well as selecting infants to potentially wean oxygen more aggressively. It may impact decisions for discharge or transfer pending eye exam results, as those at higher risk for late oxygen use may be monitored more closely for the development of ROP.

Limitations:

Despite having such a large and complete database, there were some limitations that existed. First, we do not know the amount of oxygen administered at 36 weeks PMA, only that the infant was receiving oxygen at that time. However, if late oxygen is associated with an increased incidence of ROP and the infants receiving oxygen at 36 weeks PMA included both those with high oxygen exposure as well as low exposure, this exposure misclassification is expected to bias our results towards the null.

Second, the database captures the worst ROP stage recorded. We do not know the timing of the worst exam in relation to oxygen at 36 weeks PMA. However, in our limited chart review, the majority of infants (90%) did not have the worst ROP exam until after 36 weeks PMA. Despite the small number of medical records reviewed, we feel that this random subset likely represents the database as a whole and that the worst eye exam is generally not the initial eye exam – representing the influence of factors during phase II. Also, if infants had the final eye exams after discharge and were found at that time to have severe ROP (not recorded in the VON database) then this would also bias our results towards the null and our increase in severe ROP would actually be higher.

Thirdly, our exclusion criteria excluded those who did not have data recorded for an eye exam or oxygen therapy at 36 weeks PMA. The majority were discharged or transferred prior to 36 weeks, however, 182 infants excluded died before 36 weeks. The mean gestational age of these infants was lower, putting them at risk for developing ROP. The infants who died tended to be the

most immature, were likely more critically ill overall, and therefore more likely to have risk factors for ROP. Given that they died prior to ROP exam, not including them in our analysis may lead to a negative bias in our results. The infants who were transferred to a community nursery or discharged home before 36 weeks were more mature at birth and were less likely to have risk factors for severe ROP. Not including these infants in our analysis may bias our results by increasing our calculated associations when the true value might be lower.

Finally, there is a risk of residual and unmeasured confounding. The magnitude of confounding of this association is likely to be fairly large given the crude OR for late oxygen use was reduced rather substantially by adjustment from 4 to 2. Therefore, it stands to reason that the odds ratio of 2 might contain some residual bias. This could be due to incompletely measured variables or, more likely, unmeasured variables not accounted for in our adjustment, as propensity scores do not account for unmeasured confounders. Given this possible residual confounding, our observed OR might actually be reduced even further.

Strengths:

Despite the limitations of using the Vermont Oxford Registry, this study had several strengths. First, it included a large cohort of infants between 23-28 weeks gestation at birth for which the data was prospectively collected. Second, three different centers were included. Using multiple centers enhances the generalizability of the results. Thirdly, the database was complete and standardized between the centers.

In addition, using propensity scores helped to eliminate possible confounding by indication. Observational studies are encountered frequently in neonatology because they are relatively easy to perform and are usually far less expensive to conduct than multicenter randomized clinical trials in such a specific population. Although these factors make such studies attractive, observational studies can be wrought with bias. Investigators have no control over treatment assignment and large differences can exist between the treated and control

groups, which may lead to biased estimates of treatment effects[15]. By using propensity score adjustments for observed covariates, we have attempted to minimize confounding in this observational study.

Conclusion:

In this hypothesis generating study, we conclude that late oxygen use at 36 weeks PMA during phase II of ROP is associated with an increased risk of developing severe ROP in extremely premature infants, in contrast to our original hypothesis. This might be due to the generation of reactive oxygen species and associated inflammation from oxygen exposure which alters retinal vessel development. Infants who receive oxygen at 36 weeks PMA might therefore, need to be examined more frequently for the development and/or progression of ROP. Future studies using other datasets to confirm our findings, as well as prospective studies that closely monitor oxygen saturation as well as FiO2 of supplemental oxygen are needed to better elucidate the relationship of oxygen and severe ROP.

TABLES:

Table 1: Clinical characteristics in those diagnosed with severe ROP (SROP) and those without severe ROP. p<0.05 indicated in bold.

Variable	No Severe ROP (n=813)	Severe ROP (n=190)	P value
Gestational Age (wks)	26.3 ± 1.4	24.9 ± 1.2	**<0.0001**
Birth Weight (grams)	911 ± 223.3	702.3 ± 125.4	**<0.0001**
Apgar Score 1 minute	4.7 ± 2.5	4 ± 2.4	**0.0002**
Apgar Score 5 minutes	7 ± 2	6.4 ± 2	**0.002**
Prenatal Care	797 (98%)	188 (99%)	0.39
Antenatal Steroids	698 (85.9%)	168 (88.4%)	0.35
Vaginal Delivery	274 (33.7%)	67 (35.3%)	0.68
Sex (Males)	438 (53.9%)	98 (51.6%)	0.57
Multiple Birth	249 (30.6%)	59 (31.1%)	0.91
Delivery Room Oxygen	806 (99.1%)	188 (99%)	0.8
Oxygen on DOL 28	630 (77.5%)	185 (97.4%)	**<0.0001**
Early Bacterial Sepsis	11 (1.4%)	2 (1.1%)	0.74
Severe IVH	74 (9.1%)	28 (14.7%)	**0.02**
PDA	347 (42.7%)	126 (66.3%)	**<0.0001**
PDA Ligation	86 (10.6%)	57 (30%)	**<0.0001**
NEC	40 (4.9%)	19 (10%)	**0.007**
NEC Surgery	24 (3%)	17 (9%)	**<0.0002**
RDS	740 (91%)	188 (99%)	**0.002**
Pneumothorax	53 (6.5%)	18 (9.5%)	0.15
Late Bacterial Sepsis	111 (13.7%)	50 (26.3%)	**<0.0001**
Late Fungal Sepsis	21 (2.6%)	12 (6.3%)	**0.009**
Late Oxygen 36 weeks PMA	376 (46.3%)	151 (79.5%)	**<0.0001**

*continuous variables by 2 sample t-test, categorical variables by chi square

Table 2: Propensity Score Model* for late oxygen use at 36 weeks PMA

Variable	OR	95% CI	p value
Prenatal Care	1.97	0.64-6.02	0.23
Birth Weight (grams)	0.998	0.998-0.999	<0.0001
Vaginal Delivery	1.30	0.95-1.78	0.11
Sex	1.90	1.41-2.55	<0.0001
Early Oxygen on Day of Life 28	5.33	3.31-8.60	<0.0001
Severe IVH	0.60	0.36-0.99	0.04
High Frequency Ventilation	2.53	1.85-3.47	<0.0001
PDA	1.27	0.92-1.74	0.15
PDA Ligation	2.13	1.27-3.59	0.004
Late Bacterial Sepsis	2.32	1.50-3.59	0.0002
Surgery for NEC	0.41	0.19-0.89	0.02

*Model adjusted for birth year, antenatal steroids, multiple gestation, gestational age, delivery room oxygen, early bacterial sepsis, ventilator use, surfactant treatment, respiratory distress syndrome, pneumothorax, necrotizing enterocolitis, fungemia, and the following interactions: sex*RDS, gestational age*multiple birth,gestational age*early bacterial sepsis, gestational age*IVH, gestational age*ventilator use, gestational age*RDS, gestational age*NEC, gestational age*fungemia, RDS*PDA, RDS*antenatal steroids, RDS*delivery room oxygen, RDS*early bacterial sepsis, RDS*early oxygen exposure

Table 3: Multivariable Logistic Regression Model for Severe ROP with Adjusted PS Quintiles (n=1003)

Variable	OR	95% CI	p value
PS Quintiles	1.15	0.95-1.40	0.16
Late Oxygen at 36 Weeks PMA	2.00	1.29-3.09	0.002
Gestational Age (weeks)	0.77	0.65-0.90	0.001
Birth Weight (grams)	0.996	0.995-0.998	<0.0001
Patent Ductus Arteriosus	1.60	1.08-2.35	0.018

Table 4: Association between late oxygen use and severe ROP in univariable, multivariable adjusted, propensity score quintile, and adjusted propensity score quintile models (n=1003)

Model	OR (Ox36)	95% CI	p value	-2LL	c
Univariable Model	4.50	3.08-6.57	<0.0001	901.22	0.666
Multivariable Model (adjusted)	2.21	1.46-3.35	0.0002	759.45	0.823
Ox36 + PS quintiles	2.03	1.34-3.07	0.0009	823.65	0.767
Ox36 + PS quintiles (adjusted)	2.00	1.29-3.09	0.0019	757.47	0.823

Figures:

Figure 1: Patient Flow Diagram

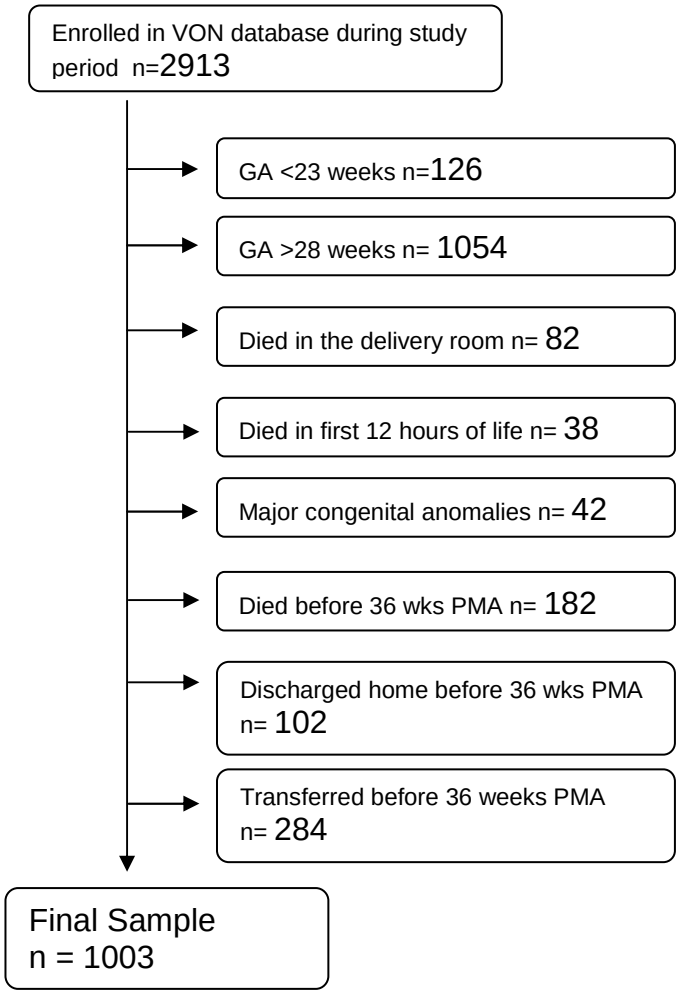
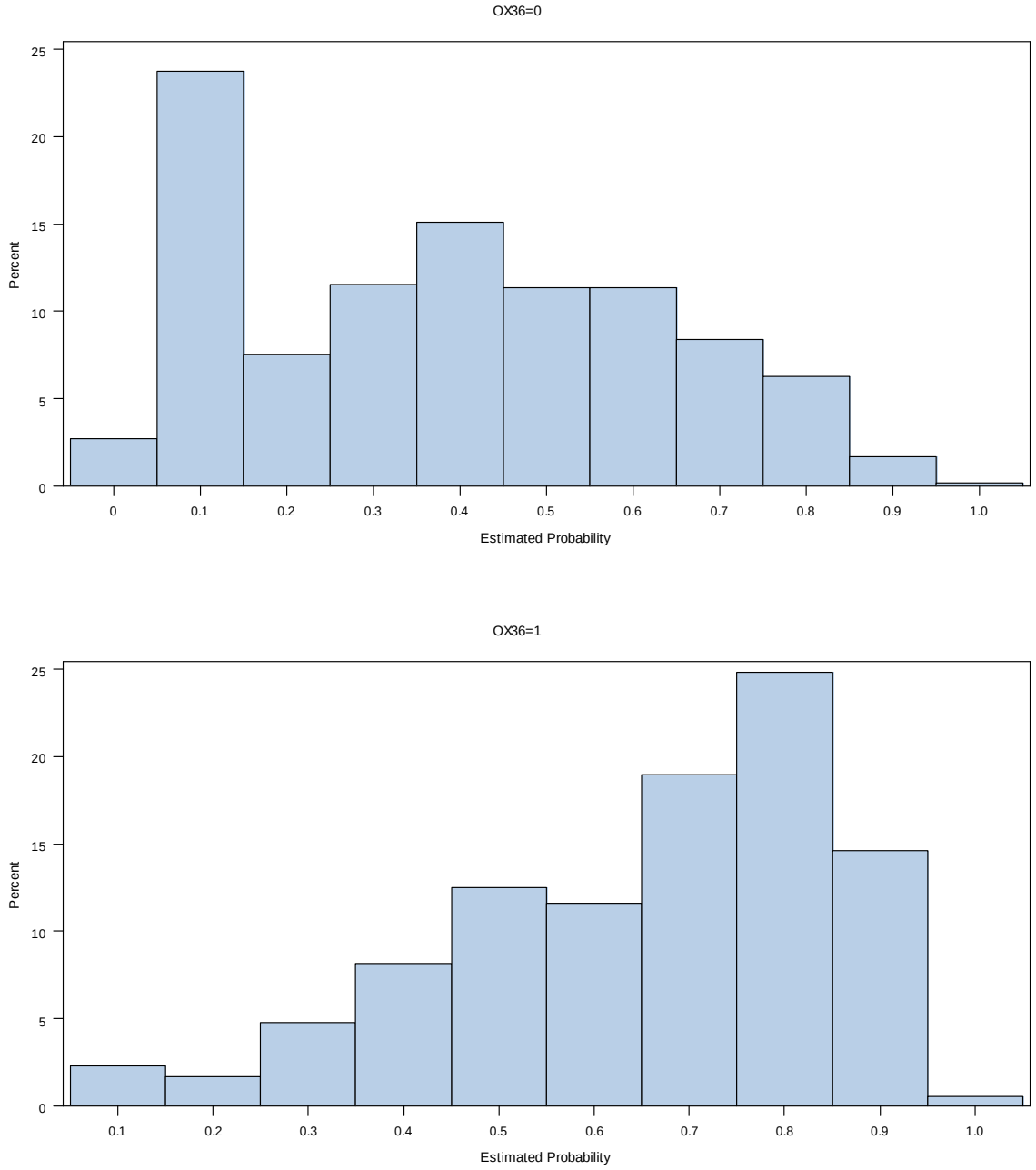


Figure 2: Distribution of Propensity Scores by Late Oxygen Use.



Appendices:

Appendix 1:

Retinopathy of Prematurity

Background:

Retinopathy of prematurity (ROP) is a vaso-proliferative disorder of the developing retina that mainly occurs in preterm newborns[21]. The long term visual outcome among children with ROP includes a prominently increased risk for blindness[22] and visual disability[23]. It is a leading cause of impaired vision and blindness throughout the world[1]. While normal retinal vasculature development occurs in utero and is completed between 36-40 weeks gestational age (GA)[2], the abnormal vessel growth of ROP begins to develop at the time of premature birth. Exposure to oxygen has been shown to be a critical factor in the development of ROP [3].

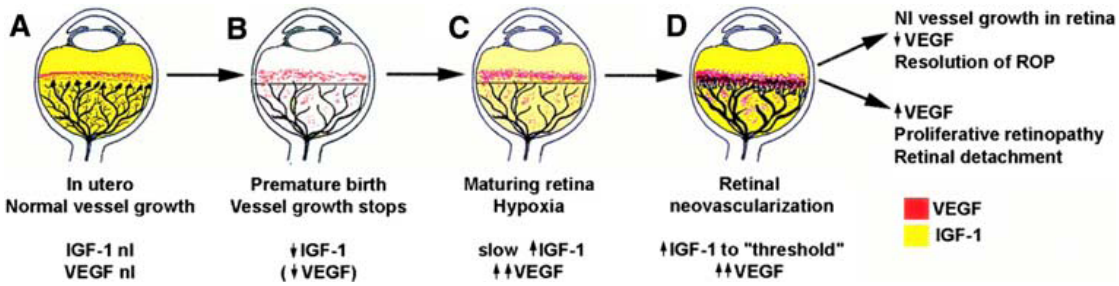
Pathophysiology:

Infants born prematurely have incompletely vascularized retinas. The normal fetal PaO2 in utero is approximately 25-30mmHg[24]. Upon birth, the developing retina is exposed to a relatively hyperoxic environment, as even room air leads to a PaO2 of 60-80mmHg[4]. In phase I, exposure to hyperoxia suppresses vascular endothelial growth factor (VEGF) expression, resulting in cessation of normal vessel growth and regression of existing vessels[3]. This occurs from the time of preterm birth to approximately 30-32 weeks PMA.

As phase II of ROP begins around 30-32 weeks, the avascular portion of the retina becomes metabolically active in the setting of a limited vascular supply, and local hypoxia develops. The hypoxia stimulates VEGF release and neovascularization occurs between the avascular and vascularized retina. This proliferation of vessels can lead to a fibrous scar and retinal detachment [4]. Also in phase II ROP, insulin like growth factor I (IGF-1) contributes to the abnormal retinal neovascularization. When IGF-I is decreased, the retinal vessels stop growing and the avascular retina becomes hypoxic, increasing VEGF. As IGF-I

rises in the setting of high levels of VEGF, rapid growth of new blood vessels is triggered[2].

Figure 1: Fig. 1 Schematic representation of IGF-I/VEGF control of blood vessel development in ROP. (A) In utero, (B) Premature birth, (C) Retinal maturation, (D) Retinal neovascularization. NI: normal



Chen J, **Smith** LE. Retinopathy of Prematurity. Angiogenesis. **2007**;10(2):133-40. Epub **2007** Feb 27. Review.

i) Vascular Endothelial Growth Factor (VEGF)

VEGF is an important regulator of retinal angiogenesis and plays a crucial role in both phases of ROP pathogenesis [25]. VEGF is expressed in response to hypoxia. During normal retinal development, the neural retina develops anterior to the vasculature and the subsequent localized hypoxia stimulates production of VEGF anterior to the developing vessels. Phase I of ROP begins after premature birth. The sudden postnatal hyperoxia relative to in utero levels suppresses VEGF mRNA expression and normal VEGF-driven vascular growth. Apoptosis of vascular endothelial cells and vaso-obliteration occur. During phase I, the neural retina continues to develop and the non-vascularized retina becomes hypoxic as it grows. This stimulates increased production of VEGF, and at a postmenstrual age (30–31 weeks) in combination with IGF-1, induces an uncontrolled neovascularization that may lead to retinal detachment[26].

In addition, inhibition of VEGF bioactivity by a neutralizing antibody to VEGF reduces VEGFR2 signaling and inhibits intravitreous neovascularization, retinal tortuosity, and dilation, without interfering with ongoing intraretinal neovascularization. However, concerns remain because VEGF is a survival factor for neurons and endothelial cells, so use in preterm infants requires careful study[27].

ii) Insulin Like Growth Factor 1:

IGF-1 is a maternally derived growth factor that is provided by the placenta and amniotic fluid. It is a polypeptide hormone that functions as a somatic growth factor and increases with increasing birth weight and gestational age, particularly during the third trimester[28]. Preterm birth causes plasma levels of IGF-1 to fall due to the loss of the placental and amniotic fluid source of the factor[29]. Maternal systemic inflammation might also contribute to the development of ROP in preterm infants by decreasing levels of IGF-1[30]. Prior studies have shown that low IGF serum levels can be used as a screening factor for predicting ROP[31]. The lack of insulin-like growth factor I (IGF-I) in mice prevents normal retinal vascular growth despite the presence of vascular endothelial growth factor.[31] Additional studies have shown that using IGF-1 receptor antagonists can suppress the proliferative phase II ROP neovascularization[4,32].

iii) Inflammation, Infection, and ROP:

It has recently become evident that the $VEGF_{164}$ isoform leads to leukocyte recruitment in pathologic hypoxia-induced retinal neovascularization[33]. Oxidative stress, which can be a consequence of inflammation[34], has also been implemented in ROP etiology[35]. Moreover, the authors of one recent study that reported a reduction of pathological retinal angiogenesis by omega-3-polyunsaturated fatty acids write that "the protective effect of omega-3-PUFAs and their bioactive metabolites was mediated, in part, through suppression of tumor necrosis factor-alpha"[36], one of the major pro-inflammatory cytokines. Taken together, these data suggest that inflammatory processes might be part of the retinal neovascularization process, the hallmark of ROP.

Low birth weight preterm infants are particularly susceptible to infection, as rates of infection increase with decreasing birth weight and gestational age. Postnatal infection is associated with significant morbidity and neonatal complications, prolonged hospitalization, and death[37]. It has been suggested for quite some time that exposure of the preterm newborn to infection and inflammatory responses is associated with an increased risk for ROP. In a large

cohort study, infants who developed early onset sepsis were associated with an increased risk for severe ROP[38]. In the ELGAN study, there was an increased risk for ROP mainly with late neonatal sepsis[39]. Multiple other studies support the idea that neonatal sepsis not further specified[40-43] and candida infection[44] are risk factors for severe ROP.

Fungal infection has been linked to chorioretinitis and ROP[45]. *Candida* is known to interact with vascular endothelial cells via multiple mechanisms, including release of proinflammatory cytokines which injure the developing retinal blood vessels[46]. *Candida* sepsis has been independently associated with increased severity of ROP as well as need for surgical therapy for advanced ROP[44]. A systematic review and meta-analysis of 8 studies found that systemic fungal infection in very low birth weight infants was significantly associated with ROP and severe ROP[47].

At least part of the sepsis-associated risk increase for ROP might be due to circulating products of inflammation. In preterm infants with early-onset sepsis, studies suggest there is a relationship between high plasma levels of cytokines IL-6, IL-8, and tumor necrosis factor-α (TNF-α) in the first days of life with the later development of ROP severe enough to treat[48]. Recent findings from a very large cohort study, suggest that levels of circulating proinflammatory cytokines are elevated at multiple timepoints after birth in preterm infants who later develop ROP when compared to controls[49].

iv) Oxidative Stress and ROP:

Under normal conditions, a delicate balance exists between the production of reactive oxygen species (ROS) and the antioxidant defenses that protect cells *in vivo*. The balance may be disturbed by increased ROS production or an inability to quench production because of inadequate antioxidant defenses. The premature infant is especially susceptible to ROS-induced damage for two major reasons. First, adequate concentrations of antioxidants may be absent at birth. Increases in antioxidant capacity occur in the latter part of gestation in preparation for the transition to extrauterine life. Second, the ability to increase synthesis of antioxidants in response to hyperoxia or other oxidant challenges is

relatively impaired. This can lead to an increased risk for the development of ROS-induced diseases of the newborn, such as ROP [50].

The developing retina in premature infants is particularly susceptible to damage mediated by ROS, as evidenced in animal studies. Oxygen fluctuations can induce cells to express NADPH oxidase, which leads to increased ROS and apoptosis of endothelial cells, contributing to the avascular retina. N-acetylcysteine (NAC) has been shown to decrease lipid hydroperoxide (LHP) in a rat model, but was not found to significantly reduce avascularity or clock hours of neovascularization[51]. Repeated oxygen fluctuations also increased retinal vascular endothelial growth factor (VEGF) and ROS. Neutralizing VEGF bioactivity reduced neovascularization and tortuosity, and inhibiting ROS with the NADPH oxidase inhibitor apocynin reduced the avascular retina by interfering with apoptosis [52].

v) Oxygen:

As mentioned above, even room air is relatively hyperoxic compared to the oxygen levels in utero. Hyperoxia suppresses VEGF and causes cessation of vessel growth during phase I of ROP. In phase II, local hypoxia stimulates VEGF production, and in the presence of sufficient IGF-I, neovascularization occurs. In lab studies, supplying oxygen during the pre-proliferative stage of ischemic retinopathy, after a period of retinal ischemia, prevented the development of abnormal neovascularization. Hyperoxia therapy also promoted the recovery and repair of damaged capillary beds within the retina, offering the possibility of significantly improving visual outcomes compared with current antiangiogenic or retinal ablative interventions[53]. Titrating supplemental oxygen during phase II to avoid hypoxia and aberrant vessel growth, while also avoiding hyperoxia leading to production of damaging reactive oxygen species may decrease severe ROP development.

A systematic review was performed in 2003 to determine in preterm or low birth weight infants with prethreshold ROP, if targeting higher as compared to normal oxygen levels or pulse oximetry levels when using supplemental oxygen would reduce the progression of ROP to threshold disease and improve visual

outcome without any adverse effects. Only one trial was included and the results did not show a statistically significant reduction in the rate of progression to threshold ROP with supplemental oxygen treatment. It called for additional research to determine whether infants without plus disease are more likely to respond to supplemental oxygen therapy than those with plus disease[10]. A recent systematic review and meta analysis, including ten studies determined that among preterm infants with a gestational age of ≤32 weeks, early low and late high oxygen saturations were associated with a reduced risk for severe ROP[11].

In a kitten model of ROP, supplemental oxygen administration significantly reduced proliferative vasculopathy of ROP[54]. This was further explored in the supplemental therapeutic oxygen for prethreshold ROP (STOP-ROP) study. STOP-ROP was designed to test the hypothesis that supplemental oxygen, given to attain a pulse oximetry range of 96% to 99% saturation, would reduce the proportion of infants who progress from moderate ROP (prethreshold) to severe (threshold) ROP requiring peripheral ablative surgery[18]. Use of supplemental oxygen did not cause additional progression of prethreshold ROP but also did not significantly reduce the number of infants requiring peripheral ablative surgery. A subgroup analysis suggested a benefit of supplemental oxygen among infants who have prethreshold ROP without plus disease, but this finding requires additional study. Although the relative risk/benefit of supplemental oxygen for each infant must be individually considered, supplemental oxygen did not appear to exacerbate active prethreshold ROP[18].

Research Question:

It is common practice to keep tight control of oxygen during the first weeks of life in premature infants to prevent hyperoxia and development of inflammation and reactive oxygen species. Preventing hyperoxia during phase I of ROP helps to diminish VEGF down-regulation and promote normal vessel growth. But, when does the oxygen administration pendulum swing from risk to benefit? It is unclear from current research whether oxygen during phase II of ROP, at 31-32

weeks postmenstrual age, can also help to decrease severe ROP by preventing local hypoxia and the resulting neovascularization.

By examining a cohort of infants with a variety of clinical conditions, this study attempts to answer the question of whether oxygen exposure after 32 weeks PMA, during phase II of ROP, is associated with a decreased risk of severe ROP while controlling for other variables.

Appendix 2:

Propensity Scores

Research in special populations, such as in newborns requiring intensive care is difficult, time consuming, and expensive. The chronic conditions that exist are frequently rare disorders in small populations, making it difficult for large scale comparative research. Given the difficulty in attaining appropriate sample sizes for rigorous neonatal RCTs, observational studies lacking randomization is a more feasible option.[55] However, observational studies must be analyzed and interpreted with caution, as they are prone to bias and confounding by indication based on differences in patient characteristics that influence treatment decisions.

Propensity score analysis was introduced by Rosenbaum and Rubin in 1983[56]. The propensity score is a measure of the likelihood that a person would have been treated on the basis of only his or her covariate scores. It is the probability that a patient is in the "treated" group given his/her background (pretreatment) characteristics[57]. Propensity scores are a statistical technique for dealing with selection bias in observational studies. Selection bias arises when certain types of patients are more or less likely to receive treatment owing to possible confounding by indication[58]. The treatment groups may markedly differ with respect to the observed pretreatment covariates measured on patients. These differences could lead to biased estimates of treatment effects. The propensity score for an individual, defined as the conditional probability of being

treated given the individual's covariates, can be used to balance the covariates in the 2 groups and thus reduce this bias[57].

In randomized controlled trials, the randomization of subjects to different treatments minimizes the chance of differences on observed or unobserved covariates. However, in nonrandomized observational studies, systematic differences can exist between treatment groups. To control for this potential bias, information on measured covariates can be incorporated into the study design or into estimation of the treatment effect. However, such methods of adjustment can often use only a limited number of covariates and are highly dependent on using the right model, whereas adjustments that use propensity scores do not have such limitation[57].

Rosenbaum and Rubin have described the role of propensity scores in observational studies. The propensity score is defined as the conditional probability of assignment to a particular treatment given a multivariable vector of observed covariates[56]. The propensity score aims to balance or ensure similarity between the treated and untreated groups based on measured confounders under the assumption that treatment assignment is ignorable[59]. Comparing treatment groups conditional on the propensity score yields unconfounded estimates of the treatment effect, given that there are not important unmeasured confounders[60].

Four different methods have primarily been employed utilizing propensity scores. These include stratification based on the propensity score, matching on the propensity score, covariate adjustment using the propensity score, and inverse probability of treatment weighting using the propensity score[14]. When building the propensity score model, only covariates that occur pretreatment are included.

Subclassification or stratification into approximately five groups (quintiles) on the basis of the propensity score adjusts for most of the covariates that went into its estimation[61]. The key feature of stratification is to make participants within a stratum as homogeneous as possible in terms of observed covariates. It is assumed that within-stratum differences based on covariate x are ignorable.

When treatment and control groups overlap in their covariate distributions, comparisons using five subclasses typically removed over 90% of the bias.[59]

Another common method is matching based on the propensity score. Based on the chosen degree of matching on PS, there may be a significant number of participants that cannot be matched, and therefore, not used in analysis. This ensures the matched pairs are similar, but the results might be less generalizable, given many subjects are not included in the final analysis.

For this analysis, logistic regression was used to create a propensity score for the treatment of late oxygen at 36 weeks PMA. This was then used to determine effects on the outcome of severe ROP. The primary analysis was stratification on PS into quintiles. We chose this method over matching given the significant amount of overlap noted in our analysis, as well as the benefit of using all of the data, without having to discard subjects that could not be matched. A secondary analysis using the PS as a covariate was also performed.

Appendix 3:

Additional Tables

Table 1: Incidence of ROP by stage

Table 2: Random subset chart review

Table 3: Variables included in PS Model

Table 4: Distribution of PS quintiles

Table 5: Univariable Logistic regression severe ROP

Table 6: Multivariable Logistic regression severe ROP

Table 7: Univariable Logistic Regression for ROP Surgery

Table 8: Multivariable Logistic Regression for ROP Surgery

Table 9: ROP Surgery in univariable, multivariable adjusted, propensity score quintile, and adjusted propensity score quintile models

Table 1: Prevalence of ROP by Stage and ROP Surgery

Stage	Frequency	Percent
0	359	35.8
1	147	14.7
2	307	30.6
3	186	18.5
4	4	0.4
ROP Surgery	157	15.7

Table 2: Subset of Data for ROP Exams

Birth Year	Late Oxygen Use	GA Worst Eye Exam	ROP Exam #
2002	Yes	36 4/7	6
2002	No	40 2/7	6
2004	Yes	36 07	6
2004	No	40 3/7	5
2006	Yes	36 6/7	3
2006	No	32 5/7	3
2008	Yes	37 4/7	5
2008	No	No ROP	3
2010	Yes	No ROP	5
2010	No	37 5/7	6

Table 3: Variables Used to Create Propensity Score

Pregnancy Characteristics	Prenatal Care
	Multiple Gestation
	Antenatal Steriods
Birth Characteristics	Birth Year
	Vaginal Delivery
	Delivery Room Oxygen
	Surfactant
Infant Characteristics	Gestational Age
	Birth Weight
	Sex
	Early Sepsis
	Late Sepsis
	Fungal Sepsis
	Necrotizing Enterocolitis (NEC)
	NEC Surgery
	Patent Ductus Arteriosus (PDA)
	PDA Ligation
	Ventilator
	High Frequency Ventilation
	Respiratory Distress Syndrome (RDS)
	Pneumothorax
	Oxygen at 28 Weeks
	Intraventricular Hemorrhage

Table 4: Distribution of PS Quintiles

		Estimated Probability			
	N	Min	Mean	Max	OR (Ox36)
Rank for Variable PS					
0	200	0.03	0.13	0.27	<0.001
1	201	0.27	0.38	0.47	2.72
2	201	0.47	0.56	0.64	3.01
3	200	0.64	0.71	0.78	2.7
4	201	0.78	0.85	0.96	0.89

Table 5: Univariable Logistic Regression for Severe ROP

Variable	OR	CI	Pr>chiSq
Gestational Age (weeks)	0.50	0.44-0.56	<.0001
Birth Weight (grams)	0.994	0.993-0.995	<.0001
Sex	0.91	0.67-1.25	0.568
Respiratory Distress Syndrome	9.27	2.26-38.13	0.0002
Early Bacterial Sepsis	0.78	0.17-3.53	0.743
Patent Ductus Arteriosus	2.64	1.90-3.68	<.0001
Early Oxygen at Day of Life 28	10.75	4.36-26.53	<.0001
Necrotizing Enterocolitis	2.15	1.21-3.80	0.009
Antenatal Steroids	1.26	0.77-2.05	0.355
Prenatal Care	1.89	0.43-8.28	0.399
Late Bacterial Sepsis	2.26	1.55-3.30	<.0001
Fungemia	2.54	1.23-5.26	0.009
High Frequency Ventilation	4.20	2.92-6.03	<.0001
Severe IVH	1.73	1.08-2.75	0.022
Late Oxygen at PMA 36 Weeks	4.50	3.08-6.57	<.0001

Table 6: Multivariable Logistic Regression for Severe ROP*

Variable	OR	CI	Pr>chiSq
Gestational Age (weeks)	0.74	0.63-0.86	.0002
Birth Weight (grams)	0.996	0.995-0.997	<.0001
Patent Ductus Arteriosus	1.73	1.20-2.50	.0036
Late Oxygen at PMA 36 Weeks	2.21	1.46-3.35	.0002

*adjusted for sex, respiratory distress syndrome, early oxygen at day of life 28, necrotizing enterocolitis, high frequency ventilation, late bacterial sepsis, fungal sepsis, and severe IVH

Table 7: Univariable Logistic Regression for ROP Surgery

Variable	OR	95% CI	p value
Gestational Age (weeks)	0.46	0.40-0.53	<0.0001
Birth Weight (grams)	0.993	0.992-0.995	<0.0001
Sex	0.94	0.67-1.33	0.74
Early Bacterial Sepsis	0.45	0.06-3.45	0.44
Patent Ductus Arteriosus	2.90	2.02-4.18	<0.0001
Early Oxygen at Day of Life 28	14.37	4.53-45.56	<0.0001
Necrotizing Enterocolitis	2.33	1.29-4.20	0.005
Antenatal Steroids	1.26	0.75-2.14	0.38
Prenatal Care	1.49	0.34-6.55	0.6
Late Bacterial Sepsis	2.74	1.85-4.07	<0.0001
Fungemia	2.08	0.95-4.57	0.067
High Frequency Ventilation	4.36	2.92-6.5	<0.0001
Severe IVH	1.67	1.01-2.75	0.045
Late Oxygen at PMA 36 Weeks	4.31	2.86-5.05	<0.0001

Table 8: Multivariable Logistic Regression for ROP Surgery*

Variable	OR	CI	Pr>chiSq
Gestational Age (weeks)	0.68	0.57-0.81	<0.0001
Birth Weight (grams)	0.996	0.994-0.998	<0.0001
Patent Ductus Arteriosus	1.95	1.30-2.92	0.001
Late Oxygen at PMA 36 Weeks	1.82	1.15-2.88	0.01
Late Bacterial Sepsis	1.58	1.01-2.47	0.045

*adjusted for sex, respiratory distress syndrome, early oxygen at day of life 28, necrotizing enterocolitis, high frequency ventilation, fungal sepsis, and severe IVH

Table 9: ROP Surgery in univariable, multivariable adjusted, propensity score quintile, and adjusted propensity score quintile models

Model	OR (Ox36)	95% CI	p value	-2LL	c
Univariable Model	4.31	2.86-5.05	<0.0001	811.98	0.661
Multivariable Model (adjusted)	1.82	1.15-2.88	0.01	660.18	0.843
Ox36 + PS quintiles	1.75	1.11-2.75	0.016	726.17	0.779
Ox36 + PS quintiles (adjusted)	1.68	1.04-2.71	0.033	660.65	0.84

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