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Blood Gases and Retinopathy of Prematurity: The Extremely Low Gestational Age Newborn Study

A Thesis Submitted to the

Yale University School of Medicine

in Partial Fulfillment of the Requirements for the

Degree of Doctor of Medicine

by

Alisse K. Hauspurg

2012

BLOOD GASES AND RETINOPATHY OF PREMATURITY: THE EXTREMELY LOW GESTATIONAL AGE NEWBORN STUDY. Alisse K. Hauspurg and Richard A. Ehrenkranz. Department of Pediatrics, Yale Univeristy School of Medicine, New Haven, CT.

This study tested the hypothesis that preterm infants who had a blood gas derangement on at least 2 of the first 3 postnatal days are at increased risk for more severe retinopathy of prematurity (ROP). 1,042 infants born before 28 weeks' gestational age (GA) were included. An infant was considered at risk if his/her blood gas measure was in the highest or lowest quartile for GA on at least 2 of the first 3 postnatal days. Multivariable models adjusting for confounders indicate that exposure to a PCO_2 in the highest quartile predicts ROP stage 3, 4 or 5: (OR = 1.6, 95% CI = 1.1–2.3); zone 1: 2.0, 1.1–3.6; prethreshold/threshold: 1.9, 1.2–3.0; plus disease: 1.8, 1.1–2.9. Estimates are similar for a low pH for zone 1 (2.1, 1.2–3.8), prethreshold/threshold (1.8, 1.1–2.8), but did not quite achieve statistical significance for ROP stage 3, 4, or 5 (1.4, 0.9–2.0) and plus disease (1.5, 0.9–2.4). A PaO_2 in the highest quartile for GA on at least 2 of the first 3 postnatal days was associated with a doubling of the risk of ROP in zone 1 (2.5, 1.4–4.4) and of prethreshold/threshold disease (2.1, 1.4–3.3), a 70% risk increase for plus disease (1.7, 1.04–2.8), while a 40% risk increase for ROP stage 3 or higher did not achieve statistical significance (1.4, 0.96–2.0). Infants who experienced high PCO_2, low pH and high PaO_2 during the first three prenatal days appear to be at increased risk of more severe ROP.

Acknowledgements

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Introduction

Extremely Low Gestational Age Newborns (ELGANs), or infants born at less than 28 weeks gestational age (GA), are at a high risk of developing retinopathy of prematurity (ROP), a developmental vascular disorder that is one of the most common causes of pediatric blindness in the United States (1,2). The smallest, most preterm infants, who have an increased survival rate with recent advances in neonatal care, also experience the most severe forms of ROP (3).

The history of ROP dates back to its initial discovery by Dr. Theodore L. Terry in 1942. Terry described an infant with grey, blood vessel-covered membranes behind its pupil, and hypothesized that "some new factor has arisen in extreme prematurity to produce such a condition." As the number of described cases increased, the condition became known as retrolental fibroplasia (RLF). One of the factors Terry originally considered in his early case reports was the excess exposure of premature infants to light, leading to early activation of pupillary and ciliary body musculature **(**4). Other factors he suggested could be contributing to the development of RLF included genetic factors, premature closure of the ductus arteriosus and foramen ovale, hyperoxia, hypoxia, hypothermia or endocrine factors (5).

It was noted to be increasingly common in premature infants, and it was recognized that infants were not born with RLF but rather developed it after birth

(6). Researchers continued to suggest various theories to explain and attempt to prevent this epidemic of blindness, including maternal factors, multiple gestations, infection, poor nutrition, iron deficiency, and other vitamin deficiencies (7).

As multiple risk factors leading to RLF were examined and dismissed, the prominent role of oxygen in its development was discovered with the first multicenter randomized controlled trial conducted by Kinsey and Hemphill (8). This study challenged the standard practice of administering 50% oxygen to premature infants in the first 4 weeks of life to prevent apnea of prematurity. Instead, infants randomized to the experimental group received room air oxygen unless they became cyanotic, at which point oxygen administration was increased, however, never to above 50%. Infants who received lower levels of oxygen developed RLF at much lower rates and with much less severity. Many did not develop RLF at all. The results of this trial were the first evidence of the role that oxygen played in the development of RLF. Following this, clinicians and researchers alike attributed the development of RLF to excess oxygen exposure alone, and thus, oxygen use was restricted in nurseries (8).

During this time, many researchers observed that in some cases RLF occurred with similar frequency in infants who did not receive oxygen. These cases were initially thought to be exceptions to the rule, and many were

dismissed as anomalies. In fact, many authors during this time called attention to the weakness of the oxygen hypothesis, but their criticism was largely ignored (7). Throughout the 1950s and 1960s, RLF nearly disappeared. Medical students were hardly taught about it, as it was thought to be a condition of past mistakes in neonatology. However, it is worth noting that despite tight control on oxygen administration to preterm infants, there were still a number of cases of RLF described.

During this time the “40% rule” arose in many clinical centers, which emphasized that 40% oxygen was safe for the eyes of premature infants, but any higher level of oxygen was dangerous. Despite the seeming protection of infants’ eyes from RLF with the curtailing of oxygen, neonatal mortality actually rose, as did spastic diplegia (9). Later investigations estimated that for every case of blindness prevented with control of oxygen therapy, approximately 16 infants died secondary to inadequate oxygenation (10). Following these developments, researchers began to advocate more “judicious” use of oxygen in the mid-1960s.

However, it warrants mention that during this time, there were no intravenous fluids, and no ventilators, thus there were very few interventions performed by neonatologists during these years. As these modalities of care were implemented in neonatal intensive care units in the mid-1970s, survival of preterm infants significantly improved. With improved survival at increasingly low

gestational ages, however, came a reemergence of RLF at extremely high rates, which could no longer be solely attributed to high oxygen exposure (7).

Animal studies conducted during this period seemed to suggest that it was not only oxygen, but also the degree of prematurity of the eye that was among the critical risk factors for development of the disease. Secondary to these findings, RLF was renamed retinopathy of prematurity (ROP). It was subsequently found that the greater the degree of immaturity of the eye (or prematurity of the infant) the greater the risk of ROP (7). Techniques began to develop to provide optimum oxygen amounts to maximize survival without damage to the brain, while attempting to minimize the risks of development of ROP. However, during this period, a second epidemic of ROP occurred, in spite of attempts to carefully control oxygen therapy. Researchers estimated that during this second epidemic, the number of infants blinded by ROP each year was comparable to the number blinded during the first epidemic, which spanned ten years between 1943 and 1953 (7).

In spite of the seemingly widespread recognition of the existence of ROP and the extensive research into predisposing factors for its development, there was no universal method of classifying the progression of ROP or its severity until the early 1980s. The classification system, International Classification of ROP (ICROP) was developed by neonatologists and ophthalmologists together

and first described in the literature in 1984. The ICROP system utilizes several different components to classify ROP, including position (zone), severity (stage), extent (clock hours) and presence or absence of plus disease (12). For a visual depiction of the ICROP classification system, see figure 1.

In terms of zones of the retina, ICROP describes three zones; with vessels growing from the optic disc initially crossing zone I, then zone II, and finally crossing zone III to reach the ora serrata. The blood vessels grow from the optic disc beginning at approximately 16 weeks GA, as such, the more premature an infant is, the less progress the vessels will have made towards the ora serrata. Thus, ROP seen in zone I initially has a much worse prognosis than that of ROP seen in zone III, which generally has a more benign, milder course (12).

Similarly, classification of stages of ROP is based on the overproduction of vessels at the transition between the vascularized and avascular retina. The abnormal vascular response at the junction of the vascularized and avascular retina is classified as stage 1 through stage 5, with stage 5 representing the most severe disease. Stage 1 is characterized by a thin demarcation line separating the vascularized retina anteriorly from the avascular retina posteriorly. There is abnormal branching of vessels leading up to the demarcation line. In stage 1 ROP, the line is relatively flat, white and is within the same plane as the retina. Stage 2 ROP is characterized by the progression of the demarcation line in stage

1 into a ridge. The ridge has height, width and extends above the retinal plane, in contrast to the demarcation line, which, as previously mentioned is relatively flat. In stage 3 disease immature vessels break through the retina, and this neovascularization extends from the ridge into the vitreous. For a visual depiction of the progression of the demarcation line from stage 1 through stage 3 ROP, see figure 2. Stage 4 disease is characterized by partial retinal detachment. Finally, stage 5 ROP, the most severe stage, represents complete detachment of the retina (13). For more detailed descriptions of specific retinal findings associated with each ROP stage, see table 1.

The clock hours, or extent classification describes the number of clock hours of the zone that contains the stage of ROP identified, and further serves to describe the degree of ROP present (12). For a visual depiction of the clock hours classification, see figure 1.

Finally, “plus disease” is encompassed by a “waviness” of the retinal vessels, which represents increased venous dilatation and arteriolar tortuosity of the posterior retinal vessels (see figure 2). This finding is concerning, because it is thought that the most severe type of ROP that leads to retinal detachment always progresses through plus disease prior to retinal detachment (12). It can later increase in severity to include iris vascular engorgement, a rigid pupil and a vitreous haze (13).

Subsequently, in clinical trials testing the efficacy of cryotherapy for ROP, further definitions of ROP were developed. “Threshold” ROP is defined as ROP in either zone I or zone II, at least 5 continuous or 8 composite clock hours of stage 3 and plus disease. This classification was found to be clinically useful in that untreated threshold ROP progresses to retinal detachment about half the time. “Prethreshold” ROP represents less severe disease and is used more as a marker for identifying infants at risk for progression to threshold disease and requiring more frequent eye examinations (12).

There are two other classifications of the disease that were described as recently as 2005 (13), and reflect the changing nature of our understanding of the progression of ROP. Pre-plus disease is defined as vascular abnormalities of the posterior pole that demonstrate more arterial tortuosity than normal but do not meet the diagnostic criteria for plus disease. Over time, the abnormalities of pre-plus disease may progress to plus disease as the vessels become more tortuous (13).

A less common, but rapidly progressive, severe form of ROP was also first defined in the literature in 2005 (13), and is termed aggressive posterior ROP (AP-ROP). It had been previously referred to as “type II ROP” or “Rush disease,” but it was not included in the initial publication of ICROP. This form of ROP is characterized by its posterior location, the prominence of plus disease and a

poorly defined nature of the retinopathy. Further, the diagnosis can be made on a single eye examination, and does not require evaluation over time. AP-ROP is most commonly observed in zone I, and may not progress through the classic stages 1 to 3. Finally, as AP-ROP can appear as only a flat area of neovascularization at the featureless junction between vascularized and nonvascularized retina, it can easily be overlooked by a lesser experienced clinician or examiner (13).

The natural history of ROP is very clearly linked to GA, as infants born at less than 27 weeks almost always develop some degree of ROP. ROP is rarely seen prior to 4 weeks after delivery, and it occurs even later in infants born at lower gestational ages. Thus, ROP is most commonly seen after 31 weeks postmenstrual age (i.e. gestational age at birth plus chronological age). Once it begins, it typically involves only a few clock hours at stage 1 or 2, which eventually progress over time, however, the zone involved typically doesn't change (12). Treatment for ROP involves cryoablation of the immature, nonvascularized retina at the periphery or laser photocoagulation to ablate the peripheral retina. Both interventions have been shown to be equally effective at reducing unfavorable visual outcomes (14).

There is also evidence of the proven benefits of timely treatment of ROP in reducing the risk of visual loss, which has raised questions regarding optimal

screening guidelines (14). The American Academy of Pediatrics (AAP) recently published a policy statement outlining ROP screening guidelines for preterm infants. Recommendations address conditions for screening, timing of screening and treatment, follow-up and conditions for discontinuing screening (14).

In terms of conditions for screening, infants born at less than 32 weeks or a birth weight of less than 1500 grams, or infants with an unstable clinical course should have a screening exam. One examination is appropriate only if it undoubtedly shows full retinal vascularization in both eyes. An ophthalmologist with sufficient knowledge and experience with ROP to enable accurate identification should perform exams with pupillary dilation. Initiation of screening is based on postmenstrual age (PMA), with most screening recommended between 31 and 36 weeks PMA (14).

Follow-up screening exams are based on the severity of ROP noted on initial exam. Infants with stage 1 or 2 ROP in zone I or stage 3 ROP in zone II should have much closer follow-up, with a repeat exam occurring within 1 week or less. Infants with immature vascularization in zone I but no ROP, stage 2 ROP in zone II or regressing ROP in zone I should be reexamined within 1-2 weeks. Two-week follow-up can occur for infants with stage I or regressing ROP in zone II. Finally reexamination in 2-3 weeks is appropriate for infants with immature

vascularization with no ROP in zone II, stage 1 or 2 or regressing ROP in zone III (14).

Infants that require treatment with peripheral ablation rather than follow-up screening include those with any plus disease, zone 1 ROP at any stage with plus disease or at stage 3 with no plus disease or zone II, stage 2 or 3 with plus disease. The AAP recommends treatment within 72 hours of identifying treatable disease to minimize progression to retinal detachment. Infants should continue to be screened at the aforementioned intervals until they have attained findings that are appropriate for conclusion of screening. Those conditions include attainment of zone III retinal vascularization without previous zone I or II ROP, full retinal vascularization, PMA of 45 weeks and no prethreshold disease, or regression of ROP (14).

The basis for the current screening and treatment guidelines comes from a number of clinical trials conducted over the previous two decades. Stemming from Terry's initial hypothesis regarding the exposure of premature infants to excess light, the LIGHT-ROP trial (15) tested the long-held belief that hospital-nursery lighting contributes to the development of ROP. This study found that a reduction in ambient-light exposure did not in fact change the incidence of ROP (15). The Multicenter Trial of Cryotherapy for Retinopathy of Prematurity or the CRYO-ROP trial (16) began in 1986. This trial was conducted to evaluate the

safety and efficacy of cryotherapy for ROP and also to determine the long-term outcomes of eyes with threshold ROP that were either treated with cryotherapy or left untreated. The authors found that cryotherapy reduces the risk of unfavorable retinal and functional outcomes from threshold ROP. Visual outcomes at ten years of age supported the long-term safety and efficacy of cyrotherapy for threshold ROP (16).

The authors of The Early Treatment for Retinopathy of Prematurity or the ETROP study (17) took CRYO-ROP a step further and sought to determine whether there was any benefit to early versus conventionally timed ablation of the peripheral retina. In this study, infants with bilateral high-risk prethreshold ROP had one eye randomized to early retinal ablation while the other eye was managed conservatively. This study found a significant reduction in unfavorable outcomes in high-risk prethreshold eyes that were treated earlier (17), and these results form the basis for current practice recommendations.

There are also a number of studies evaluating optimal oxygenation for prevention of severe ROP. The Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity, or STOP-ROP trial (18) evaluated the safety of supplemental oxygen for infants with prethreshold ROP in reduction of progression to threshold ROP and need for eventual ablation. The study found that supplemental oxygen did not cause additional progression of prethreshold

ROP, but also did not significantly decrease the number of infants requiring ablation. The authors concluded that clinicians do not need to be concerned that supplemental oxygen will exacerbate prethreshold ROP (18).

The most recent trial evaluating optimal oxygenation in prevention of ROP is the SUPPORT trial (19), published in 2010. This trial randomized infants to target oxygen saturations with one group attaining values ranging from 85 to 89% and the other group to target saturations from 91 to 95% and studied whether there was a difference in severe ROP between the two groups. The authors found that the infants in the lower target range of oxygenation (85 to 89%) group did have a decrease in severe ROP among survivors; however, there was an increase in mortality among these infants (19). This increase in mortality is concerning given the present goals of lower target oxygen saturation to prevent ROP. These results call to mind the mistakes of neonatologists in the past, who were so restrictive with their use of oxygen that each case of blindness they prevented came at a cost of 16 infants' lives (10).

The pathophysiology of ROP has been extensively researched since the earliest studies linking its development to high oxygen levels. The most current understanding of the factors leading to the development of ROP come from both animal models and human studies, and are constantly being expanded upon.

The pathogenesis of ROP is biphasic, with an initial phase of retinal blood vessel degeneration followed by a second phase of neovascularization (20).

The first phase begins when retinal vascular growth ceases after premature birth. It is during this time when vessels are particularly susceptible to injury and can be obliterated by stressors such as oxygen supply and imbalance of growth factors. Notably, during this time, decreased vascular endothelial growth factor (VEGF) levels lead to suppression of vasoproliferation. This downregulation of VEGF is a direct result of the higher oxygen levels infants are exposed to after birth compared to the relative hypoxia in utero. VEGF expression is triggered by hypoxia, and its downregulation results in vasoobliteration of the retinal capillaries. This event is one of the first initiating events in the development of ROP (21).

Subsequently, the relatively vascular-deplete retina becomes increasingly hypoxic – mainly secondary to its lack of an adequate blood supply as well as increased metabolic demands during its development. This hypoxia triggers the second, or vasoproliferative phase of ROP. During this phase there is an overproduction of hormones and growth factors in order to increase perfusion to the hypoxic retina. Specifically, there is an increase in production of VEGF, and other growth hormones, such as insulin-like growth factor 1 (IGF-1). These factors lead to the formation of new vessels in a non-organized, excessive

manner, and can lead to invasion of the vitreous, causing traction on the retina and also bleeding (21). This abnormal growth of vessels can produce a fibrous scar, which can retract and separate the retina from the retinal pigment epithelium, resulting in retinal distortion or detachment (22).

Infants developing ROP are at risk not only for the retinal changes above but also for a multitude of other ocular abnormalities, including myopia, astigmatism, anisometropia and strabismus. Further, increased severity of ROP is associated with increased prevalence and amount of myopia (23-25).

Low gestational age and low birth weight are the strongest risk factors predicting ROP (26, 27). Others include low IGF-1 levels (28), varying VEGF levels (29), systemic infection (30), inflammation (31), and genetic predisposition (32, 33). Intraventricular hemorrhage appears to be associated with ROP, probably because the same risk factors predispose very premature infants to both conditions (1, 34).

Respiratory distress syndrome, and chronic *in utero* hypoxia also appear to be associated with an increased risk of ROP, presumably through the effects of respiratory dysfunction on arterial blood gases (35, 36). Despite this, the role of blood gases and development of ROP is still unclear. Historically, as discussed earlier, high arterial oxygen levels were implicated in the development of ROP (37). However, more recently, widely fluctuating arterial oxygen tensions

and timing of high or low blood oxygen concentrations were better predictors of development and severity of ROP (38, 39).

Hypothesis

We hypothesized that ELGANs who have a blood gas extreme (abnormality) on at least two of the first three postnatal days are at a higher risk for more severe forms of ROP than infants who did not.

Methods

The ELGAN study was designed to identify characteristics and exposures that increase the risk of structural and functional neurologic disorders in ELGANs (40-46). During the years 2002-2004, women delivering before 28 weeks gestation at one of 14 participating institutions in 11 cities in 5 states were asked to enroll in the study. The enrollment and consent processes were approved by the individual institutional review boards.

Mothers were approached for consent either upon antenatal admission or shortly after delivery, depending on clinical circumstance and institutional preference. 1249 mothers of 1506 infants consented. A total of 464 of these infants died, were discharged before 36 weeks post-menstrual age, did not have

blood gas assessments on at least 2 of the first 3 postnatal days or did not have a retinal examination. The remaining 1042 infants comprise our sample for this report (Table 2).

Demographic and pregnancy variables

After delivery, a trained research nurse interviewed each mother in her native language using a structured data collection and following procedures contained in a manual. The mother's report of her own characteristics and exposures, as well as the sequence of events leading to preterm delivery were taken as truth, even when her medical record provided discrepant information.

Shortly after discharge, the research nurse reviewed the maternal chart using a second structured data collection form. The medical record was relied on for events following admission. The clinical circumstances that led to each maternal admission and ultimately to each preterm delivery were operationally defined using both data from the maternal interview and data abstracted from the medical record (46).

Newborn variables

The GA estimates were based on a hierarchy of the quality of available information. Most desirable were estimates based on the dates of embryo

retrieval or intrauterine insemination or fetal ultrasound before the 14th week (62%). When these were not available, reliance was placed sequentially on a fetal ultrasound at 14 or more weeks (29%), last menstrual period (LMP) without fetal ultrasound (7%), and GA recorded in the log of the neonatal intensive care unit (1%). The birth weight Z-score is the number of standard deviations the infant's birth weight is above or below the median weight of infants at the same gestational age in a standard data set (47).

Placentas

Placental histology and bacterial presence was documented in an effort to characterize the relationship between placental characteristics, ROP severity and blood gas derangements. Delivered placentas were placed in a sterile exam basin and transported to a sampling room. Eighty-two percent of the samples were obtained within 1 hour of delivery. The microbiologic procedures are described in detail elsewhere (48, 49).

Blood gases

We classified ELGANs by their extreme blood gas measurements on postnatal days 1, 2, and 3. On each of these days, we collected information about the lowest, modal, and highest P_aO_2, PCO_2, and pH. In our sample, the

blood gas measurement that defined the extreme quartile varied by gestational age and by postnatal day (Table 3). We therefore classified infants by whether or not their extreme value each day was in the extreme quartile for their gestational age (23-24, 25-26, and 27 weeks). Further, in order for an infant to be classified as having a blood gas in an extreme quartile, the blood gas value had to be less than (in the case of lowest quartiles) or greater than (in the case of highest quartiles) the defined value for each quartile. Because an extreme measure on one day could reflect a fleeting event, we required that an infant be in the extreme quartile on at least two of the three days to be considered “exposed” to such extremes.

Our not having blood gas measurements on all three of the first three postnatal days might be an example of informative missingness. In essence, children who did not have a blood gas on postnatal day 3 were more likely to be physiologically stable than infants who had a set of blood gas measurements that day. These children who did not have day 3 measurements are also much less likely than others to have postnatal day 2 measurements that are in any extreme quartile. Assigning these non-extreme measurements to postnatal day 3 seems eminently reasonable, and allows us to include these children in this sample, and thereby avoids our inflating odds ratios inappropriately.

Eye examinations

Participating ophthalmologists helped prepare a manual and data collection form, and then participated in efforts to minimize observer variability. Definitions of terms were those accepted by the International Committee for Classification of ROP (12). In keeping with guidelines (50), the first ophthalmologic examination was within the 31st to 33rd post-menstrual week. Follow-up exams were as clinically indicated until normal vascularization began in zone III.

Data Analysis

The generalized form of the null hypotheses which we evaluated is that children who have a blood gas extreme (abnormality) on two of the first three postnatal days are not at higher risk of any form of ROP than children who did not have that blood gas extreme on two of the first three postnatal days. We evaluated five blood gas extremes (lowest quartile of P_aO_2, highest quartile of P_aO_2, lowest quartile of PCO_2, highest quartile of PCO_2, lowest quartile of pH) in models that included the three of the other four blood gas extremes as well as other potential confounders. All conditional logistic multivariable models included a hospital cluster term (to account for the probability that infants born at a particular hospital are more like each other than like infants born at other

hospitals) (51). The contributions of relevant variables are presented as risk ratios with 95% confidence intervals.

In this sample, children who had a PCO_2 in the highest quartile tended to have a pH in the lowest quartile more commonly than expected if their co-occurrence was independent of the other. As expected, models with both of these variables provided evidence that these two variables shared discriminating information. Consequently, we present two multivariable models for each ROP classification, Model 1 invariably includes a variable for low pH, but not a variable for high PCO_2. Model 2, on the other hand, has a variable for high PCO_2, but not a variable for low pH.

We summarize some of our data with box and whiskers displays of the central tendency and dispersion of blood gases in ROP groups (Figure 3). The central tendency is indicated by the line close to the middle of the box, which is the median, and by the top and bottom of each box, which indicate the 25^{th} and 75^{th} centiles. The dispersion of blood gases is indicated by the length of the vertical lines that emanate from the box, as well as by the block dots, which identify outliers.

Others involved in the study conducted the initial data collection for the larger database. For this project, I played a significant role in the study design along with Drs. Olaf Dammann and Alan Leviton. I analyzed the raw data from

the ELGAN database along with Elizabeth Allred. I complied the tables and figures, identified confounders and developed all the models described below along with Ms. Allred.

Results

Blood gas extremes were associated with an increased ROP risk on each postnatal day (Figure 3). We observed an upward trend for each postnatal day for oxygen and carbon dioxide extremes, with higher levels of each blood gas associated with more severe forms of ROP. Infants with more severe forms of ROP show a trend towards lower pH levels on each postnatal day.

Maternal characteristics, illness and medication use (Table 4)

Infants of mothers who used aspirin had a considerably increased risk of developing more severe stages of ROP—nearly half of those infants developed stage 3+ ROP. Further, these infants also were more likely to experience an exposure to lower arterial blood oxygen and less likely to be exposed to higher arterial blood oxygen. Mothers with a white blood cell (WBC) count higher than 20,000 within the interval before delivery to 48 hours post delivery were more likely to have infants with a more severe stage of ROP, with 35% of those infants

developing severe ROP. However, their infants were not at an increased risk of experiencing blood gas extremes. While maternal fever was not associated with having an infant at an increased risk of any stage of ROP, it was associated with increased exposure to CO_2 levels in the highest quartile.

Delivery characteristics (Table 4)

Mothers with preeclampsia or a fetal indication for preterm delivery were more likely to have infants with more severe stages of ROP. In addition, infants of preeclamptic mothers were at a considerably greater risk for exposure to higher PCO_2 levels. Infants delivered due to fetal indication were more likely to experience exposure to not just higher PCO_2 levels, but also lower arterial oxygen levels and lower pH levels.

Placenta bacteriology and histology (Table 5)

The presence of any mycoplasma in the placenta or thrombosis of fetal stem vessels was not associated with an increased risk of an infant's development of ROP. However, infants with mycoplasma in their placentas were more likely to experience an exposure to lower CO_2 and less likely to be exposed to higher CO_2 levels. Infants with placentas with thrombosis of fetal stem vessels were more likely to be exposed to lower pH levels. Decidual hemorrhage or fibrin

deposition was associated with an increased risk for development of more severe ROP, with 32% of those infants developing stage 2 ROP, and 35% developing severe ROP. Decidual hemorrhage or fibrin deposition, however, were not associated with an increased risk of exposure to blood gas extremes.

Infant characteristics (Table 6)

Within each gestational age stratum, the blood gas values that defined the highest and lowest quartiles were consistently more extreme on the first three postnatal days for infants of lower gestational age (Table 3). Lower gestational age and lower birth weight Z-score was highly correlated with increased development of more severe forms of ROP. Fifty-four percent of infants born at 23-24 weeks GA developed stage 3+ ROP compared to 31% of infants born between 25-26 weeks and just 11% of infants born at 27 weeks GA. Infants of lower birth weight and birth weight Z-score also developed more severe forms of ROP at a considerably increased rate. Nearly half of infants (49%) with a birth weight Z-score less than -2 developed stage 3+ ROP. These infants with the lowest birth weight Z-score were also much more likely to experience exposure to a lower pH or higher CO_2 levels. The same relationships hold true for infants with head circumference Z-scores, with infants with the lowest Z-scores more

likely to develop more severe forms of ROP and more likely to experience exposures to lower pH and higher PCO_2.

ROP severity classifications and blood gases (Table 7)

Infants with stage 3 or higher ROP were more likely than their peers to be exposed to a high PaO_2. Further, infants with plus disease were more likely than their peers to be exposed to a high PCO_2 or low pH. Stratifying newborns by the presence/absence of zone 1 or prethreshold/ threshold disease resulted in even more prominent differences in hyperoxemia frequency, as well as prominent differences in frequencies of hypercapnia and acidemia. This increased difference is probably due to the fact that 'stage 3 or higher' includes ROP in all zones, thereby diluting the effect.

Multi-variable analyses (Table 8)

After adjustment for confounders, our multivariable models indicated several significant relationships between blood gas extremes and ROP, which were more prominent when ROP was dichotomized by zone or pre-threshold/threshold than by stage. First, higher arterial O_2 levels were significantly associated with zone 1, pre-threshold and threshold ROP, and the

presence of plus disease, while the association with stage 3 or higher disease did not reach statistical significance (both models). Second, high PCO_2 levels were invariably significantly associated with increased risk of ROP stage 3 or higher, zone 1, pre- threshold and threshold ROP and the presence of plus disease (model 1). Third, very similar effects were present with exposure to low pH (model 2). Infants who experienced high PCO_2, low pH and high PaO_2 appear to be at an increased risk for development of more severe ROP, as will be further described below.

Infants who experienced the highest PaO_2 levels were 40% more likely to develop stage 3 or higher ROP in both models; model 1 (1.4, 0.95-2.0) and model 2 (1.4, 0.96-2.0). These infants were also significantly more likely to develop zone I disease; model 1 (2.5, 1.5-4.5) and model 2 (2.5, 1.4-4.4). They were also more than two times more likely to develop pre-threshold or threshold disease; model 1 (2.1, 1.3-3.3) and model 2 (2.1, 1.4-3.3). Finally, infants with a PaO_2 in the highest quartile were 70% more likely to develop plus disease; model 1 (1.7, 1.02-2.7) and model 2 (1.7, 1.04-2.8).

Likewise, infants who experienced the highest PCO_2 levels were also much more likely than their peers to develop severe ROP in model 1. These infants were 60% more likely to develop stage 3 or higher (1.6, 1.1-2.3). They were significantly more likely to develop zone I disease (2.0, 1.1-3.6), and pre-

threshold or threshold disease (1.9, 1.2-3.0). Finally, infants exposed to the highest quartile of PCO_2 levels were 80% more likely to subsequently develop plus disease (1.8, 1.1-2.9).

Similarly, infants exposed to the lowest pH levels were also much more likely to develop severe ROP in model 2. These infants were more likely to develop stage 3 or higher ROP (1.4, 0.9-2.0), although non-significantly. They were significantly more likely to develop zone I disease (2.1, 1.2-3.8). Infants with the lowest pH levels were 80% more likely to develop pre-threshold or threshold disease (1.8, 1.1-2.8). Finally, these infants were also more likely to develop plus disease (1.5, 0.9-2.4), although again, non-significantly.

Discussion

This is the first large-scale epidemiologic study showing an association between blood gas disturbances and risk of ROP development in ELGANs. Our main finding is that exposure to high blood oxygen, high carbon dioxide concentrations or low pH is significantly associated with an increased risk of severe forms of ROP. These relationships remain statistically significant even after adjustment for confounders.

Hypercarbia and both metabolic and respiratory acidosis increase retinal neovascularization in animal models (52, 53). These models propose that hypercarbia affects the retinal vasculature through vessel dilation, which can lead to increased oxygenation and increased retinal blood flow (54), which in turn may contribute to abnormal retinal vascularization (37).

However, prior clinical studies assessing the role of blood gases in ROP development have yielded inconsistent results (39, 55). In a study of 91 mechanically-ventilated infants of mean gestational age 29 weeks, a measurement of hypercarbia or hypocarbia on the first three postnatal days was not associated with an increased risk of ROP development (55). The authors suggested that cumulative exposure integrated over time might be a better way to capture potentially adverse events than single measurements. Similar results were found in another underpowered study, of 25 infants less than 30 weeks gestational age, in which mean transcutaneous PCO_2 was correlated to ROP development (56). Both studies cited the need for further investigation with larger cohorts. Additionally, effects of pH on development of ROP have not been fully investigated in clinical studies, despite an abundance of evidence in animal models suggesting acidosis as a risk factor for ROP (52, 53).

In contrast to the studies mentioned above, our study defined an exposure as a blood gas measure in the highest or lowest quartile for gestational age on

two of the first three postnatal days, rather than defining an upper limit, or using mean values. In this way, we are able to better identify infants with an exposure to a blood gas extreme during the first three postnatal days.

The infants in our study experienced PCO_2 extremes that exceed the values defined as hypercarbia in previous studies (55). We found that exposure to a PCO_2 in the highest quartile was greatly associated with increased risk of severe ROP.

Current clinical studies have not fully investigated or shown that decreased pH is associated with increased ROP severity. Our model showed that decreased pH is highly correlated with an increased risk of more severe forms of ROP. We speculate that there could be a potential role for early sepsis in the development of blood gas extremes and ROP severity.

Finally, our study adds to our knowledge about arterial oxygen levels and ROP development. We found that exposure to higher arterial oxygen levels in the first three postnatal days is strongly associated with increased ROP severity. This is consistent with many current studies and the current clinical practice advocating the benefits of lower blood oxygen saturation during the first few weeks of life (57, 58). Several recent studies have found that lowering oxygen alarm limit parameters leads to a decreased risk of more severe forms of ROP (59), and better visual outcomes (60, 61).

While the studies discussed above have not found a significant link between hypercarbia and ROP development, nor fully investigated the effects of pH extremes on development of ROP, they have alluded to the need for a larger sample size to generate more powerful data. Our study included 1042 ELGANs, of whom, 800 (77%) developed any stage of ROP. Compared to previous studies with sample sizes less than 100 infants, this is a huge increase in power. While most prior studies did not fully address confounding, we created a multivariable model adjusting for confounders. Consequently, we expect our results to provide a better understanding of the relationship between blood gas extremes and severity of ROP.

The weaknesses of our study are those of all observational studies. We are unable to distinguish between causation and association as explanations for what we found by looking at the data only. Further, we assume that an extreme blood gas measure on two of the first three postnatal days constitutes an "exposure." This definition was necessary considering the limits of our data, however, there is no published evidence for this definition. Finally, we are unable to differentiate between respiratory and metabolic acidosis, a clinically relevant distinction that is worth further investigation in future studies.

In light of the biologic plausibility of our findings, we suggest that blood-gas-associated ROP risk be considered in discussions of clinical practice. While

oxygenation has dominated most of the recent ROP literature, the role of hypercarbia and acidosis in severe ROP may warrant further clinical consideration and study. This is especially important in light of recommendations about the safety and efficacy of 'permissive hypercarbia' (62). Permissive hypercarbia involves the adjustment of blood gas targets to allow higher than normal PCO_2 levels in premature infants to prevent lung injury. While permitting higher PCO_2 levels may indeed reduce ventilator-associated lung injuries, our findings concerning the effects of hypercarbia on development of severe ROP justify concerns raised in current discussions (63).

Conclusion

In summary, we found that independent exposure to high PCO_2, high P_aO_2 or low pH during the first three days of life significantly increases risk of development of more severe forms of ROP. Further research might help better characterize such relationships and to develop more effective clinical parameters for safe blood gas levels in ELGANs.

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Table 1. Description of retinal findings associated with each ROP stage on ophthalmologic examination (12).

ROP Stage	**Retinal Findings**
No ROP	Vessels fade from vascularized retina into avascular retina imperceptibly
Stage 1	Distinct line or flat demarcation (usually white or yellowish) between transition from vascularized, posterior retina to avascular, peripheral retina
Stage 2	Line (as described in stage 1 findings) becomes thicker in height and width, more three-dimensional now known as a ridge. Mass of growing immature vasculature, but contained within retina
Stage 3	Vessels (as described in stage 2 findings) break through the retina into the vitreous space, also known as extraretinal neovascularization. This is source of arterio-venous shunting and contractile elements that can potentially lead to retinal detachment.
Stage 4	Retina begins to become detached, lifted or pulled off choroidal bed
Stage 5	Complete retinal detachment

Table 2. Sample description.

	Yes	**No**
Enrolled	1506	
Survived to day 7 and had a head ultrasound	1414	92
Had blood gas assessments on 2 days of days 1-3	1172	242
Had a retinal examination	1042	130

Table 3. The values that define the highest and lowest quartiles of each blood gas displayed on the left on each day in each gestational age group. An infant is classified as having a blood gas in an extreme quartile, the blood gas value had to be less than (in the case of lowest quartiles) or greater than (in the case of highest quartiles) the defined value for each quartile.

Blood Gas	Postnatal Day	Gestational Age (weeks)		
		23-24	25-26	27
Lowest Quartile P_aO_2 (mmHg)	1	40	42	40
	2	43	45	44
	3	43	45	43
Highest Quartile P_aO_2 (mmHg)	1	152	145	143
	2	98	100	92
	3	103	95	93
Lowest Quartile PCO_2 (mmHg)	1	27	29	29
	2	33	35	35
	3	33	33	35
Highest Quartile PCO_2 (mmHg)	1	65	67	63
	2	60	64	60
	3	58	57	56
Lowest Quartile pH	1	7.15	7.20	7.22
	2	7.14	7.17	7.22
	3	7.16	7.19	7.22

Table 4. The distribution of ROP and infants who had a P_aO_2, PCO_2, or pH in the extreme quartile for gestational age listed at the top of each column on at least two of the first three postnatal days in categories of maternal characteristics, maternal illness and medication use and delivery characteristics. These are row percents.

Maternal/Delivery Characteristic		Blood Gas Extreme						ROP Stage				
		Lowest P_aO_2	Highest P_aO_2	Lowest PCO_2	Highest PCO_2	Lowest pH	N	None	1	2	3+	N
Pre-pregnancy BMI	< 18.5	21	18	26	28	26	76	29	26	20	25	91
	≥ 18.5, < 25	24	19	21	23	21	559	26	23	23	28	580
	≥ 25, < 30	19	27	21	18	21	224	28	21	23	28	230
	≥ 30	21	22	23	21	24	256	26	19	23	32	248
Conception assistance	Yes	24	18	17	28	23	232	28	21	21	30	246
	No	21	22	24	20	22	897	26	22	23	28	916
Fever	Yes	25	24	20	35	23	71	27	22	26	26	74
	No	22	21	23	21	22	1058	26	22	23	29	1088
Aspirin use	Yes	31	13	23	21	25	61	21	18	18	44	62
	No	21	22	22	22	22	1062	27	22	23	28	1095
Highest WBC*	> 20K	24	23	18	21	16	233	23	22	20	35	241
	≤ 20K	21	20	23	22	23	913	27	22	23	28	936
Initiator of delivery	PTL	19	20	21	18	18	531	25	25	22	28	537
	PPROM	26	21	26	21	23	240	32	20	20	28	261
	Pre-eclampsia	26	29	22	31	26	154	23	18	24	37	154
	Abruption	12	20	26	24	21	122	25	25	25	25	126
	Cervical insufficiency	23	22	16	19	23	73	30	17	27	27	71
	Fetal indication	40	17	13	35	37	52	20	14	32	34	50

***** White blood cell count (WBC) within the interval from before delivery to 48 hours post delivery

Table 5. The distribution of ROP and infants who had a P_aO_2, PCO_2, or pH in the extreme quartile for gestational age listed at the top of each column on at least two of the first three postnatal days in categories of placental histology and organism presence. These are row percents.

Placenta Characteristic		Blood Gas Extreme						ROP Stage				
		Lowest P_aO_2	Highest P_aO_2	Lowest PCO_2	Highest PCO_2	Lowest pH	N	None	1	2	3+	N
Any Mycoplasma	Yes	19	19	30	15	21	112	26	21	21	32	111
	No	22	21	21	23	22	947	27	22	23	29	968
Thrombosis of fetal stem vessels	Yes	29	16	23	27	32	62	35	21	16	28	57
	No	22	22	21	21	21	1009	26	22	23	29	1040
Decidual hemorrhage/ fibrin deposition	Yes	22	13	19	22	21	182	16	17	32	35	179
	No	22	23	22	21	22	893	28	23	21	28	925

Table 6. The distribution of ROP and infants who had a P_aO_2, PCO_2, or pH in the extreme quartile for gestational age listed at the top of each column on at least two of the first three postnatal days in categories of infant characteristics. These are row percents.

Infant Characteristic		**Blood Gas Extreme**						**ROP Stage**				
		Lowest P_aO_2	Highest P_aO_2	Lowest PCO_2	Highest PCO_2	Lowest pH	N	None	1	2	3+	N
Gestational age (weeks)	23-24	22	21	22	23	24	321	5	14	27	54	248
	25-26	22	20	22	21	20	536	23	22	24	31	556
	27	23	23	22	23	23	315	44	27	18	11	395
Birth weight (grams)	≤ 750	24	22	22	26	27	523	10	13	30	47	443
	751-1000	20	20	22	17	16	481	28	28	20	24	519
	> 1000	20	22	21	21	19	168	53	26	15	7	237
BW Z score*	< -2	23	22	27	27	38	78	12	17	22	49	65
	< -1	29	25	25	32	28	173	13	17	31	39	155
	≥ -1	21	20	21	20	19	921	29	23	22	26	979
Head circumference Z score*	< -2	25	24	21	28	29	105	15	13	32	40	91
	< -1	23	20	28	23	25	253	22	19	26	33	258
	≥ -1	20	22	21	20	19	773	29	23	21	27	807

*Birth weight (BW) and head circumference Z-scores based on Yudkin et al. standard

Table 7. The percent of children classified by their ROP status (on the left) who satisfied the blood gas criterion at the top of each column. These are row percents.

ROP severity		Blood Gas Extreme					Row N
		Lowest quartile P_aO_2	Highest quartile P_aO_2	Lowest quartile PCO_2	Highest quartile PCO_2	Lowest quartile pH	
Stage 3, 4, or 5	Yes	20	27	24	26	25	331
	No	23	19	21	20	20	711
Zone 1	Yes	20	37	19	30	35	89
	No	22	20	22	21	20	953
Pre-threshold or threshold	Yes	25	31	21	33	32	174
	No	21	19	22	20	20	868
Plus disease	Yes	28	28	22	36	31	134
	No	21	20	22	20	20	908
Overall		22	21	22	22	22	
Column N		227	221	230	229	225	1042

Table 8. Odds ratios[1] (and 95% confidence intervals) of the association between blood gas extremes (defined as a blood gas measure in the highest or lowest quartile for gestational age) and the risk of ROP. The referent group for each set of analyses consists of all children who did not have the ROP classification listed at the top of each set of columns.

	Stage 3, 4, or 5		Zone 1	
Blood gas	Model 1	Model 2	Model 1	Model 2
Lowest PO_2	0.9 (0.6, 1.4)	1.0 (0.7, 1.5)	0.6 (0.3, 1.1)	0.6 (0.3, 1.2)
Highest PO_2	1.4 (0.95, 2.0)	1.4 (0.96, 2.0)	2.5 (1.5, 4.5)	2.5 (1.4, 4.4)
Lowest PCO_2	0.8 (0.6, 1.2)	0.8 (0.6, 1.2)	0.7 (0.4, 1.5)	0.7 (0.4, 1.4)
Highest PCO_2	1.6 (1.1, 2.3)		2.0 (1.1, 3.6)	
Lowest pH		1.4 (0.9, 2.0)		2.1 (1.2, 3.8)

	Pre-threshold or threshold		Plus disease	
Blood gas	Model 1	Model 2	Model 1	Model 2
Lowest PO_2	1.1 (0.7, 1.7)	1.1 (0.7, 1.8)	1.4 (0.9, 2.3)	1.5 (0.9, 2.4)
Highest PO_2	2.1 (1.3, 3.3)	2.1 (1.4, 3.3)	1.7 (1.02, 2.7)	1.7 (1.04, 2.8)
Lowest PCO_2	0.8 (0.5, 1.3)	0.8 (0.5, 1.3)	0.8 (0.5, 1.4)	0.8 (0.5, 1.4)
Highest PCO_2	1.9 (1.2, 3.0)		1.8 (1.1, 2.9)	
Lowest pH		1.8 (1.1, 2.8)		1.5 (0.9, 2.4)

[1] All models are adjusted for maternal BMI > 30, maternal use of aspirin during pregnancy, WBC > 20K within 48 hours of delivery, delivery for preeclampsia or fetal indication, decidual hemorrhage/fibrin deposition in placenta, gestational age (23-24, 25-26, 27 weeks) and birth weight Z-score < -1, definite early sepsis, definite late sepsis and all of the other blood gas extremes in the model. These models include a hospital cluster term to account for the possibility that infants born at a particular hospital are more like each other than like infants born at other hospitals.

Figure 1. Diagram of eyes showing zones and clock hours to describe location and extent of ROP

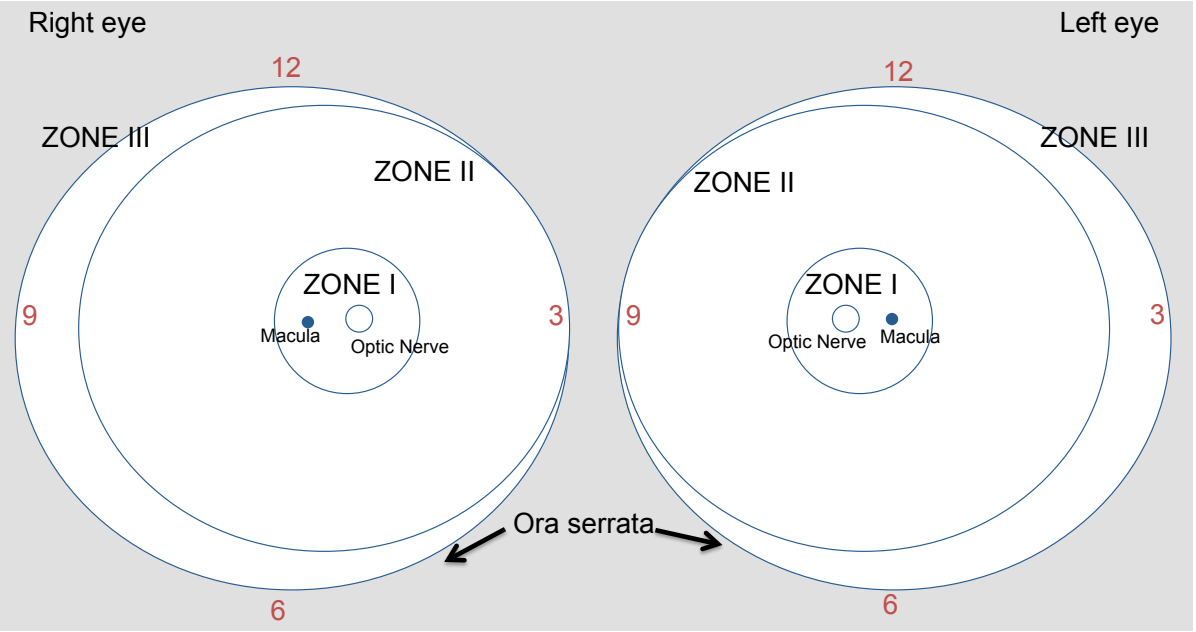
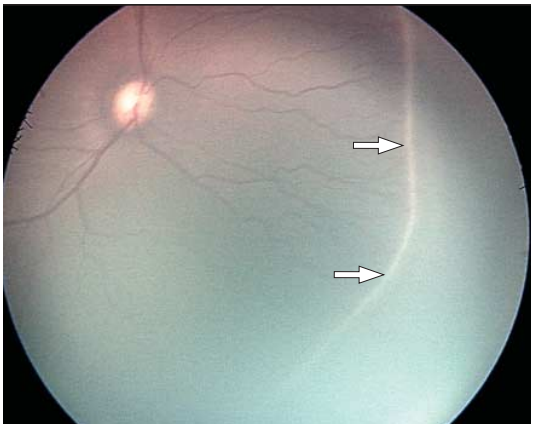


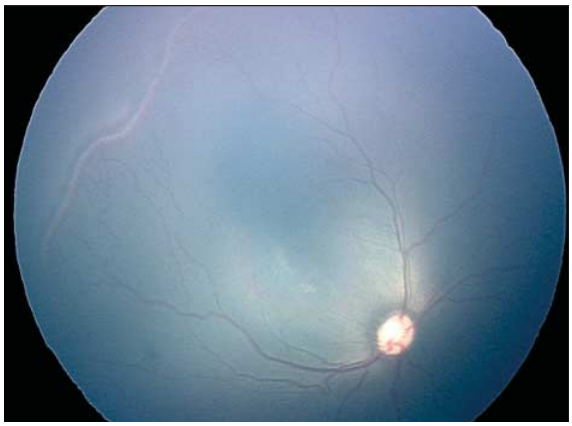
Diagram depicting premature retina with demarcated zones. Zone I surrounds optic nerve head. Also denoted around perimeter are clock hours (in red) to document extent of disease. Retinal changes are recorded on a document such as the one above. Adapted from figure 1 in American Academy of Pediatrics Policy Statement: Screening Examination of Premature Infants for Retinopathy of Prematurity (14).

Figure 2. Photographs of ROP stages 1, 2 and 3, and plus disease, reproduced with permission from figures 3,4, 6 and 10 in International Classification of Retinopathy of Prematurity Revisited (13).

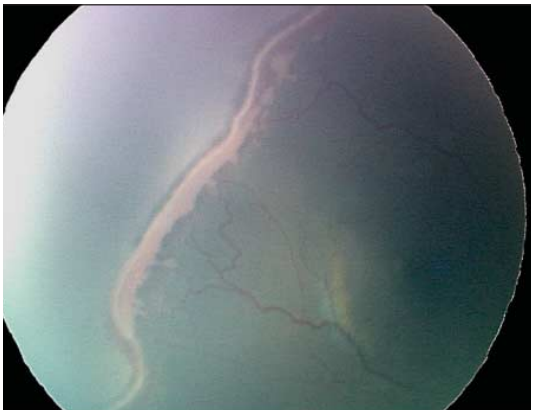
(a) ROP Stage 1



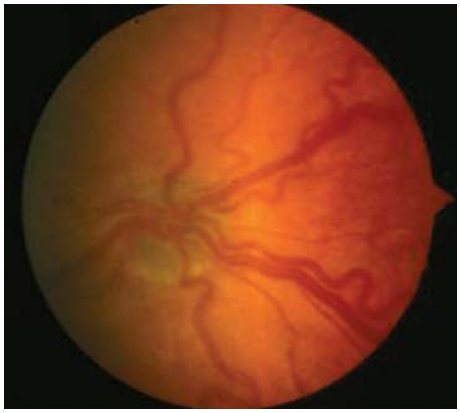
(b) ROP Stage 2



c) ROP Stage 3



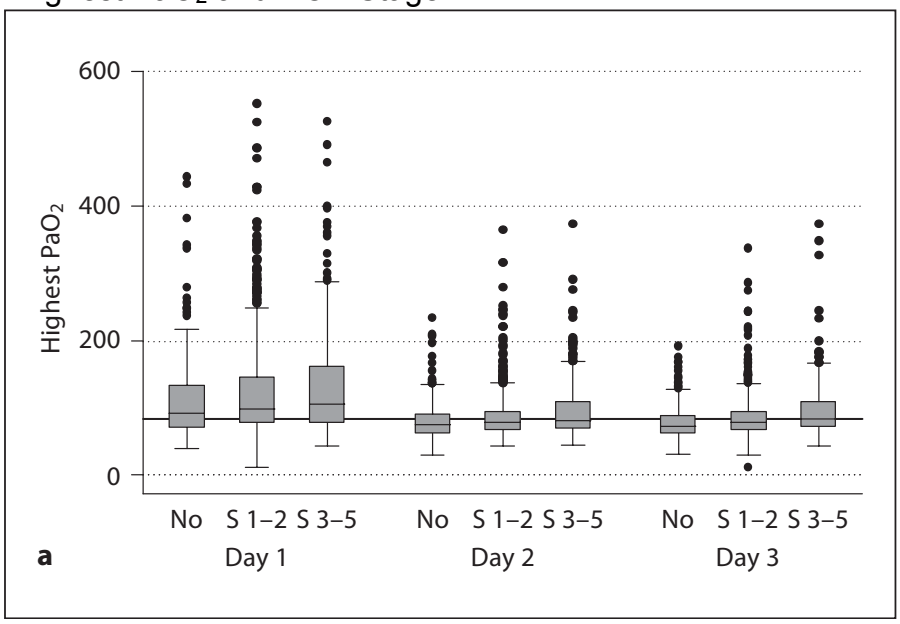
(d) Plus disease



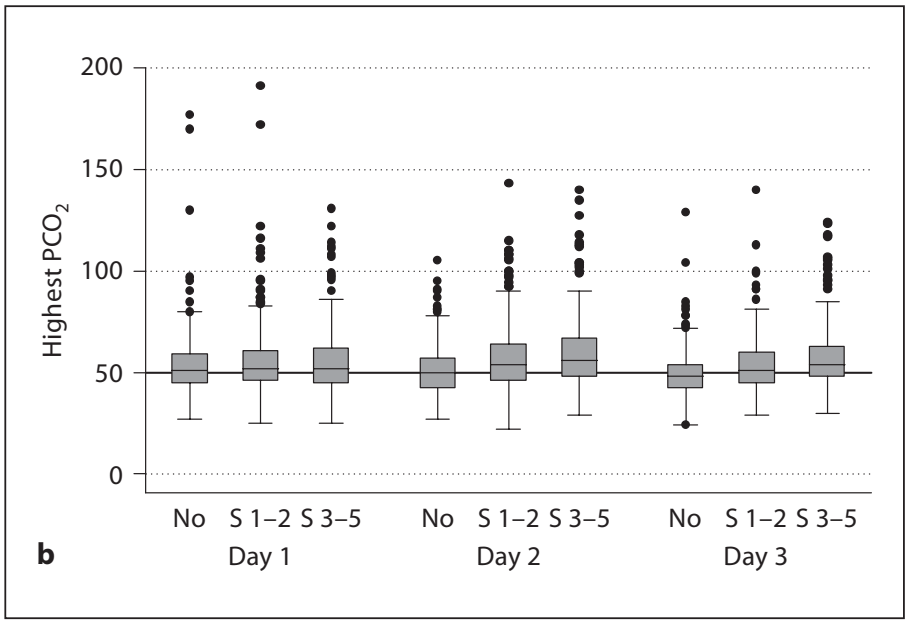
Pigmented fundus photographs (a, b, c) and fundus photograph (d) depicting stage 1 (a), stage 2 (b), stage 3 (c) ROP and plus disease (d). White arrows (a) indicate demarcation line between vascularized and avascular retina in ROP stage 1. Note progression of line into three-dimensional ridge (b) signaling development of stage 2 ROP and progression of stage 2 (b) to stage 3 ROP (c). Image (d) depicts posterior venous dilatation and arteriolar tortuosity characteristic of plus disease.

Figure 3. Correlation between highest PaO_2, highest PCO_2 and lowest pH to ROP stage

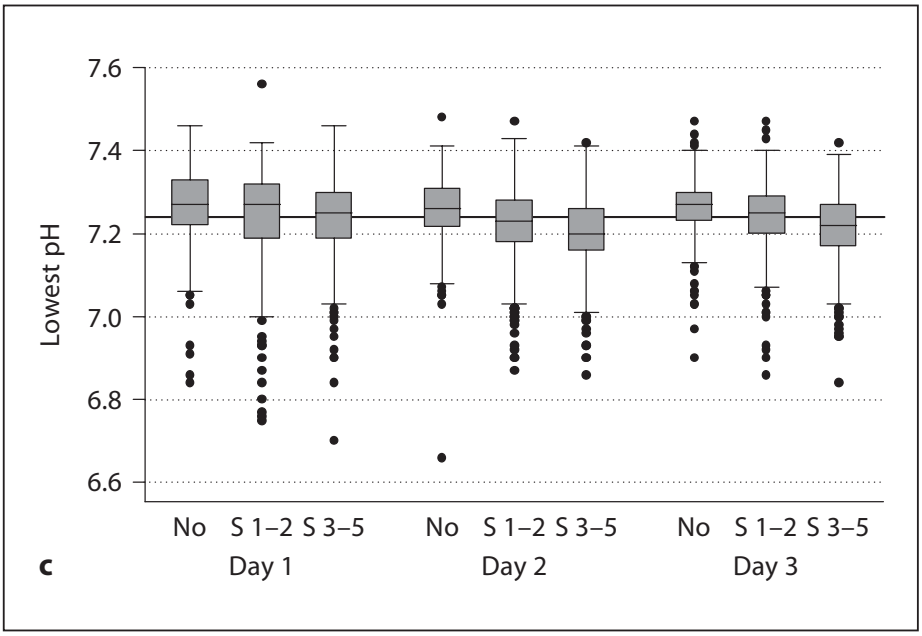
(a) Highest PaO_2 and ROP Stage



(b) Highest PCO_2 and ROP Stage



(c) Lowest pH and ROP Stage



Box and Whisker plots comparing highest PO_2 (a), highest PCO_2 (b), and lowest pH (c) to ROP stage, on each postnatal day. The median is indicated by the line closest to the middle of the box, which indicates the 25th and 75th centiles. The dispersion of blood gases is indicated by the length of the vertical lines emanating from each box, as well as the black dots, which identify outliers.