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Chapter 1

RETINOPATHY OF PREMATURITY: ETIOLOGY AND MODIFICATION OF RISK FACTORS

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ABSTRACT

Not only is severe retinopathy of prematurity (ROP) the dominant cause of severe visual impairment in childhood in the US and Canada, but milder forms of the disease are also associated with significant visual sequelae. Extreme prematurity and low birth weight are major risk factors and over 80% of infants below 28 weeks gestation will be affected to varying degrees. A key question, therefore, is how to modify the multiple risk factors these vulnerable infants are exposed to.

After preterm birth, the normal process of retinal vascularization is halted and there is relative retinal hyperoxia following delivery. As the preterm infants begins to grow the oxygen demand by the retina increases and this leads to production of growth factors and neovascularization. This process can be associated with abnormal blood vessel development and ultimately retinal haemorrhage and detachment. Excessive oxygen

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use has been known to be linked with ROP for many decades. There have been a number of approaches to mitigate this. The use of invasive respiratory support has undergone a major change in recent years with increasing use of continuous positive airway pressure (CPAP) and potentially reduced oxygen exposure. Optimizing oxygen exposure by defining oxygen saturation ranges is challenging and has been the subject of numerous randomized studies which have recently been published. While targeting lower ranges was associated with a reduction in ROP, overall mortality was increased. It is possible that clinicians may now target higher ranges, increasing the risk of hyperoxia and ROP. Reducing periods of hypoxia followed by hyperoxia after apnea and desaturation events is recognized as a contributing factor to ROP. Blood transfusion for anaemia of prematurity is common amongst preterm infants and associated with oxidative stress and linked to ROP. Measures such as delayed cord clamping and use of erythropoietin to reduce exposure to transfusion are therefore important. Optimal nutrition, including protein intake and the association with insulin growth factor-1 (IGF-1) levels and ROP is interesting and an avenue of ongoing exploration. As oxidative stress is a prominent feature of illnesses affecting preterm infants, it follows that use of antioxidants might be beneficial.

Overall, ROP remains a challenging and multifactorial condition associated with prematurity. While progress has been made in understanding the pathogenesis, reducing the incidence of severe disease has proved to be difficult. Furthermore, balance between competing outcomes is required, as has been well demonstrated by the recent large multicentre studies of oxygen saturation targeting.

INTRODUCTION

The retina is incompletely vascularised following preterm birth. Vessels grow from the optic disk toward the periphery, the process being completed as term approaches [1]. Many of the conditions associated with prematurity, such as adapting from the relatively hypoxic environment in utero, the need for respiratory support, the frequent use of oxygen, apnea, anaemia, sepsis, and the like, all have the potential to affect retinal tissue oxygenation and retinal blood vessel growth. Retinopathy of prematurity (ROP) is a condition characterized by abnormal proliferation of retinal blood vessels during the months following preterm birth. The more preterm the infant, the higher the incidence and severity of the condition [2]. Commonly, there is a progression of vessel growth through a series of stages from Stage 1 to 5 of retinopathy. In stage 1 there is demarcation between vascular and avascular parts of the retina.

In Stage 2 the demarcation line has an elevated ridge, while in Stage 3 there is neovasularisation. Stage 4 occurs when there is retinal detachment and Stage 5 when the retina is completely detached. ROP Stage 3 can progress to visual impairment and blindness [3]. Fortunately, treatment during Stage 3, usually with laser therapy or bevacizumab can prevent advancement of the condition and preserve sight. Many preterm infants will develop stage 1 or 2 but this usually regresses spontaneously. More severe stages ('severe ROP') are clinically significant and criteria are in use to determine which infants should be treated [4]. Most neonatal units have a programme of screening for ROP with repeated retinal examinations until the retinal vascularization process is complete.

There have been three so called epidemics of the disease [5]. The first occurred in the 1940s and 50s when oxygen use was relatively unrestricted. The second followed the development of neonatal intensive care units in the 1970s and retinal ablative therapy with cryotherapy and then laser surgery were established. There is now a third epidemic in middle income countries as neonatal care systems are put in place and more preterm infants survive. While the incidence varies widely, a study from the USA indicated that almost 60% of infants <28 weeks had some degree of ROP and 16% had severe ROP [6].

In many ways, the story of ROP is that of neonatology and neonatal intensive care in general, because there are so many inter-related aspects of care which affect diverse outcomes at a local tissue level (e.g. the retina, lungs and intestine) and a more general level like neurodevelopment and mortality.

PATHOGENESIS

Vascularization of the retina begins with development of supporting structures followed by vasculogenesis between 12-20 weeks gestation and is complete by 36 weeks to term [7, 8]. The stimulus for new vessel formation is oxygen demand by the developing retina [9]. Retinal tissue has the highest oxygen consumption of any tissue in the body and once the developing neurones outstrip oxygen supply from the choroid, new vessel formation is stimulated [10]. Vascular endothelial growth factor (VEGF) is the main growth factor responsible for the wave of vascularisation, with insulin growth factor-1 (IGF-1) playing a supportive role [11].

This normal process is interrupted by preterm birth. The fetus in utero is exposed to relatively low oxygen saturations (60-70%), while after birth saturations of 100%, even when breathing air, are not unusual [12]. This

removes the hypoxic stimulus for ordered vessel development. In addition, IGF-1 levels drop following preterm birth. IGF-1 has a permissive role in VEGF signalling via Akt and MAPK pathways which promote endothelial cell proliferation and growth [9]. The removal of these stimuli result in a hypoproliferative phase of retinal vessel development known as Phase 1 ROP [13]. Experimental use of VEGF, placental growth factor or IGF-1 prevent this process. Understanding of these events has been greatly aided by animal models of retinopathy in mice, rats, kittens and dogs [9, 11, 14].

Around 32weeks corrected gestation the oxygen demand by retinal tissue increases. Hypoxia promotes release of vasculogenic factors such as VEGF, IGF-1 and erythropoietin (EPO). In addition, IGF-1 levels rise with recovery after preterm birth. Depending on the severity of the stimulus, disordered vessel growth up to the junction of hypoxic and perfused parts of the retina may result [9]. This proliferative phase is known as Phase 2 ROP. These events can be blocked by intravitreal injection of antibodies to VEGF and small interfering molecules of RNA targeted at VEGF or IGF-1 [15, 16, 17].

Reactive oxygen and nitrogen species contribute to the pathogenesis of ROP. Both hypoxic and hyperoxic conditions can be responsible for their generation. The effects of their production include lipid peroxidation (the retina being at risk for this because of its high lipid content), damage to DNA, apoptosis and, through signalling pathways, to vasculogenesis. The relative paucity of antioxidant systems in preterm infants contributes to their susceptibility to retinal damage [10].

OXYGEN USE AND ROP

In 1942, a study was published which indicated that preterm infants given oxygen suffered from less periodic breathing [18]. This led support to the practice of widespread and relatively unrestricted and unmonitored use of oxygen. It was common to nurse preterm infants in 50% incubator oxygen for 28 days [19]. Severe ROP known as retrolental fibroplasia was also described in 1942 [20], and some years later a link with oxygen use was suspected [21]. Between 1942 and 1954 there were an estimated 10 000 cases of blindness [22].

A series of clinical trials showed that reduced oxygen use (compared to the 50% described above) resulted in fewer cases of retrolental fibroplasia [19]. Following this, use of the gas was severely limited. There followed a

marked reduction in severe ROP but an increase in mortality such that it was estimated that for every case of blindness prevented, 16 infants died [23].

Monitoring of Oxygen Levels

Initially, this was carried out using intermittent arterial blood gas sampling and directly measuring arterial oxygen tension (PaO2) with oxygen electrodes. No definite PaO2 was established to minimize development of retinopathy [24]. Using transcutaneous oxygen sensors to obtain continuous readout of oxygen tension, it was shown that targeting a level of 50-70mm Hg reduced the incidence of retinopathy (though not significantly) compared to standard practice which was intermittent monitoring during the acute stage of care [25]. When data from this study was further analysed, it was found that there was a link between time spent with a PaO2 of greater than 80mmHg and the severity of ROP. Subsequently, it become standard practice to target PaO2 of 50-80mmHg [26].

Following this, in the 1980s, pulse oximetry (SpO2) became the usual method of monitoring oxygen therapy. The haemoglobin oxygen dissociation curve has been well studied and it is known that many factors including the percentage of fetal haemoglobin, pH and levels of 2,3 diphosphoglycerate determine oxygen unloading to the tissues [27, 14]. The practice of targeting lower oxygen saturation levels (at a lower limit of 85%) has been shown to be associated with PaO2 in the range of 29 to 67mmHg with 95% confidence [28]. The lower confidence limit is considerably below that targeted with transcutaneous oxygen levels (see above).

It became common practice to target lower saturation ranges following observational studies indicating a reduction in ROP [29].

Oxygen Saturation Targets and ROP

While the optimal range of SpO2 has not been defined, a meta-analysis of observational and randomized controlled trials (RCTs) was carried out by Chen et al. 2010 [30]. Targeting lower saturation levels of 70-96% in the first weeks of life was associated with a marked reduction in severe ROP rates (by 52%). This reduction was even greater amongst studies that targeted saturations of $\leq$ 83% compared with those aiming for saturations above this level in the first weeks of life (RR 0.34 95%CI 0.18-0.65). In contrast, a higher

saturation target of 94-99% at a post menstrual age of ≥32 weeks reduced severe ROP by 46%. No increase in mortality was observed, although not all studies reported this outcome. There was heterogeneity between studies in terms of saturation targets and the type of study (observational or RCT). With regard to ROP, it appeared that different saturation ranges might be appropriate at different postnatal ages, with lower ranges being beneficial during phase 1 ROP and higher ranges during the proliferative phase 2 ROP.

Two of the larger RCTs, STOP ROP and BOOST, are described in more detail.

Oxygen Saturation Trials

STOP-ROP (Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity) was a large study of almost 650 preterm infants conducted at 30 sites over a 5 year period in the USA [31]. Infants with pre-threshold retinopathy in at least one eye were eligible if their median oxygen saturation over a 4 hour period was <94%. They were randomized to a conventional arm where the SpO2 target was 89-94% or a supplemental arm where SpO2 of 96-99% was targeted until study endpoints were reached (ie progression to threshold or worse or regression of the retinopathy). Progression was 49% in the lower target group compared to 41% in the higher target range (adjusted OR 0.72 95% CI 0.52-1.01). However, in the higher target group there were more infants in oxygen (47% vs 37%; p=0.02) and more in supplemental oxygen at 3 months (12.7% vs 6.8%; p=0.012). From the study it appeared that once ROP was established there was slower progression with higher saturations (and presumably higher oxygen levels), but pulmonary outcomes were less favourable.

The HOPE-ROP (High Oxygen Percentage in Retinopathy of Prematurity) study followed up the infants not eligible for the STOP-ROP study because their median SpO2 over the 4 hour period was >94% [32]. In these infants there was also less progression of ROP (compared to the conventional arm in the STOP-ROP study, but the results were not statistically significant (OR 0.6 CI 0.36-1.03).

The first Benefits Of Oxygen Saturation Targeting (BOOST) study [33] examined the effects of targeting different oxygen saturation ranges on the primary outcomes of growth and neurodevelopment at 12 months of age. Infants <30weeks were eligible if still receiving oxygen at 32 weeks corrected gestation. Target saturations were 91-94% in the standard group and 95-98%

in the high target group. Oximeters were programmed to read 2% higher in the standard group and 2% lower in the high target group, whilst caregivers were asked to target 93-96%, thus allowing blinding of treatment allocation. The mean gestations in both groups were 26 weeks at birth, while 70% were <28 weeks. There were no significant differences in the primary outcomes although in the high saturation group 64% of infants were still in oxygen at 36 weeks compared to 46% in the standard group ($p<0.001$). In addition, significantly more infants in the high saturation group received home oxygen. In the high SpO2 group fewer infants were treated with laser surgery (11% compared to 6%), although this difference was not statistically significant (RR 0.54 CI 0.27-1.1). In keeping with the STOP-ROP study, targeting higher saturation levels resulted in less progression of ROP to surgery, but at the expense of worse respiratory outcomes.

These RCTs led to the concept that higher oxygen saturations later in the time course of ROP may slow its progression.

NEOPROM STUDIES

NEOPROM (Neonatal Oxygenation Prospective Meta-Analysis)

Following on from this, a series of large, independent multicentre international trials were conducted in attempt to determine optimal saturation ranges [34]. Infants were randomized to SpO2 target ranges of 85-89% or 90-94%. Masimo Radical Pulse oximeters were programmed to read 3% lower or 3% higher than the actual readings and caregivers asked to target 88-92%, thus concealing treatment allocation groups. Saturations below 85% and above 94% were true readings but the change, for example from 85% to 83%, was gradual, preserving blinding. Each study group used the same type of offset monitors and the primary outcome was the 2 year neurodevelopmental status. As it was planned to carry out a combined meta- analysis, the studies were referred to as the NEOPROM (Neonatal Oxygenation Prospective Meta-analysis) trials. The SUPPORT, BOOST II and COT studies were part of NEOPROM (see below). To help mitigate against the known difficulty and varying success with attaining target saturations, it was planned to provide individual caregivers with feedback as to the percentage of time in each saturation range.

SUPPORT (Surfactant Positive Pressure and Pulse Oximetry Randomised Trial)

The SUPPORT study was a 2x2 factorial design randomized controlled trial carried out by the National Institutes of Child Health and Development in the USA [35].

Infants were randomized to the high or low saturation target described above and also to CPAP or intubation and surfactant. The primary outcome was a composite of ROP at threshold or surgery for ROP or use of bevacizumab or death before discharge.

The study enrolled 1316 infants. There was reasonable separation between the median oxygen saturations in the two groups, although it was found that the medians in both groups were at the upper end of the target range (91% and 94% respectively). There was no significant difference between groups in the primary composite outcome. However, the group randomized to the lower target had a decreased incidence of severe ROP (as defined above) from 17.9% to 8.6 % with a relative risk (RR) 0.52 (95% CI 0.37-0.73). However, the mortality was increased in the low target group (19.9% vs 16.2%; RR 1.27 CI 1.01-1.6).

The specific cause of the increased mortality could not be determined; rather there was an increase in all-cause deaths. Stating the results another way round, for every case of death, 2 cases of severe ROP were prevented. On the basis of the increased mortality, there was criticism of the SUPPORT study consent process in that advocacy groups argued that the potential for adverse outcomes were not sufficiently highlighted in the information given to parents. However, in the context of prevailing knowledge and practices it was felt this was a model study in how to make medical progress [36]. In light of the requirement to obtain antenatal consent, the infants randomized were generally those whose mother's had spent time on the antenatal wards. This resulted in a somewhat skewed population from those normally found in newborn nurseries [37].

The follow up results of the infants in SUPPORT at 18-22 months [38] showed that the composite rate of mortality or neurodevelopmental disability was 30% in the low saturation target group compared to 27.5% in high target group (RR 1.12 ; 95% CI 0. 94-1.32). Once again, mortality was significantly increased (22% vs 18%) and the RR was 1.25 (95% CI 1.0-1.55).

BOOSTII (Benefits of Oxygen Saturation Targeting II)

During the BOOST II United Kingdom (UK) study it became apparent that there were fewer saturation readings in the 87-90% range. This was found to be due to a shift up in the calibration curve so that in the range 87-96% displayed readings were 1 to 2 % higher. This had the effect of reducing the target range and decreasing separation between groups. At this point, the Masimo monitors had the original software replaced with an updated algorithm. New Zealand (NZ) centres had already completed enrolment for the study, and like SUPPORT, only Masimo monitors with the original software were used [39].

In the NZ BOOSTII study, 340 infants were randomized and the primary outcome was death or major disability [40]. The 2 year follow up showed no significant difference in the primary outcome. Similarly the secondary outcomes of ROP and chronic lung disease were not significantly different.

In 2010, the Data and Safety Monitoring Committees of the BOOSTII studies carried out an interim analysis of data from the BOOSTII UK, Australia and NZ studies [41]. At that time 1260 infants had been randomized to the original software and 1055 to the updated algorithm. With the improved software there was an increased mortality at 36 weeks ($p<0.001$), and the UK and Australian studies were closed (The NZ study was already finished). By this time 1187 infants were managed with the new algorithm and the mortality was 23.1% vs 15.9% ($p=0.002$), but there were no significant differences in rates of ROP or necrotizing enterocolitis (NEC).

For all data combined, there was no difference in mortality but there was a difference in severe ROP, favouring the lower target group (10.6% to 13.5%), but with an increase in severe NEC in the same group. Median saturation levels were 89% and 92% in BOOST II compared to 91 and 94% in the SUPPORT study. While there was better targeting of saturation ranges with the newer software, there was no increased time with saturations below 85% with the original software, indicating that this was unlikely to be the cause of the increased mortality [42, 43]

The differences in outcomes noted between old and new software was unexplained, but could be due to different end organs responding preferentially to different saturation levels.

COT (Canadian Oxygen Trial)

The Data and Safety Monitoring Committee of COT, however, found no reason to terminate the study early. There were 1147 infants analysed for the primary outcome which was death or neurodevelopmental disability at 18 months. There were no significant differences in the primary outcome (p=0.54), and secondary outcomes were not different in the high and low target groups. In addition, whether the new or old software was used did not affect outcomes. In contrast to the BOOSTII UK study, saturation targeting was no better with the new algorithm [44].

Meta- Analysis of NEOPROM Outcomes

To date, BOOST II UK and Australia have not reported the neurodevelopmental data, while SUPPORT, COT and BOOSTII NZ have. Overall there was no difference in the primary outcome of death or major disability where this outcome has been reported. In terms or mortality, the BOOST UK and Australia studies reported rates at discharge while COT and SUPPORT reported results at 18 months. With the revised software and using the definitions of mortality above, there was a 40% increase in mortality in the group targeting lower saturation levels (see Table 1 below). Although less pronounced, considering results of all the studies regardless of which software was used, there was still an 18% increase [45].

Table 1. Results of meta-analysis for NEOPROM trials

Outcome	Relative Risk	95% confidence intervals
Mortality (revised software)	1.41	1.14-1.74
Combined mortality (all data)	1.18	1.04-1.34
Retinpathy of prematurity	0.74	0.59-0.92
Necrotizing enterocolitis	1.25	1.05-1.49
Bronchopulmonary dysplasia	0.95	0.86-1.04
Patent ductus arteriosus	1.10	0.95-1.08
Brain injury	1.02	0.88-1.19

There were some differences between studies in the reporting of ROP, but overall there was a 26% reduction in severe ROP in the low saturation target group. Cases of severe NEC were 25% more frequent in the low target group. Bronchopulmonary dysplasia (BPD) according to the physiological definition, was not significantly altered, while based on the definition of oxygen dependence at 36 weeks, it was significantly decreased by 14% in the low saturation target group. There were no differences in patent ductus arteriosus treatment or brain injury (defined on the basis of head ultrasound appearances).

Summary of Current Oximeter Trials

Overall, it is apparent that there are competing outcomes in different organ systems in defining optimal saturation levels. The observed increase in mortality has meant that it is currently unacceptable to target saturations in the mid to high 80s.

EFFECT OF DESATURATION EVENTS

It is common for preterm infants to experience periods of SpO2 desaturation below 80%. Using short averaging times with SpO2, DiFiore et al., 2010 [46] showed that the number of hypoxemic events (saturations ≤80% for ≥ 10 secs and ≤3 mins) increased with illness severity, decreasing gestational age, male sex and increasing postnatal age. Infants receiving laser therapy for ROP (LaserROP group) had greater numbers of events than those not receiving laser (NoLaser ROP). Results were corrected for covariates. There was divergence between the LaserROP and NoLaserROP groups in the frequency of desaturations after 2 weeks of age which increased with time and persisted through 6-8 weeks postnatal age. This suggested that repeated hypoxemic events could contribute to disordered retinal neovascularisation. Work carried out with experimental models has indicated that exposure to fluctuating oxygen levels and hypoxic events led to increased VEGF production and worse retinopathy [47].

Interestingly, in the DiFiore study [46], infants who had higher saturations (>95%) most notably between 4-8 weeks, were significantly less likely to be treated with laser (no distinction was made between infants receiving

supplemental oxygen or breathing room air). These results are in keeping with the results of the HOPE-ROP study [32].

AUTOMATIC ADJUSTMENT OF OXYGEN ACCORDING TO OXYGEN SATURATIONS

It follows that avoiding hypoxic events and fluctuating oxygen levels is important but this is difficult to achieve in practice. The AVIOx study [48], aimed to document SpO2 levels in preterm infants <28 weeks in the first 4 weeks of life, the saturation levels recorded were compared with local guidelines indicating best practice. Only periods where oxygen therapy was regarded as modifiable were analysed. Infants were within target only 16-64% of the time and above range 20-73% of the time. Considerable variability was noted amongst different infants and in different centres.

There have been studies using automated fractional inspired oxygen (FiO2) control. In a randomized crossover study by Urschitz et al., 2004 [49], infants on nasal continuous positive airway pressure (CPAP) with an oxygen requirement had periods of either routine manual control of FiO2 or automated FiO2 control and these were compared with "optimal" FiO2 control by a fully dedicated caregiver. The target range (87-96%) was achieved in 81% in the routine group compared to 90% for automatic control ($p=0.01$) and 91% for fully dedicated control. A recent study by the same group demonstrated similar findings [50].

Likewise, a study in mechanically ventilated preterm infants with frequent desaturation episodes showed improvement in attaining target saturations between 87-93% with an automated system. The percentage of time in target increased from 32% with manual routine control to 40% ($p <0.001$). There was, however, a significant increase in time with saturations below 87% in the group with automatic FiO2 adjustment [51].

RECOMMENDATIONS FOR OXYGEN SATURATION TARGETS

Many authors have suggested targets from 90-94% [40]. Others have stressed the importance of strictly enforcing alarm limits between 85-95% and possibly selecting optimal limits based on whether there is a high mortality or

high ROP rate [52]. On the other hand, it seems prudent to avoid saturations above 94% because of the increased risk of retinopathy [40]. In keeping with the STOP ROP and BOOST studies, it may still be prudent to target higher ranges with increasing postnatal age.

The planned individual patient meta- analysis from the NEOPROM studies will likely also provide additional information on outcomes at different saturations. Even if an optimal saturation range is found, our current ability to effectively target such a range is limited. Methods to automatically adjust oxygen levels depending on saturations are being developed, as described above, and will likely be followed by a new series of saturation targeting studies.

TYPE OF RESPIRATORY SUPPORT

In the CAP (Caffeine for Apnea of Prematurity) study, severe ROP was decreased in the group receiving caffeine. This study arm was also exposed to less oxygen use and less mechanical invasive ventilation [53, 54]. On the other hand, meta-analysis of RCTs comparing early use of CPAP with intubation in infants <32 weeks showed no difference in severe ROP [55]. Likewise, an observational study examining the effects of a change of practice to that of predominant CPAP use, did not demonstrate any reduction in ROP [56]. While it seems likely that the respiratory support strategy used could affect ROP rates, more evidence is needed.

ANAEMIA AND BLOOD TRANSFUSION

Risk factors for ROP include anaemia and blood transfusion [57]. Haemoglobin values influence tissue oxygen delivery and therefore could influence retinal vascularisation. In addition, blood transfusion (BTF) in preterm (but not term) infants, is associated with an increase in free iron in the plasma and this in turn is a potent source of reactive oxygen species which could exacerbate retinal injury [58].

There are few randomized studies that have reported associations between BTF and ROP rates, even where the latter were considered as a secondary outcome. Four trials are included in the Cochrane Neonatal Review [59] and, of these, 2 are relatively large and the other 2 are small (fewer than 20

patients). The studies are reported on the basis of whether restrictive or liberal transfusion guidelines were followed. The guidelines used to determine the use of transfusion are reasonably similar to those used in clinical practice in many hospitals. Around 90% of infants in the liberal and restrictive transfusion groups had at least one transfusion. In addition, infants in both groups received a mean of 5-6 transfusions, although there was an average difference of approximately one transfusion between liberal and restrictive groups. This means infants in both groups had significant exposure to blood transfusion. There was no difference in ROP of any stage or of stage 3 or more ROP between groups. For the reasons described above, it is difficult to draw any firm conclusions in regard to ROP rates in these studies. Brooks et al., [60] performed a RCT of different protocols for blood transfusion. Limiting transfusions in the restrictive group to those with symptom based guidelines of anaemia significantly reduced the total number of transfusions given, but did not affect the incidence of ROP. Nevertheless, nearly all the infants in the study received EPO, and this could have affected the results.

A number of observational studies have looked at whether there is an association between BTF and ROP. These studies are subject to the same limitations as the randomized trials, and to the additional problem that there are multiple factors affecting ROP outcomes and these are difficult to control for.

Hesse et al., [61] noted that amongst very low birth weight infants, there was a highly significant relationship between the volume of blood transfused and ROP. There was, however, no independent relationship between measures of iron status and ROP. Cooke et al., [62] noted a 9% increased risk for ROP with each BTF, while Brown et al. (see below) found a 37% increase in risk following each BTF [63]. Dani et al., [64] reported that gestational age, blood volume transfused and iron intake were all independently associated with ROP.

Erythropoietin

EPO has been used as an alternative to blood transfusion in anaemia of prematurity. As a glycoprotein hormone, it has many and varied effects, including stimulation of red blood cell production, vasculogenesis and neurogenesis [65]. EPO levels decrease after birth following loss of the relatively hypoxic in-utero environment [66]. These lower levels could contribute to decreased vascularization in Phase 1 ROP. Indeed, exogenous

EPO minimizes experimental retinal vessel loss [10]). However, late EPO promotes pathologic neovascularisation in the proliferative phase of ROP in a mouse model. In contrast, in a rat model, early high dose EPO had no effect on the development of ROP [65]. Whilst it appears the effects of EPO may vary depending on the species used, it is also likely that the timing of exogenous EPO may determine its effects on retinal vessel growth.

The experimental effects noted in the mouse model are somewhat borne out by results of clinical studies in preterm infants. A series of meta-analyses of EPO trials is reported in the Cochrane neonatal data base [67, 68, 69]. Before discussing these studies in detail, it is worth noting that although EPO trials have reported the utility of EPO in reducing transfusions in over 1100 patients, the number in whom ROP outcomes is reported is relatively small (around 500 per group at best) and none of the trials were designed with the primary outcome of ROP in mind. For a relatively uncommon outcome such as severe ROP, much larger studies are required. In addition, firm conclusions are hampered by different ages of commencement of EPO and use of different doses. All studies used supplemental iron, the effects of which on ROP are unknown.

Early use of EPO in the first 7 days of life compared to placebo did not increase the rate of any stage of ROP [67]. There was no significant heterogeneity between studies and the RR was 0.99 (95% CI 0.81-1,21). For ROP stage 3 or more, again there was no significant difference.

Early EPO use (as defined above) compared to later use (7-to 28 days) was associated with an increased risk of ROP of any stage (RR2.4 CI 1.05-1.86). Of note, there were fewer than 100 patients assessed for this outcome and there was no increase in the incidence of Stage 3 or more ROP [68].

Compared to placebo, late EPO (d7-28) showed an increase in ROP of any stage [69] which was of borderline statistical significance (around 200 patients had this outcome reported). For Stage 3 or more the differences were not significant (RR 1.73 CI 0.92-3.24). It was noteworthy, however, that EPO use at any stage ie early or late, was associated with ROP stage 3 (RR 1. 48 CI 1.02 2.13).

OBSERVATIONAL STUDIES OF EPO AND ROP

Because of the relative paucity of results from randomized trials, evidence from observational studies continues to be published. The results of these studies are also conflicting, with some showing increased stage 3 or more ROP

and others showing no difference in ROP rates between controls and infants receiving EPO. Perhaps these varying results are not surprising in view of the potential confounding factors to which preterm infants are exposed during their hospital course. One such observational study was that of Brown et al. [63]. EPO doses from 750-1200U/kg/week were used from day 10 with an average total dose of 3500U. In this study there was an association between the cumulative EPO dose and the risk of ROP as shown below (Table 2).

Table 2. Risk factors associated with severe retinopathy of prematurity noted in observational studies

Risk factor	Relative Risk
EPO dose (500U/kg/week)	1.27 (1.04-1.55)
Gestational age (per week)	0.75 (0.61-0.91)
Blood transfusion (each)	1.36 (1.15-1.60)
Oxygen exposure (per week)	1.66 (1.22-2.30)
Ventilation for 30 days or more	6.8 (2.4-19.4)
EPO >20doses	4.3 (2-9.3)

As it would not be unusual for infants such as those studied to receive weekly transfusions, it follows that the risks of ROP following a week of EPO treatment or a transfusion were similar. One of the drawbacks of the study was that only 8 infants did not receive EPO, so the number of controls was very small, leading to potential bias. The EPO dose used was also relatively high.

Suk et al. [70] recorded the rate of ROP in 264 infants before and after the introduction of EPO. They used a dose of 1200U/week (also relatively high) and noted an increased risk for ROP treated with laser therapy after receipt of 20 doses of EPO (see Table 2 above).

Some aspects of the clinical management described in this study would not be common practice eg high upper oxygen saturation levels (98%) were targeted, the rate of BPD was high (66%), infants received a large number of BTFs (8-12)and the median days of ventilation was also high (30 days).

Schneider et al. [71] also reported ROP rates in a group of 138 preterm extremely low birth weight infants receiving EPO and matched controls. ROP stage 3 or more occurred in approximately 20% of infants in both groups (not significantly different between groups, but nevertheless a high rate).

In contrast, Manzoni et al. [72] noted an increase in proliferative ROP associated with EPO use.

In summary, there are concerns with the use of EPO in terms of the genesis and progression of ROP. It seems prudent to use the lowest possible dose for the shortest time and to watch closely for excessive retinal vessel proliferation

POSTNATAL WEIGHT GAIN

As noted previously, risk factors for development of severe ROP are many and varied [73, 46, 47, 54]. Most of these factors are associated with poor postnatal weight gain and in turn, this has been shown to have a high degree of correlation with IGF-1 levels. IGF-1 levels drop following preterm birth and increase as postnatal growth recovers [74]. In fact, logistic regression models have demonstrated that postnatal weight gain on its own allows for better prediction of ROP severity than models considering other multiple risk factors described above [73]. As noted previously, IGF-1 plays a role in promoting VEGF activity which in turn influences retinal vasculogenesis. Low levels following preterm birth correlate with retinal hypoxia in phase 1 ROP and higher levels associated with better postnatal weight gain could be linked with the proliferative stage of ROP [11, 13].

Predicting ROP Outcomes Based on Postnatal Weight Gain

In terms of predicting ROP outcomes a number of studies have developed models using postnatal weight gain, either alone or in combination with other factors [73]. WINROP was the first of these predictive models [74]. In the study, the infant's actual weight was compared with the growth curve for infants who did not develop severe ROP. Weekly measurements were done and a cumulative risk obtained. Birth weight and gestation were also included in the model. A very high sensitivity for severe ROP was demonstrated with a number of retrospective studies based on these criteria, with sensitivities of 100%.

ROPScore [75] used a regression equation to estimate risk. The equation was calculated on a single occasion based on birth weight and gestation and postnatal weight gain at 6 weeks of age as a proportion of birth weight. There were also terms for blood transfusion and the use of oxygen during mechanical ventilation in the first 6 weeks of life.

PINT ROP [76] developed a model using data from infants in the PINT study (Preterm Infants in Need of Transfusion). This predictive approach was updated and a nomogram developed called CHOP ROP [77]. All the studies indicated a very high sensitivity for detecting severe ROP in countries with sophisticated neonatal facilities.

Interestingly, however, when WINROP prediction was applied in Brazil and Mexico, where neonatal facilities are being developed, it was less predictive of severe ROP (90% and 55% in the two countries respectively). The ROPScore, which includes other terms as noted above, was more predictive. This may relate to the fact that higher gestational age infants are at risk of ROP in these countries and the pathogenesis may depend more on exposure to high oxygen levels than poor postnatal growth.

Improving Postnatal Weight Gain

Faltering postnatal weight gain is a common problem encountered amongst preterm infants [78]. Studies have investigated the effects of increased nutrient intake and observed improved weight gain as a result. The increase in intake can be achieved by both parenteral and enteral means [79]. As weight gain is such a powerful predictor of ROP outcomes, does improved nutrition reduce severe ROP? Nutrient intake was studied retrospectively in a group of extremely preterm infants and ROP risk was found to be associated with calorie and lipid intake, but not protein intake [80]. In other studies, decreased growth in head circumference was associated with ROP [81]. Improved nutrition including protein intake resulted in better growth in head circumference a RCT [82]. To date, the effects of these strategies on severe ROP rates have not been reported to any significant extent.

ANTIOXIDANTS

The importance of reactive oxygen species in ROP pathogenesis and the relative paucity of preterm antioxidant systems [10], implies that use of antioxidants could help prevent development of ROP.

Meta -analysis of Vitamin E studies has shown that supplementation may reduce severe ROP by over 50% [83, 84]. Lactoferrin (LF), an anti-infective and anti-inflammatory component present in breast milk was shown to reduce ROP progression in a multicentre RCT. In this study, bovine LF was given

with the aim of reducing late onset sepsis and ROP was reported as a secondary outcome [85]. Subsequently, an observational study indicated a significant reduction in stage 3 or more ROP following routine introduction of LF supplementation in preterm infants [86]. However, such studies can be difficult to interpret owing to the multifactorial nature of ROP as well as other concurrent changes in clinical practice. Lutein, although a potent antioxidant, was not shown to decrease severe ROP in a RCT where lutein supplementation was compared to placebo [87, 88]. Omega 3 long chain polyunsaturated fatty acid suppresses neovascularization in a mouse model [89]. Other potential antioxidant candidates include melatonin, edaravone a free radical scavenger, and EPO [10]. Suk

GENETICS

In view of the critical interplay between inflammatory pathways, vasculogenesis, oxidative stress and antioxidant systems, it would be surprising if genetic polymorphisms did not affect the incidence and severity of ROP [90]. Indeed the distribution of VEGF alleles at VEGF −634 was significantly different between a group of preterm infants who developed severe ROP and those who did not ($p = 0.03$). Homozygotes for the G allele, associated with higher VEGF production, were twice as likely to have threshold ROP [91]. Norrie disease gene polymorphisms have also been linked to more severe ROP [92].

EXPOSURE TO LIGHT

As exposure of the retina to light results in photo oxidation [10], it follows that preterm infants may experience development and/or progression of ROP. Whether light exposure affects ROP was the subject of a Cochrane Review which found a lack of association, however, the number of patients with severe ROP was small [93]. In a more recent RCT in which one group received ocular protection until 35 weeks corrected gestation, there was no link [94]. These findings were confirmed in a more recent meta-analysis [95].

CONCLUSION

It is apparent that many factors are involved in the pathogenesis of ROP. There are, therefore, many potential interventions such as defining target ranges for saturations and oxygen use, automated oxygen adjustments, attention to hypoxic desaturation events, blood transfusion (and factors which affect it such as delayed cord clamping and use of Epo), use of antioxidants and attention to nutrition. These interventions all affect general aspects of neonatal care. A simplistic goal is to optimise ROP outcomes as well as outcomes for all other neonatal conditions. It is apparent that this goal may not be achievable at present without some compromise. Competing outcomes have been clearly demonstrated between ROP, mortality, NEC, and to a lesser extent for BPD. Is it possible that in future, local interventions involving the retina itself to prevent vasoproliferation (eg through use of locally targeted antibodies), may be possible without altering other aspects of care? In this way different local conditions could be present, for example in the retina, compared to the rest of the body.

In the meantime, higher oxygen saturations are likely to be targeted because of the increased mortality noted in the latest studies, and this may well result in an increase in ROP rates. Further analysis of NEOPROM may hold some of the answers regarding target saturation levels, and these levels may be different for individual babies, depending on postnatal age and ROP stage.

Further refinements to screening for and treatment of established ROP are likely. The use of retinal photography to provide a permanent retinal record is already established in many centres. This allows for better monitoring of disease progression. Overall, ROP will continue to be a fruitful area for research for the foreseeable future.

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