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Chapter 1

Introduction

Retinopathy of Prematurity (ROP) is an important cause of partial sight or blindness in prematurely born infants. Worldwide assessment in 2010 estimated that 21.8% of preterm born infants born has some degree of ROP.[1] Incidence figures for ROP and visual disability due to ROP differ however per country, region or continent. Worldwide ROP accounts for 17.5% of visual impairment in prematurely born babies.

In middle income countries like many Latin American and Asian countries and the Former Socialist Countries of Eastern Europe, ROP is emerging as a major cause of blindness (also referred to as the "third epidemic").[2] Possible reasons for this epidemic are:

- birth rates and rates of premature birth are increasing;
- neonatal care may be compromised as a result of limitation of resources, resulting in higher rates of severe ROP not only in extremely premature infants but also in bigger, more mature infants; and
- no nationwide implementation of screening and treatment programs due to the lack of awareness, skilled personnel and/or financial resources.[3]

For example, a third of all children under the age of ten in schools for the visually impaired in Vietnam and more than 40% of under-5 years of age in similar schools in Mexico are blind as a result of ROP.[4] In lower income countries the incidence of ROP is low as neonatal care is not well developed, so less premature infants survive.[5]

Permanent visual damage will increase with increasing prematurity of the infant. Over the past two decades changes in neonatal care have increased the survival rate of very small and very preterm infants, also in the Netherlands. Significant progress in health care practice like the introduction of surfactant, increased use of antenatal steroids, resuscitation of infants with lower gestational ages, improved nutrition and prophylactic use of indomethacin have contributed to this increased survival. Consequently an increasing number of infants is at risk, not only for visual impairment (VI) caused by ROP or cerebral visual impairment (CVI), but also for non-visual disabilities.[6-10] Termote et al found a significant increase in behavioral abnormalities in infants with visual impairment due to ROP, born between 1994 and 2000.[11]

The last national survey in the Netherlands showed that 8-10 infants per year become visually disabled or blind as a consequence of ROP.[11] Visual impairment due to ROP can be decreased by timely screening, thorough follow up and prompt treatment of neonates who are at risk. So a dedicated screening program is indispensable. However, as reported, incidences of ROP strongly depend on study cohort, level of care and country, so screening guidelines cannot be applied uniformly in different countries. For that reason it is important to define and to inventorize the population at risk for potentially blinding ROP and to provide evidence for a quality guideline per country.

HISTORY

ROP was first reported in 1942 by Terry, who described the histological findings of what would now be considered end-stage, cicatricial disease but back then was called retrolental fibroplasia (RLF).[12]

The first ROP epidemic, occurring in the USA from the 1940s until late 50s, was caused by administering high doses of oxygen to premature infants without monitoring; pulse oximetry was not yet available.[13-19] As a reaction to this, attempts were made to curtail oxygen use in the premature nursery by administering oxygen only at times of clinical need, as short a period as possible and at concentrations less than 40%.[20] Although rates of ROP decreased, cerebral palsy and death increased, blocking the implementation of a restricted oxygen regimen.[17;18]

With the introduction of pulse oximetry in the 1970s, oxygen administration became more controlled and it was anticipated that ROP would disappear. Research by Patz[14] and by Kinsey et al[16], and laboratory experiments by Ashton[21], suggested that ROP could be entirely preventable by thorough oxygen regulation. Unfortunately, this proved not to be the case.

In the 1970's ROP incidence once again increased, referred to as the second epidemic, now as a consequence of increased survival rates of extremely premature babies.[22] In those days ROP screening was performed by neonatologists who performed external examination of the eyes as well as direct ophthalmoscopy. However, by the time pathological retinal changes were evident to the neonatologist the disease was usually very advanced. This called for examination by experienced ophthalmologists. In 1976 the AAP (American Academy of Pediatrics) issued the first screening guideline and recommended that screening examinations for ROP should be performed at the time of discharge from the nursery and at three to six month of age.[23] The median length of stay in the neonatal intensive care unit (NICU) at that time was 61 days (almost 9 weeks) for those with a birth weight (BW) of 751-1000gr, and 30 days for those with BW 1000-1250gr. If there were no fundus abnormalities on discharge from the hospital, no follow-up eye examinations were planned.

Palmer found that the optimal age to detect acute RLF was at age 7-9 weeks and that, for optimal ROP detection, the initial examination should not only occur at about 6 weeks of life but should also be performed by an ophthalmologist.[23] Ideally the fundi should at least twice be classified as normal with an interval of 4 weeks before final discontinuation of screening. Palmer et al[24] also suggested that, if high-risk infants (<1700gr) were discharged or transferred to another hospital prior to an age of 6 weeks, screening should be postponed and not be performed at discharge. He reasoned that parents could have a false sense of security from a normal eye examination and could wrongly decide to refrain from further screening. When this first examination was postponed, the

numbers of infants lost to follow up would be smaller and ROP could still be detected in time to be able to treat infants with severe disease.

In the late '60s treatment of acute ROP with Xenon-arc photocoagulation and cryotherapy were introduced.[25] Cryo-ablation therapy of the avascular retina, freezing from the external ocular surface, affecting the sclera, choroid, and the full thickness of the retina, emerged as the standard treatment for acute phase ROP in the 1980's.[26]

In 1988 the Cryotherapy for Retinopathy of Prematurity Cooperative Group trial (CRYO-ROP) showed the beneficial effect of cryoablation of the peripheral avascular retina and significantly reduced the progression of threshold ROP, which if left untreated carries a risk of blindness up to 50%.[27] The 10-year follow-up showed that 44.4% treated vs 62.1% untreated had visual acuity of 20/200 or worse. At 15 years, the rate of unfavorable outcomes was 30% for treated eyes and 51.9% ($p<0.001$) for eyes that were observed without cryotherapy.[28;29]

In the 1990's Laser (light amplification by stimulated emission of radiation) therapy evolved as the primary modality of treatment, in which a laser is applied through the dilated pupil to the internal retinal surface. Compared to cryotherapy, fewer complications were described. Given the greater ease of treatment with laser a randomized, controlled trial was elaborated in 2003 to prospectively assess the effects of early laser treatment for ROP: The Early Treatment of ROP (ETROP) study.[30] This study confirmed the efficacy of treatment for severe ROP and redefined the indications for treatment.[31] The clock hours and the terminology of threshold and pre-threshold were abandoned and a new categorization of ROP was introduced in which ROP was defined in two types.

Also during the mid-1980s, after decades of discussion regarding the nature of the pathogenesis and the clinical course of ROP, a group of ophthalmologists representing 11 countries and sharing a common interest in ROP, decided that an international classification of ROP was required. They developed the International Classification for ROP (ICROP).[32] Later, in 1988, they added a section on the classification of retinal detachment.[33]

In 2005 the ICROP needed revision as new presentations of ROP occurred in infants with GA of 25 weeks or under and the role of the retinal vessels for the decision to treat had changed as a result of the ETROP study. An extra stage and a definition of (pre-)plus disease were added, clock hours were no longer included.[34]

CLASSIFICATION

The revised ICROP uses the following criteria to score ROP:

- location of retinal involvement specified as zone I-III
- the degree of peripheral vascular abnormalities
  - o stages 1-5, with stage 5 being the most severe (figure 1)
  - o aggressive, posterior ROP (APROP), an aggressive form of ROP, not following the classical stages 1-5
- plus- and pre-plus disease to describe specific features of the retinal vessels. The designation 'plus' is added to the ROP stage number

Zones to define the location of the disease:

Zone I: the area within a circle with a radius of twice the distance from the optic disc to the center of the macula.

Posterior Zone II: the area within the dotted circle with a radius of three times the distance from the optic disc to the center of the macula.

Zone II: extends from the edge of zone I and with the peripheral border the edge of a circle with a radius equal to the distance between optic disc and nasal ora serrata.

Zone III: extends from the edge of zone II. It is only entered with certainty when the nasal retina is fully vascularized.

The zone that is affected by the disease is important because it reflects the extent of the outgrowth of the retinal vessels. The lower the zone, the larger the avascular area resulting in the highest risk for severe ROP. Zone I or posterior zone II are therefore associated with a bigger chance of progression of disease or need of treatment.[35]

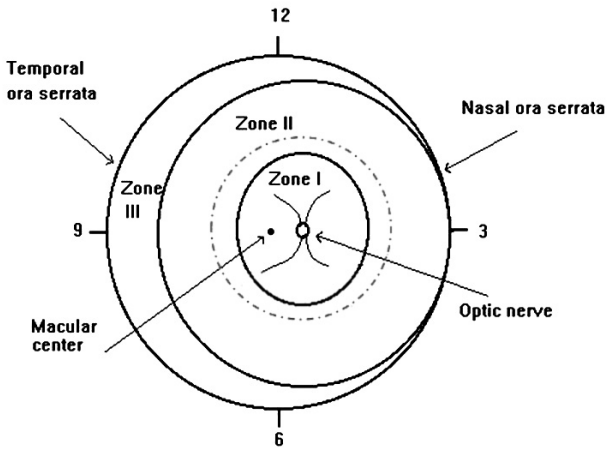

Figure 1 Retinal zones

Stages to classify the severity of the disease:

Stage 1: Characterized by a demarcation line: A thin, flat white line, seen between the vascular and avascular retina.

Stage 2: Characterized by a ridge: an elevation or thickening of the retina in the region of the demarcation line.

Stage 3: Characterized by a ridge with extraretinal fibrovascular proliferation.

Stage 4: Characterized by partial retinal detachment and subdivided in;

Stage 4a: Partial detachment not involving the foveal region

Stage 4b: partial detachment involving the foveal region

Stage 5: Characterized by total detachment of the retina, forming a funnel shape. The funnel is divided in an anterior and posterior part, for descriptive purposes. And both parts can be open or closed.

Anterior	*Posterior*
Open	Open
Narrow	Narrow
Open	Narrow
Narrow	Open

APROP: Characterized by neovascular fronts that lay flat on the retinal surface. No ridge is seen in these eyes, and yet the AV shunting, which occurs within the ridge tissue in more typical ROP, is seen throughout the posterior pole. Vessels are dilated and tortuous in a syncytial pattern. ROP is confined to zone 1 or posterior zone 2.
Most critical feature: may progress directly to severe ROP, without interval findings typical of stage 1 or 2 ROP.

Pre-plus disease: A description of an intermediate level of plus disease (pre-plus) between normal aspect of posterior pole vessels and frank dilation as in plus disease.

Plus disease: Defined as an increased dilation of venules and tortuosity of arteries of the retina in at least 2 quadrants in the posterior pole, whether or not accompanied by engorgement of iris vessels, pupil rigidity and vitreous haze. Plus disease implies that the ROP process is (highly) active and progression may be rapid.

Stages 1 and 2 and any other phase without plus disease are usually considered mild since most cases resolve spontaneously without major visually disabling sequelae.[36] ROP with plus disease and stages 3 - 5 are considered severe, as they have a significant risk of poor visual outcome. Stage 4a eyes that remain stable usually maintain reasonable

vision but progression to stages 4b and 5 (being associated with retinal detachment involving the macular area) always carry a poor prognosis for vision.

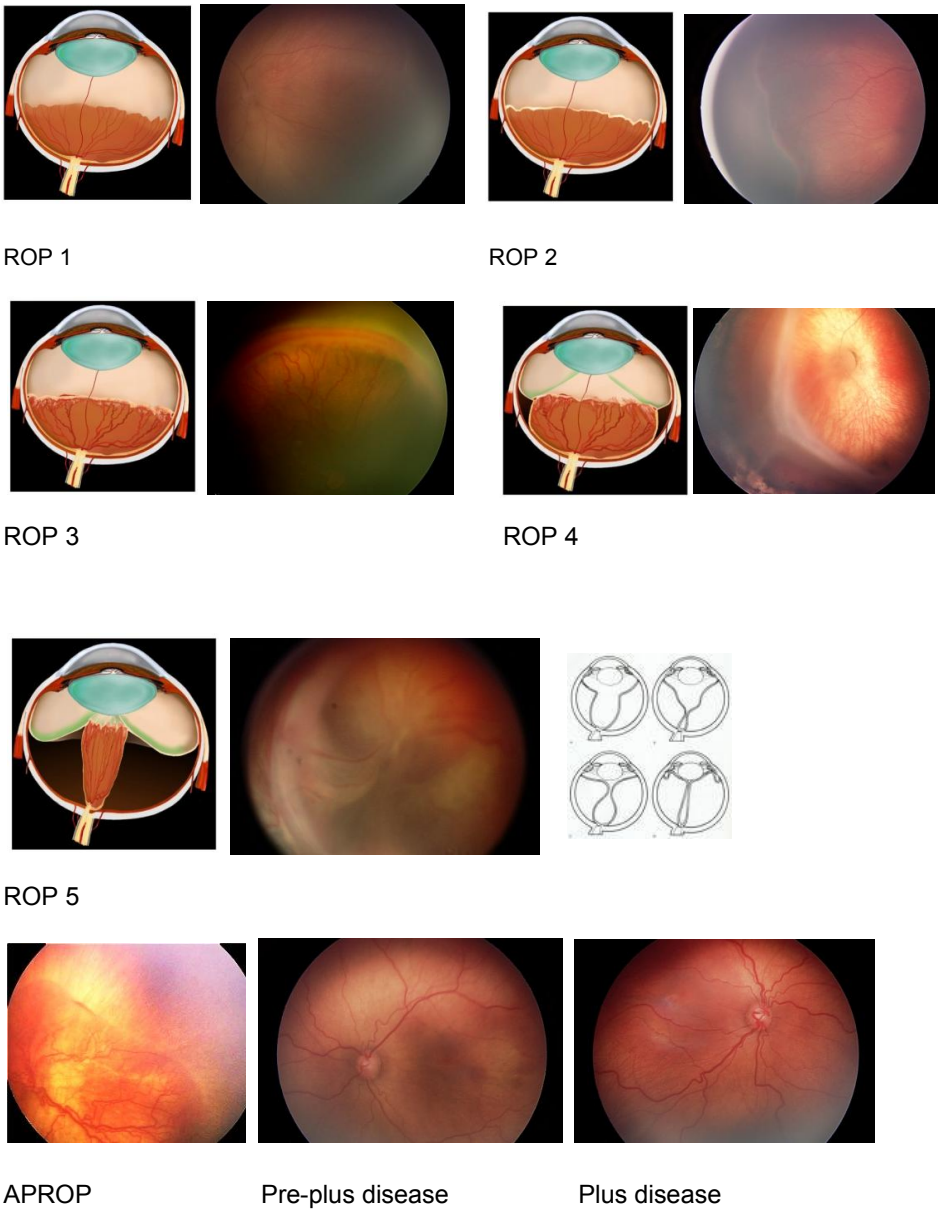

Figure 2 Photo's depicting the different stages of ROP
Schematic pictures are published with approval of ROPARD.
Retcam images from MMC Veldhoven and LUMC.

PATHOFYSIOLOGY

From the 16th week of gestation the retinal vessels start growing from the optic disc to the periphery of the retina. The retina is fully vascularized at the nasal side at 32 weeks and the temporal side around 40 weeks of gestation.[37] Infants born prematurely have incompletely vascularized retinas with a peripheral avascular zone.[38] Two processes underlie vascular development. The first process, termed vasculogenesis, involves the de novo establishment of a rudimentary vascular plexus, and is completed at 21 weeks GA. The second process is termed angiogenesis and involves the development of new vessels from already existing vessels and is initiated by 17–18 weeks GA.[39] When there is a significant disruption in the angiogenesis, ROP may develop.

After premature birth, there is not only a change in oxygenation of the retina but also a loss of maternally derived factors that contribute to normal retinal vessel formation.[40] This may compromise normal vascular development.

Retinopathy of prematurity is a biphasic disease, consisting of an initial phase of vessel growth retardation called the vaso-obliterative phase, followed by a second phase the vaso-proliferative phase. The vaso-obliterative phase is initiated upon birth of the premature infant. The extra-uterine environment is relatively hyperoxic for the infant, and by use of additional oxygen this hyperoxia increases. In response to the elevated levels of oxygen, expression of hypoxia-driven angiogenic factors, like vascular endothelial growth factor (VEGF) and Erythropoietin (EPO) are downregulated: vessel growth ceases and already formed vessels constrict and retract.[41]

The vaso-proliferative phase usually develops after 32 week GA. Because of maturation of the retina, there is an increase in the metabolic activity in the incompletely vascularized areas causing hypoxia. This hypoxia leads to stabilization and translocation of hypoxia-inducible factors (HIFs). HIF-1 is rapidly degraded during normoxia, but in hypoxic conditions, its half-life is prolonged, promoting its nuclear accumulation. Of the hypoxia-inducible factors, HIF-1α is considered the primary hypoxic signaling molecule, leading to stimulation of transcription of angiogenic factors like VEGF and EPO that stimulate new vessel growth.[42] The reduction of HIF-1 is essential for the initiation and progression of the first phase of ROP, whereas its increase is essential for the second phase.[43]

VEGF is necessary for normal vascular development and maintenance of growth. Its production is oxygen-regulated and is found to be important in both phases of ROP.[44] VEGF is likely to be the factor with the strongest influence on vascular activity in ROP eyes.[45]

EPO has a main function in erythrocyte formation in the bone marrow and plays a role in angiogenesis, independent of VEGF.[45] The exact role of EPO in retinal neovascularization is still not well elucidated but it has been reported that it stimulates cell proliferation, migration, tube formation and permeability, just like VEGF.[46]

Hypoxia stimulates overproduction of VEGF and EPO, inducing angiogenesis that can lead to the formation of new vessels of bad quality (neovascularization) at the verge of avascularized and vascularized retina, that can regress if adequate oxygenation is provided to the avascular retina.
Also other molecules with angiogenic or anti-angiogenic properties appear to play an important role either by inter-acting with VEGF or by acting independently as is seen with IGF-1.[47]
Nitric oxide (NO•) and nitric oxide synthase (NOS) are oxidative and nitro-oxidative stress-dependent mediators that are increased in retinal hypoxia. Nitric oxide (NO) is a key signaling molecule that mediates neurotransmission, vasodilatation and host cell defense. NO• triggers the gene expression of several angiogenic, cell-migration and proliferation-inducing factors, including VEGF.[48]
Hypoxic injury increases retinal mRNA and protein expression of endothelial nitric oxide synthase (eNOS), leading to increased NO production and, consequently, vasodilatation and angiogenesis.[49]

Maternally derived factors that play a role in the pathogenesis of ROP are insulin-like growth factor–I (IGF-I) and omega-3 polyunsaturated fatty acids (PUFA).
Insulin-like growth factor (IGF-I) is produced by the placenta and an important factor in normal retinal vascular development.[50] Under normal conditions the concentration of IGF-I in the foetus increases during the third trimester of pregnancy, but in case of preterm birth, IGF-I concentration decreases rapidly, as the placenta can no longer supply IGF-I.[51] As the infant matures, IGF-I levels slowly increase, and metabolic activity of the nonvascularized retina increases, leading to tissue hypoxia. For normal vascular development and outgrowth, concentrations of VEGF and IGF-1 need to be in balance with each other. If the balance is disrupted ROP can develop (fig 3), if the balance is established than regression of ROP can be seen and normal vessel outgrowth can develop.

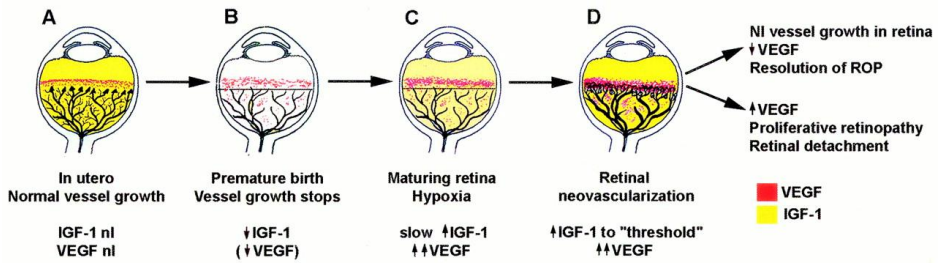

Figure 3 Schematic representation of IGF-I/VEGF control of blood vessel development in ROP.
Adapted from: Hellström et al, Proc Natl Acad Sci USA 2001; 98: 5804-8.

Another maternally derived factor is PUFA that potently protects against neovascularization. Retinal lipids have a very high content in long-chain poly-unsaturated fatty acids (PUFA). The major PUFA's found in the retina are omega-3 and omega-6. These lipids are essential fatty acids. They have to be of exogenous origin since the human body cannot synthesize their precursors.[48] Omega-3 PUFA is mainly transported in the third trimester of pregnancy and infants born before miss that infusion of specific lipid. Deficiency of omega-3 PUFA causes retardation of retinal vessel growth.[52]
It may be evident that a large number of mediators are involved in the pathogenesis of ROP. Every step in a better understanding of the complex molecular mechanisms underlying the pathogenesis of ROP will hopefully allow the development of new therapies to prevent or treat ROP.

ETIOLOGY

The etiology of ROP is multifactorial, but the most important risk factors are birth weight and gestational age of the premature born infant.[53-57] Other frequently found risk factors are: length of stay on a neonatal intensive care unit (NICU)[58], duration of artificial ventilation (AV)[59;60], administration of postnatal glucocorticoids[59;60], duration of oxygen supplementation and fluctuations in oxygen saturation levels.[57;61] [55] Perinatal infection/inflammation or sepsis and necrotizing enterocolitis (NEC) are published as a risk factor for (severe) ROP.[62-66] Another study found that male gender has a significantly increased risk to develop ROP.[67] More recently published risk factors are hyperglycaemia[68;69], slow weight gain during the first 3-4 weeks after birth[56] or poor growth.[70]
From the 1940s onwards, the toxity of oxygen in relation to ROP has been studied. Hypoxia induced oxidative stress to the developing vessels is not only caused by supplemental oxygen but also mediated by the hypoxanthine-xanthine system generating oxygen radicals. This causes capillary damage through toxic effects on endothelial cells, vaso-obliteration, shunt formation and subsequent neovascularization of vessels.[21;71]
The role of blood transfusions and the association with the development of ROP has been confirmed in several studies.[63,72,73] A Dutch cohort study showed that more than three blood transfusions, given in the first 4 weeks after birth, increased the risk to develop ROP significantly.[74] Red blood cell (RBC) transfusions are associated with an increased risk for ROP as they increase retinal oxygen levels by an increase in oxygen carrying capacity and a decrease in oxygen affinity of the red blood cell. The latter is caused by the fact that infants are usually transfused with adult hemoglobin, which has a reduced oxygen affinity compared to fetal haemoglobin.[75] An additional explanation may be that blood transfusions increase the free, non-protein bound iron load. Non protein bound iron may react with various intermediates of oxygen. It converts less reactive radicals

such as hydrogen peroxide and superoxide to the highly reactive, free hydroxyl radical. Free oxygen radicals are assumed to play an important role in the pathogenesis of ROP as they cause direct, irreversible damage to the developing retinal vessels by damaging the endothelial cell membrane, its mitochondrion and its nucleus.[76] An unintended side effect of RBC transfusions could be the concomitant administration of IGF-1. Hübler et al[77] showed that the IGF-1 load in RBC transfusions is equivalent to a single dose of 1 µg/kg, which is 5-10% of the adult dose. IGF-1 is an important mediator for the development of retinal vessels. Hellström et al showed that in a period of rapid increase of IGF-1 in combination with high levels of VEGF, rapid growth of new vessels takes place.[78] Therefore, supplemental administration of IGF-1 via RBC transfusions may thus trigger a rapid growth of neovascularizations as seen in the development of severe ROP.
Regarding transfusions, infants with twin-twin transfusion syndrome (TTTS) are of interest. Due to severe anaemia, donor infants need high numbers of RBC transfusions facilitating the development of severe ROP.[79]
All above mentioned risk factors have in common that they are indicators for the severity of illness of the infant.[55;80]

PROTECTIVE FACTORS

A few factors are associated with a reduced risk for ROP, and thus related with a lower incidence of ROP: prenatal glucocorticoids, female gender, surfactant and oxygen.[67;81]

Prenatal glucocorticoids
The beneficial role of antenatal glucocorticoids on the severity of ROP is described by Higgins et al.[81] Given before birth, maturation of the fetal lung is stimulated, resulting in a reduction in respiratory distress syndrome (RDS), associated with decreased morbidity and mortality.[82;83]

Female gender
Darlow et al[67] identified gender as a risk factor for ROP finding a significant increase of ROP in boys. The study of Binet et al[84] found no difference in the rates of ROP between the two gender groups. But males had poorer respiratory outcome, were more likely to have an adverse neonatal outcome or had a higher mortality than females. They hypothesize that antenatal glucorticoids do not benefit males as much as females.[84] Several studies describe an advantage in the survival of girls among premature infants supposedly related to differences in hormonal milieu and illness severity.[85;86]

Surfactant

Respiratory distress syndrome (RDS) is caused by a deficiency or dysfunction of pulmonary surfactant. Surfactant lines the alveolar surface and prevents atelectasis by reducing surface tension. Its concentration is often decreased or even lacking in the lungs of very preterm babies and therefore surfactant replacement therapy has been used for the treatment of RDS since the eighties. Beneficial effects on ROP can be explained by earlier stabilization of the RDS, less RDS-related morbidity, a decrease in duration of supplemental oxygen, a decrease in mean fraction inspired oxygen administration and less fluctuations in oxygen saturation. The influence of surfactant therapy on overall incidence and the incidence of severe ROP has been investigated. Some studies found a decrease in severe ROP, others did not find a protective effect.[87-90] To evaluate if the timing of administration of surfactant influences mortality and morbidity a Cochrane review has been performed.[91] Early (within the first two hours of life) versus delayed surfactant administration to infants with established RDS requiring assisted ventilation showed a decreased risk of acute pulmonary injury (decreased risk of pneumothorax and pulmonary interstitial emphysema) and a decreased risk of neonatal mortality and chronic lung disease compared to delaying treatment of such infants until they develop worsening RDS. No difference was found between early versus delayed surfactant administration regarding all stages or stage 3 or more ROP.[92-95]

Oxygen

Although oxygen in general is considered a risk factor for ROP, the role of oxygen to reduce the risk of severe ROP is extensively studied and will be discussed in more detail. The Supplemental Therapeutic Oxygen for Prethreshold ROP (STOP-ROP) trial found that higher oxygen saturation levels (96-99%) as compared with lower levels (89-94%) did not significantly reduce the risk of severe ROP, but a subgroup analysis suggested that infants with prethreshold disease without plus disease could benefit from these higher levels.[96] Other studies[61;97;98] describe that lower oxygen saturation levels during the first weeks of life decrease the incidence of ROP and of severe ROP, but are also associated with a higher mortality. To answer the question whether infants should be kept at low or high oxygen saturation in the period after birth, five multicenter randomized trials were recently analyzed in the Neoprom study.[99] The trials included were the SUPPORT (Surfactant, Positive Pressure and Pulse Oximetry Randomized Trial)[100;101], the three BOOST trials (Benefits of Oxygen Saturation Targeting) from the UK, Australia and New Zealand[102], and the Canadian Oxygen Trial (COT).[103] A total of 4911 infants with GA < 28 weeks were randomized to low (85–89%) or high oxygen saturation (91–95%) in the first 24 hours after birth and relative risks (RR with 95% confidence intervals) were calculated for mortality and morbidity. A RR>1.0 favored high oxygen saturation. The primary outcome of the SUPPORT was severe ROP / death before discharge, for the BOOST II

death or severe disability at 18-24 months and for the COT death before 18 months or severe neurosensory outcome. The RR for mortality (1.41; 114-174) and necrotizing enterocolitis (1.25;1.05-1.49) was significantly increased and for severe ROP (0.74;0.59-0.92) significantly decreased in low versus high oxygen saturations. No differences were found for bronchopulmonary dysplasia, patent ductus arteriosus and injury to the brain. The conclusion of the Neoprom meta-analysis was that in infants <28 weeks low saturation targets (85–89%) until 36 weeks postmenstrual age (PMA) are associated with more deaths and more NEC and higher saturation targets (91–95%) are associated with more ROP. Based on these figures the final advice of the Neoprom is to target SpO_2 at 90-95% in infants born before 28 weeks until 36 weeks PMA, although this might result in more infants developing ROP.

NATURAL COURSE

ROP is in general a symmetrical disease and both eyes are usually evenly affected. In most infants ROP regresses spontaneously. When ROP develops, it normally progresses with one stage per week, except for APROP.

In studies two different definitions for the age of an infant are used:

- PNA: post natal age: number of days or weeks after birth, and
- PMA: post menstrual age: gestational age plus the days or weeks after birth

The first signs of ROP develop between 5–7 week post natal age (PNA).

In the Natural History Study of Fielder[104] the PMA at which ROP developed ranged from 29.7 to 45.0 weeks. Furthermore it is known that severe ROP does not develop before 31 weeks post menstrual age (PMA) or 4-5 weeks PNA.[105,106]

Comparing the outcomes of the CRYO-ROP and ETROP study onset of the different stages of ROP appears to be consistent: stage 1 at 34.3 vs 34.1 weeks, stage 2 at 35.4 vs 35.1 weeks, stage 3 for both at 36.6 weeks and plus disease at 36.3 vs 36.0 weeks PMA.[107] Larsson et al[105] found a PMA for severe ROP of 36.1 (32–44) weeks. Hains et al[108] found a mean PNA at first diagnosis of stage 3 ROP at 10.8 (4–24.7) weeks and at mean PMA 36.6 (30.8–51.7) weeks.

In most cases, spontaneous regression of ROP usually occurs within 15 weeks from onset[109] without serious secondary damage in eyes with stages 1, 2 and early stage 3 ROP, while serious visual impairment or blindness results from progression to retinal detachment (stage 4 or 5) or severe distortion of the macular area.[110] The incidence of spontaneous regression of ROP stage 1 was between 86.7 - 85%, stage 2 between 57.1-56% and stage 3 between 5.9-6.0%.

With vascular changes in zone III regression was detected in 95-100%, in zone II in 45-46.2% and in zone I in 0-6%.[111;112]

SCREENING

Worldwide different screening criteria are used.
The guideline of the UK developed by the Royal College of Pediatric & Child Health, recommends screening for all infants with a birth weight ≤ 1,500 g or a gestational age of ≤ 31 weeks.[113]
The American guideline advises to screen all infants with a birth weight (BW) <1.500 g or a gestational age (GA) ≤ 30 weeks, as well as selected infants between 1.500 and 2.000 g and selected infants > 30 weeks GA with an unstable clinical course, including those requiring cardiorespiratory support and those who are believed to be at high risk for ROP by their attending pediatrician or neonatologist.[114]
The Canadian guideline recommends screening of infants with a BW ≤ 30 weeks or BW ≤ 1500 g (www.eyesite.ca).
The Guideline of Germany uses the inclusion criterion of GA < 32 weeks or when GA is unsure, a BW < 1500 g.[115] Infants with a GA between 32-36 weeks and > 3 days in need of extra oxygen administration should also be screened.
The screening criterion of Sweden is GA ≤ 31 weeks.[105] The inclusion criteria for screening in the Netherlands, as defined in 1997, were all infants with GA<32 weeks and/or BW< 1500 g and all those needing >40% oxygen for more than 3 days.

The timing for follow-up screening is grossly the same in all the aforementioned countries:
Twice a week: ROP in Zone I including suspected presence of APROP.
Weekly screening: avascular retina in zone I or posterior zone II; with (pre) plus disease; ROP 3 in every zone; ROP 2 in zone II and ROP 1 in zone I.
Every 2 weeks: avascular retina or ROP 1 in zone II; ROP in regression in zone II; avascular retina in zone III with or without ROP.
Every 2-3 weeks: avascular retina in zone II; ROP 1 or 2 in zone III; regression of ROP in zone III; and when regression is seen with follow up examinations.

Last screening
When there is no risk for the development of vision-threatening ROP, screening can be discontinued. If the retina is fully vascularized ROP can no longer develop. When the vessels have grown to zone III the chance of developing severe ROP is minimal.
According to the UK guideline screening may be stopped when apparent regression is seen. The definition of regression has been described in the ICROP.[34] The German guideline advises to end the screening at 40 weeks PMA. When the condition of an infant deteriorates, a reactivation of ROP can be seen and screening should be restarted.

Screening models

Several attempts are made to define risk factor models predicting the chance to develop ROP with the ultimate goal to focus screening on those infants that are really at risk to develop ROP and reduce the overall number of screening examinations.
Preconditions for these models are:

- easy to execute,
- high predictive score so no infants with severe ROP will be missed and
- applicable for different countries.

Four ROP screening models based on postnatal course were developed: WINROP, ROPScore, Cumulative Illness Severity (CIS) and a model based on Clinical Risk Index for Babies (CRIB) score, multiple birth, race and gender.[86;116-119]

WINROP

The WINROP[116] model is an algorithm using postnatal weight measurements, as a tool for the prediction of ROP. Weight measurements are entered into the WINROP database, which triggers an alarm for an abnormal weight gain rate. Infants were classified into categories of no alarm (unlikely to develop type 1 ROP) and alarm (at risk for developing type 1 ROP). Use of WINROP requires that an infant has:

- gestational age less than 32 weeks at birth,
- weekly weight measurements,
- physiologic weight gain, and
- absence of other pathologic retinal vascular disease.

Studies to evaluate the efficacy of WINROP in predicting the need for treatment have been done in different countries: in China the predictive score was 87.5%[120], in Mexico 84.7%[121] and in a Swedish cohort 96%.[122] A retrospective cohort study in South East Scotland emphasizes that WINROP should be used as recommended i.e. using weekly birth weights. They found a sensitivity of 73% when missing BW data were extrapolated and a sensitivity of 87% when BW had been measured weekly.[123]

ROPScore

The ROPScore model is based on risk factors for the onset of ROP and includes data on BW, GA, weight gain proportional to BW measured at 6 weeks of life, the use of oxygen in mechanical ventilation and the need for blood transfusions. ROPScore is calculated automatically when data are inserted. Points are given if risk factors are present. If the score is above 14.5 there will be a higher risk to develop severe ROP than in infants with lower scores. Eckert found a sensitivity of 98% and a specificity of 56% in a cohort of 474 Brazilian infants.[117]

CIS

The third model, Cumulative Illness Severity[118], works with cumulative neonatal illness severity and illness severity fluctuation as predictors of progression from moderate to severe ROP. It is measured using daily Scores for Neonatal Acute Physiology (SNAP) for the first 28 days of life, and illness severity fluctuation as assessed by summing up changes between daily SNAP scores. The physiology-based SNAP score is calculated from 34 biological parameters including vital signs and laboratory values. Cumulative SNAP score for the first 28 days of life (SNAP28) was calculated by addition of daily SNAP scores for each subject. The cumulative SNAP score, turned out to be an independent risk factor for progression from moderate to severe ROP, but after adjustment for different risk factors, it did not enhance assessment of risk for ROP. As it is also time consuming to execute, CIS seems less applicable as easy screening tool.

CRIB

The Clinical Risk Index for Babies score (CRIB)[86] estimates illness severity using data collected in the first 12 hours after birth. The parameters are birth weight, gestational age, congenital malformation, maximum base excess and maximum and minimum appropriate FiO_2 (fraction of inspired oxygen). Yang et al investigated 5 models based on CRIB score, multiple birth, race and gender. Their primary outcome variable was ROP warranting surgery and they found that nonblack race, male gender, and higher CRIB illness severity scores were significant predictors. They also excluded BW and GA from the CRIB score and found that the CRIB-score remained a highly significant independent predictive factor for ROP warranting surgery.

All these prediction models need further validation before they can possibly replace established screening programs. They can however help to reduce the frequency of screening exams in low risk infants.

TREATMENT

The goal of treatment is to decrease the production of angiogenic factors and to stop retinal neovascularization by inactivation of the avascular peripheral retina.
The Cryotherapy for Retinopathy of Prematurity Cooperative Group trial (CRYO-ROP) showed the beneficial effect of cryoablation of the peripheral avascular retina.
Two types of ROP were distinguished, namely threshold and pre-threshold ROP.
Threshold ROP was defined as at least 5 contiguous or 8 cumulative clock hours of stage 3 ROP in zone I or II in the presence of plus disease. Pre-threshold ROP was defined in relation to the location of the disease, for zone I: any stage less than threshold and

for zone II: stage 2 ROP with plus disease, or stage 3 ROP of any amount without plus disease, or stage 3 ROP with less than 5 contiguous or 8 cumulative clock hours with plus disease. When lasertreatment started to replace cryotreatment the Early Treatment of ROP (ETROP) study was performed which confirmed the efficacy of treatment for severe ROP and redefined the indications for treatment.[30;31] The clock hours and the terminology of threshold and pre-threshold were abandoned and a new categorization of ROP was introduced in which ROP was defined in two types, where type 1 needed immediate treatment and type 2 warranted thorough observation:

- Type 1:
  - ROP in zone I, with plus disease;
  - ROP 3 in zone I, with or without plus disease;
  - ROP 2 or 3 in zone II, with plus disease.
- Type 2:
  - ROP 1 or 2, in zone I, without plus disease;
  - ROP 3 in zone II, without plus disease.

The ETROP study was a randomized, prospective multicenter trial comparing the safety and efficacy of earlier vs. conventionally timed ablation of the peripheral retina for the management of moderate to severe retinopathy of prematurity (ROP). Infants with birth weights <1251 g were screened and the total sample size for the randomized trial consisted of 401 infants. Only prethreshold eyes that had a high risk of an adverse outcome were randomized for early treatment. The primary outcome measure was rating visual acuity measured by Teller acuity card. The secondary outcome measure was retinal structure, assessed by ophthalmological examinations conducted at 6 and 9 months post-term.
At six year follow-up an unfavourable outcome was seen in type 1 ROP with early treatment in 25.1% and in type 1 ROP treated at threshold in 32.8% ($p < 0.001$). A visual acuity (VA) worse than 20/200 was found in 24.7% for those treated at pre-threshold vs 29% for those treated at threshold ($p=0.15$).[124]

Definitions of favorable and unfavorable outcome[28] of treatment according to ETROP:

Favorable:

- Essentially normal posterior pole (near periphery and zone I), including angle of vessels
- Abnormal angle of major temporal vascular arcade in the posterior pole
- Macular ectopia
- Stage 4A partial retinal detachment, also including retinoschisis, or fold in the posterior pole (fovea spared)

Unfavorable:

- Stage 4B partial retinal detachment, also including retinoschisis, or fold-all with foveal involvement
- View of macula (and presumably patient's central vision) blocked owing to partial cataract, partial retrolental membrane, or partial corneal opacity due to ROP
- Stage 5 total retinal detachment, or total retinoschisis, or retrolental membrane (blocking all view of fundus)
- Entire view of posterior pole and near periphery blocked by total cataract or total corneal opacity from ROP
- Enucleation for any reason
- Unable to grade or determine or none of the above

To calculate improved vision after treatment according to ETROP criteria vs CRYOROP criteria one has to compare no treatment vs treatment. For the CRYO-ROP study cryo-therapy vs no treatment meant 44.4 vs 62.1% = 17.7% improved vision. In the ETROP study no comparison was made between no treatment and laser as this was considered unethical. So the improved vision in this study is the difference between 25.1% vs 32.8% resulting in 7.7% less infants with VA < 20/200.
The total improved vision is determined by summing the results of CRYO-ROP and ETROP, which would result in 17.7 + 7.7= 25.4% improved vision after early treatment.
With the ETROP classification more emphasis came on the presence of (pre)plus disease, and the extent of avascularity, defined by zone. Nowadays this is the most important treatment algorithm.

With laser and cryo-therapy the treated areas of the retina are permanently damaged and lose their function. Different new possibilities for treatment are being investigated, that might save peripheral retina, the main being treatment suppressing VEGF production by intraocular injections.
To study the efficacy of anti-VEGF drugs, preclinical studies have used models of oxygen-induced retinopathy (OIR) that develop hypoxia-induced intravitreal neovascularization (IVNV).
The Bevacizumab Eliminates the Angiogenic Threat of ROP (BEAT-ROP) study is the first prospective, controlled, randomized, multicenter trial to assess intravitreal bevacizumab monotherapy for zone I or zone II posterior stage 3+ ROP compared to conventional laser.[125] The primary ocular outcome was recurrence of retinopathy of prematurity in one or both eyes requiring retreatment before 54 weeks' postmenstrual age. Recurrence of ROP was 4% in the bevacizumab group vs 22% in the laser group (p=0.002). A significant

treatment effect was found for zone I retinopathy of prematurity (P=0.003) but not for zone II disease (P=0.27). The main comment on the study is that laser treatment was not performed according to ETROP criteria, which is common practice nowadays. This might have biased outcomes of laser treated eyes.

Because of this positive result on the short term, other studies have been conducted. Concerning reports of persistent peripheral avascular retina (AVA), recurrent IVNV, and stage 5 ROP retinal detachment, even 1 year following treatment, in some of these treated eyes have been published.[126-129] Furthermore it has been reported that anti-VEGF agents are still measurable in the systemic circulation 8 weeks after treatment possibly causing adverse effects on other developing organs such as kidney, lung and brain.[130]

In an animal study, it was established that even in a controlled model in which external conditions (as for example oxygen levels, body weight, species, number of pups) were kept constant, variability in responses could be seen. The signaling effects following anti-VEGF treatment are complicated by the effects on different retinal cells, timing of anti-VEGF treatment, and dosing. It was concluded that in a human preterm infant the variability is even greater, making it difficult to determine the correct dose. They also found that weight gain was impaired in the pups who received anti-VEGF treatment.[131]

All these side effects of Anti-VEGF treatment therefore call for further investigation in multicenter, randomized controlled trials. Until then, administration of these drugs should only be done by experienced centers and after written informed consent of the parents. After all, we have to keep in mind that VEGF is necessary for normal retinal vessel development.

OUTLINE OF THIS THESIS

Changes in neonatal care have increased the survival of preterm infants and decreased the age at which preterm born infants are kept alive, resulting in an increasing number of infants at risk for ROP. Therefore up-to date information on incidence of ROP and visual impairment (VI) due to ROP was needed.

In Chapter 2 the incidence of VI due to ROP and concomitant disabilities in preterm neonates born between 2000 and 2009 in the Netherlands is reported. Data were retrieved from the Dutch Institutes for the visually impaired. Outcomes were compared with previous Dutch studies resulting in an overview of more than 30 years to determine if changes in neonatal care resulted in a different outcome in incidence of ROP and concomitant disabilities.

The last study on ROP incidence in the Netherlands was conducted in 1952 by Von Winning.[132] As no obligatory national registry for ROP exists, a deficient insight in incidence and risk factors for ROP exists, leaving several Dutch cohort studies as the only source of

information.[60;89;133] A Netherlands Perinatal Registry (PRN) is available, which is used by neonatologists and pediatricians to improve the quality of health care through insight into the perinatal care process and its outcomes. A section on ROP is included, but is often poorly filled out because correct information on ROP is not known or the PRN document is completed in a phase where information on ROP screening is not available yet.
In the Netherlands an increasing number of hospitals provides care to premature born infants, facilitating earlier transfer. Initially most premature infants were admitted to one of ten neonatal intensive care units (NICUs). Nowadays as soon as infants are respiratory and circulatory stable and intensive care is no longer required, they are transferred to one of 16 high care centers (HC) or 77 regional centers (RC).
Before, the initial screening examination was performed by ophthalmologists in a NICU. They screened a large amount of infants and were therefore very experienced in ROP screening. This resulted in careful selection of high risk infants whose transfer to a HC or RC was postponed. Nowadays, transfer from a NICU to a HC or RC often takes place before the first ROP screening has been performed. Therefore, ROP screening has to be performed by an increasing number of Dutch ophthalmologists who have less overall exposure to ROP patients due to a limited number of patients. In addition, transfer of a child can result in unintended loss of clinical information concerning the neonate as well as loss of data concerning start or follow-up of ROP screening.
All the above mentioned issues called for the necessity of an updated insight in incidence and risk factors for ROP, adherence to the screening protocol and treatment policy in our country. Therefore a prospective nationwide inventory on ROP was initialized: the NEDROP study. With these up-to-date data, our final aim was to develop a new quality guideline for screening and treatment that would fit the Dutch situation.

The NEDROP-study

In 2008 we requested pediatricians and neonatologists to report all infants born in 2009 that complied with the inclusion criteria of the then prevailing National Guideline. Furthermore we recruited the screening ophthalmologists to report all children they screened for ROP born in 2009. A code was developed to enable anonymous data transmission and coupling to the National Perinatal Registry (PRN) to link risk factors for ROP with ophthalmological data.
Permission of the Medical Ethical Committee (METC) in Leiden was obtained. Their decision was as follows: *'Since no persons are subjected to a treatment or are required to behave in a certain manner, this proposal does not require a full review by the Medical Ethics Committee according to the WMO (Medical Research involving Human Subjects Act). To protect the privacy the processing of personal data is performed according with the Wbp (Personal Data Protection Act).'*

All personal data were coded and later on further depersonalized by numbering. This discharged us from the obligation to ask permission of METC's of all participating hospitals, enlarging the chance of success enormously.

At the start of this study the active guideline in the Netherlands dated from 1997. [134] The screening criteria included infants with GA < 32 weeks and / or a BW < 1500 grams and /or preterm infants treated with more than 40% oxygen for more than 3 days. Initial screening should be between 5–6 weeks of life (PNA).

Based on these screening criteria we calculated that approximately 1650 premature infants per year would comply and were eligible to enter the NEDROP study. The results of the NEDROP study are reported in chapter 3, 4 and 5. Chapter 3 reports data on the incidence, screening schedules, treatment, transfers and logistics of ROP in the Netherlands. Chapter 4 provides insight in the risk factors found for ROP after coupling the NEDROP database to the PRN database.

With all these data we acquired a good overview of the current situation in our country. As screening for ROP is costly, discomforting for the neonate and time-consuming for the ophthalmologist, the screening guideline was further evaluated. A national ROP guideline working group investigated whether a reduction of our inclusion criteria for screening was possible, on the pre-condition that no infants with severe ROP would be left out. Whether a modification of our national screening guideline is justified is described in Chapter 5. Based on figures from the NEDROP study a cost-effectiveness study was performed, the most efficient strategy is calculated in Chapter 6. Chapter 7 is a case report of two twin pairs with severe retinopathy of prematurity in twin-twin transfusion syndrome (TTTS) after multiple blood transfusions.

Finally a summary of the results is presented and conclusions and recommendations for the future are formulated.

The addendum provides the tangible outcomes of this thesis namely the:

- Summary of the quality screening guideline 2013
- Parents information folder
- The newly developed screening form

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