Growth pattern and nutritional intake as predictors of retinopathy of prematurity

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Abstract

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Background Retinopathy of prematurity (ROP) is a sight-threatening disease that affects extremely preterm and very preterm infants. Approximately 5–10% of infants screened for ROP go on to develop severe ROP that requires treatment. To minimize unnecessary screening procedures, which can be stressful for these fragile infants, it is important to identify new risk factors and predictors that better determine which infants are at high risk for ROP. The objective of this study was to investigate growth pattern (peri- and postnatal weight gain) and nutritional intake as risk factors for severe ROP.

Methods WINROP (Weight, insulin-like growth factor 1, neonatal, retinopathy of prematurity) is a web-based surveillance system that aims to identify infants at high risk of ROP based on their birth weight (BW), gestational age (GA), and postnatal weight gain. In all cohorts that we studied, BW, GA, gender, and maximum ROP stage and ROP treatment were retrospectively retrieved from hospital records. In Paper I, we validated WINROP in a Swedish population-based cohort of extremely preterm infants (born at GA <27 weeks) (n=407). This cohort, called the EXPRESS cohort, was further evaluated in Paper IV in relation to nutritional intake and the correlation with severe ROP. In Paper II, the association between infant weight standard deviation scores (WSDS) at first sign of ROP and ROP requiring treatment was evaluated in a Gothenburg cohort screened for ROP (n=147). In Paper III, the birth weight standard deviations score (BWSDS) was calculated in 5 cohorts (n=2941) that were previously included in WINROP studies; Paper III assessed the impact of low birth weight as a risk factor for severe ROP.

Results WINROP correctly identified 96% (45/47) of infants who required treatment for ROP in an extremely preterm cohort. Low weight (p=0.001) and low WSDS at first detection of ROP (p=0.002) were risk factors for severe ROP. Low BWSDS (p<0.001) was a risk factor for severe ROP for all preterm infants; however, the impact of low BWSDS increased with increasing GA. In addition, low energy intake (p<0.01) during the first four weeks of life was associated with the development of severe ROP (p<0.01).

Conclusions Weight at birth and postnatal weight gain can be useful predictors for severe ROP as can weight at first detection of ROP. In addition, low energy intake during the first four weeks of a preterm infant's life may be associated with later severe ROP.

Keywords: birth weight, nutrition, preterm infant, retinopathy of prematurity, risk factor, weight gain.

Contents

List of publications

This thesis is based on the following studies. In the text, these are referred to by the indicated Roman numerals.

I.   **Lundgren P, Stoltz Sjöstrom E, Domellöf M, Källen K, Holmström G, Hård AL, Smith LE, Löfqvist C, Hellström A.**
WINROP identifies severe retinopathy of prematurity at an early stage in a nation-based cohort of extremely preterm infants.
PLoS One. 2013 Sep 12;8(9):e73256

II.   **Lundgren P, Wilde Å, Löfqvist C, Smith LE, Hård AL, Hellström A.**
Weight at first detection of retinopathy of prematurity predicts disease severity.
Br J Ophthalmol. 2014 Nov;98(11):1565–9

II.   **Lundgren P, Kistner A, Andersson EM, Hansen-Pupp I, Holmström G, Ley D, Niklasson A, Smith LE, Wu C, Hellström A, Löfqvist C.**
Low birth weight is a risk factor for severe retinopathy of prematurity depending on gestational age.
PLoS One. 2014 Oct 15;9(10):e109460

IV.   **Stoltz Sjöström E, Lundgren P, Öhlund I, Holmström G, Hellström A, Domellöf M.**
Low energy intake during the first four weeks of life increases the risk for severe retinopathy of prematurity in extremely preterm infants.
Submitted for publication

Sammanfattning på svenska

Bakgrund

Med för tidig födelse, menar vi födelse mer än tre veckor före beräknad fullgången graviditet (40 veckor). I världen föds årligen ca 15 miljoner barn för tidigt. Allt fler barn som föds för tidigt överlever idag världen över. Ju omognare barnet är då det föds desto svårare är det för barnet att överleva eftersom många av barnets organ inte är fullt utvecklade. Omhändertagande av barnet första levnadstiden är viktig för barnets överlevnad och kommande livskvalitet, ju bättre vi kan hjälpa barnet att anpassa sig till världen utanför mammas mage desto större chans har barnet till ett gott liv.

Prematuritetsretinopati

Ett av de organ som inte är färdigutvecklat om barnet föds för tidigt är ögat och dess näthinna. Prematuritetsretinopati (ROP) är en synhotande ögonsjukdom som kan drabba barn som föds mycket omogna. Vid ROP utvecklas inte blodkärlen i näthinnan normalt. Vid den allvarligaste formen av ROP har onormala blodkärl bildats i näthinnan. Dessa onormala blodkärl kan orsaka blödningar i ögat, dragningar i näthinnan och i värsta fall näthinneavlossning, vilket kan leda till blindhet. Det är därför viktigt att allvarlig ROP upptäcks och behandlas i tid. Den mest använda behandlingen för att förebygga näthinneavlossning är laserbehandling.

För att identifiera de barn som behöver behandlas för synhotande ROP görs upprepade ögonundersökningar, s.k. ROP screening av för tidigt födda barn. I Sverige och i många andra länder undersöks alla barn som fötts före 31 graviditetsveckor rutinmässigt i ett ROP screening program. Cirka 1000 barn per år genomgår ROP screeningen i Sverige. Barnen ögonundersöks vanligen varje/varannan vecka från graviditetsvecka 31 tills näthinnan är färdigutvecklad eller tills behandling krävs. Endast ca 5-10 % av de barn som ögonundersöks behöver behandling för allvarlig ROP. Ögonundersökningen kan vara mycket påfrestande för barnet.

Barnets omognad vid födelse är den viktigaste riskfaktorn för ROP, men även andra faktorer kan öka risken som till exempel exponering för höga och ojämna syrgasnivåer, övrig sjuklighet och infektioner.

Tidig tillväxt

Under senare år har dålig tidig tillväxt identifierats som ytterligare en riskfaktor för ROP. Ju sämre barnet växer under sina första levnadsveckor desto större risk för ROP. Vid Göteborgs universitet har ett webbaserat system, kallat WINROP (www.winrop.com), utvecklats för att identifiera de barn som växer dåligt och har störst risk att utveckla allvarlig ROP. Genom att en gång i veckan programmera in barnets vikt i WINROP, beräknar WINROP om barnet har en hög risk att utveckla allvarlig ROP och larmar om så är fallet.

Nutrition
En annan nyupptäckt riskfaktor för ROP är otillräcklig näringstillförsel under barnets första levnadsveckor. För tidigt födda barn har ett stort behov av näring för att växa. Det är dock svårt att tillgodose detta behov eftersom bl.a. magtarmkanalen är omogen hos barnet. Ett för tidigt fött barn har heller inte utvecklat någon sugreflex så näringen måste från början ges via blodet eller via en sond.

Syfte
Samtliga studier i den här avhandlingen har genomförts i syfte att förbättra identifieringen av de barn som har allra störst risk att utveckla allvarlig ROP. Syftet var framförallt att minimera antalet påfrestande ögonundersökningar för de barn som har mindre risk att få allvarlig ROP. Tidig identifiering av riskbarn medför att dessa kan följas extra noggrant.

Studiepopulation och metod
EXPRESS studien inkluderar alla barn födda extremt för tidigt, före graviditetsvecka 27, i Sverige under åren 2004-2007. EXPRESS barnen studeras i artikel I och IV samt delvis i artikel III. I artikel II studeras barn i Göteborgsregionen under åren 2011-2012 som genomgått ROP screening. I artikel III studeras 5 kohorter;
3 svenska samt 2 amerikanska, där alla barn har genomgått ROP screening och ingått i tidigare WINROP studier.

Resultat
I denna avhandling konfirmerar vi att WINROP är ett tillförlitligt hjälpmedel vid ROP screening av extremt för tidigt födda barn. Vi visar också att barnets vikt vid första tecken till ROP kan användas för att prediktera synhotande ROP. Vi klargör att låg vikt/tillväxthämning vid födelse är en riskfaktor för allvarlig ROP, men med varierande genomslagskraft, som påverkar framför allt de något "mer mogna" extrem prematur födda barnen (födda efter graviditetsvecka 26). Låg näringstillförsel under de första veckorna i livet är associerat med senare allvarlig ROP.

Slutsats
Genom att analysera det för tidigt födda barnets födelsevikt och viktuppgång efter födelse kan vi erhålla värdefull information om barnets framtida risker för att utveckla synhotande ROP. Genom att optimera barnets tidiga näringstillförsel kan vi möjligen minska risken för allvarlig ROP.

Klinisk betydelse
De ögonundersökningar som görs för att identifiera allvarlig ROP är ofta påfrestande för barnet. Kan vi individualisera ROP screening med nya metoder och screening program och med större säkerhet identifiera de barn som har störst risk att utveckla allvarlig ROP, så kan vi bespara barnen med mindre risk onödiga påfrestande ögonundersökningar.

Abbreviations

AP ROP	Aggressive posterior retinopathy of prematurity
AUC	Area under the curve
BPD	Bronchopulmonary dysplasia
BW	Birth weight
BWSDS	Birth weight standard deviation score
CHOP	Children's Hospital of Philadelphia
CI	Confidence interval
CRIB	Clinical risk index for babies
DHA	Docosahexaenoic acid
EXPRESS	Extremely preterm infants in Sweden study
GA	Gestational age
EPA	Eicosapentaenoic acid
EPO	Erythropoietin
IGF-I	Insulin-like growth factor I
IVH	Intraventricular hemorrhage
LCPUFA	Long-chain polyunsaturated fatty acids
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
OR	Odds ratio
PDA	Patent ductus arteriousus
PMA	Postmenstrual age
ROC	Receiver operating characteristic
SDS	Standard deviation score
SGA	Small for gestational age
ROP	Retinopathy of prematurity
WHO	World health organization
WINROP	Weight, insulin-like growth factor 1, neonatal, retinopathy of prematurity
WSDS	Weight standard deviation score

Introduction

The preterm infant

A preterm infant is defined as an infant that is born at less than 37 weeks of gestation. Globally, about 15 million infants are born preterm each year according to a 2012 World Health Organization (WHO) report[1]. Prematurity is the world's leading cause of newborn death within the first four weeks of life and causes about 1 million newborn deaths each year. Approximately 10% of births worldwide are considered preterm[2].

Based on gestational age (GA), preterm infants are sub-grouped as follows:
- Moderate to late preterm (GA 32 to 37 weeks)
- Very preterm (GA 28 to 32 weeks)
- Extremely preterm (GA <28 weeks)

In recent decades, the survival rate of preterm infants has improved rapidly as the result of advanced peri- and postnatal care in countries with high-quality neonatal intensive care units (NICUs)[3,4]. Medical improvements have extended the limit of viability so that infants born as early as GA 22–23 weeks can survive. Annually, approximately 5.4% or 800 000 infants are born before GA 28 weeks (extremely preterm)[5].

In the third trimester, i.e. from the 29th week of gestation, the fetus undergoes intense growth and maturation as preparation for the transition from intrauterine to extrauterine life. Therefore, infants who are born extremely preterm are often poorly equipped to manage the extrauterine environment since vital alterations have not yet occurred in their immature organs. The preterm infant's first weeks of life are frequently complicated due to conditions and diseases that are correlated with immaturity (Figure 1)[6].

Figure 1. Mio is a very preterm boy who was born at 30 weeks and 6 days of gestation with a birth weight of 1350 g. ©Stina Fahlén

Retinopathy of prematurity

The retinal vasculature is not fully developed before full term gestation at ~40 weeks. Retinopathy of prematurity (ROP), a potentially blinding disease, arises when abnormal vascularization occurs in the maturing retina. Accordingly, in order to understand the pathogenesis of ROP, it is important to first understand normal retinal development and vascularization.

Normal retinal vascularization

The fetal eye has three major vascular systems: the hyaloid, the choroidal, and the retinal vasculature. The hyaloid vasculature gradually undergoes near-complete regression in the developing eye. In the maturing fetal retina, the choroid provides the retina with nutrients and oxygen by diffusion until the retinal vascularization is fully developed. Vasculogenesis (the formation and differentiation of the embryotic vascular system) in the retina starts at ~12 weeks of gestation and is completed at ~21 weeks of gestation, while angiogenesis (the formation and differentiation of blood vessels) starts at ~17 weeks of gestation[7,8]. The primitive retinal vessels emerge from the optic disc and extend/spread outward to the peripheral retina. The retina is fully vascularized at ~36 weeks of gestation in the periphery on the nasal side and at ~40 weeks on the temporal side[9]. It has been proposed that the vascularization of the maturing retina is initiated by the increased metabolic demands of the developing neural retina. As the retinal neurons develop and thicken, the retinal demands for oxygen exceed the supply from the underlying choroid. This leads to up-regulation of vasoactive growth factors such as vascular endothelial growth factor (VEGF) and erythropoietin (EPO), which in turn stimulates new retinal vessel growth[10,11].

Pathogenesis of retinopathy of prematurity

The hypothesis of oxygen toxicity and the concept of ROP as a two-phase disease were presented by Ashton in 1954. Ashton showed through animal experiments that excessive oxygen, termed hyperoxia, leads to vessel loss (phase I) followed by hypoxia-mediated vasoproliferation (phase II)[12].

Phase I of retinopathy of prematurity

Phase I of ROP is characterized by impaired retinal vessel growth and the loss of previously formed vessels, phenomena that have been attributed mainly to hyperoxia. Specifically, the preterm infant is born into an oxygen-rich extrauterine environment that is thought to cause retinal hyperoxia. Hyperoxia leads to down-regulation of vasoactive growth factors, such as VEGF. Decreased VEGF levels and low levels of other growth factors, such as insulin-like growth factor I (IGF-I), that are the result of preterm birth result in arrested retinal vessel growth and in vessel loss[12,13]. However, retinal oxygenation has not been measured in preterm infants. Phase I of ROP occurs approximately from birth to GA 30 weeks.

Phase II of retinopathy of prematurity

Phase II of ROP occurs as the preterm infant matures. As the retinal neurons develop, their demand for oxygen increases, resulting in localized hypoxia in the avas-

cular retina. This hypoxia causes up-regulation of VEGF levels and EPO, leading to unregulated vessel growth and vasoproliferation[11,14.] Vasoproliferation can cause fibrosis, retinal traction, and, in the worst-case scenario, retinal detachment and blindness. Phase II occurs at approximately from GA 31 weeks (Figure 2).
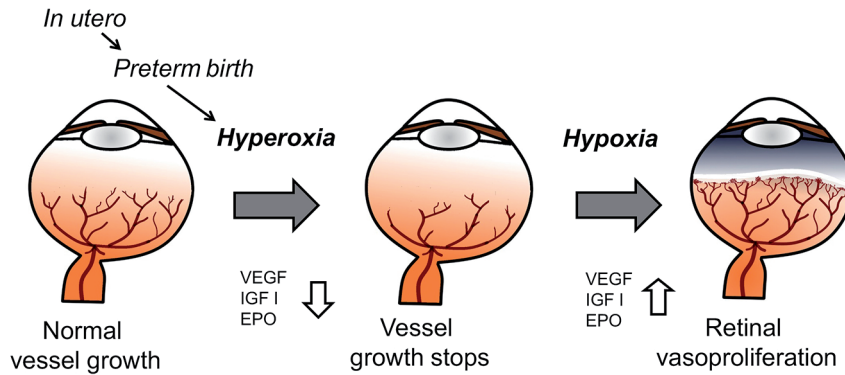

Figure 2. Development of retinopathy of prematurity (ROP). Up and down-regulation of vasoactive growth factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor I (IGF-I) and erythropoietin (EPO) due to hyperoxia and hypoxia are important actors in the development of ROP.

Classification of retinopathy of prematurity
ROP is classified by stage according to disease severity (ROP stages 0 to 5) and by zone according to the location of ROP in the retina (zones I to III).

Stages to classify the severity of disease
- Stage 1: A demarcation line, a thin and flat white line, is seen between the vascular and avascular retina.
- Stage 2: A ridge, an elevation of the retina, is seen in the region of the demarcation line.
- Stage 3: Extraretinal vasoproliferation extends from the ridge.
- Stage 4: Partial retinal partial retinal detachment.
- Stage 5: Total retinal detachment.

Zones to define the location of the disease
Zone I is the most central part of the retina, and zone III is the most peripheral part of the retina (Figure 3)[15].
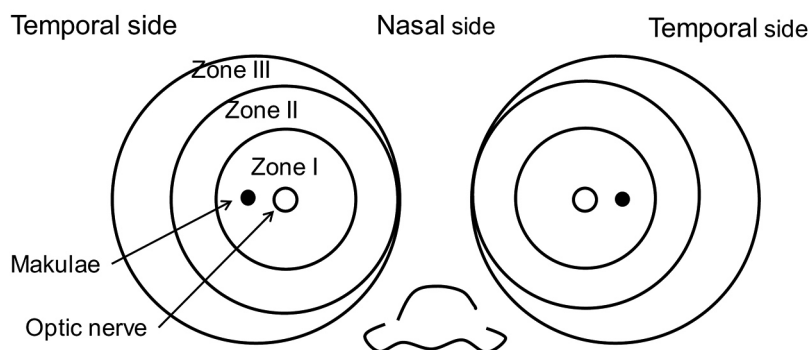
Figure 3. Retinal zones when classifying retinopathy of prematurity (ROP).

In clinical practice, ROP is often described as follows: no ROP (ROP stage 0), mild ROP (ROP stages 1–2), and severe ROP (ROP stages 3–5). Mild stages of ROP frequently regress spontaneously. When the disease progresses to ROP stage 3, a substantial proportion of cases continue to progress to sight-threatening ROP. Accordingly, treatment must be initiated as soon as possible. 'Plus disease' is a term used to describe an additional sign of ominous activity i.e. the finding that the central retinal vessels are dilated and tortuous. Aggressive posterior ROP (AP ROP) is an especially severe, rapidly progressive form of ROP that is characterized by prominent plus disease and by a flat network of neovascularization in zone I or in posterior zone II. If untreated, AP ROP usually progresses rapidly to retinal detachment, generally without developing through the classical ROP stages[15].

The international recommendations for ROP treatment are based on the following criteria: the stage of ROP, the zone in which ROP is detected, and whether plus disease is detected[16].

The term 'ROP type 1' is frequently used when any of these treatment criteria are fulfilled. Severe ROP that almost fulfills the treatment criteria is called ROP type 2. Frequent screening examinations, i.e. examinations conducted twice a week, are warranted for ROP type 2.

Retinopathy of prematurity type 1:
- ROP of any stage is present in zone I with plus disease;
- ROP stage 3 is present in zone I without plus disease;
- ROP stage 2 or 3 is present in zone II with plus disease.

Retinopathy of prematurity type 2:
- ROP stage 1 or 2 in zone I without plus disease;
- ROP stage 3 in zone II without plus disease.

Treatment for retinopathy of prematurity

For many years, laser treatment has been the treatment of choice for avascular retina. The purpose of laser treatment is to destroy the avascular hypoxic retina to reduce further up-regulation of VEGF, the treated areas of the retina are permanently damaged. Current recommendations specify that when the established treatment criteria for ROP type 1 are fulfilled, treatment should be initiated within 48 hours. In recent years, intravitreal injection of anti-VEGF antibodies has also emerged as an alternative treatment for central disease (zone 1) and AP ROP, although this remains an off-label application. Prolonged clinical follow-up may be warranted to those infants who have received anti-VEGF treatment because of the possibility of delayed recurrence of ROP[18,19]. Notably, neither the appropriate dose of anti-VEGF molecules nor the potential long-time side effects of anti-VEGF injections have been established. There have been reports that anti-VEGF agents can be measured in serum at least 8 weeks following an intravitreal injection in preterm infants and animals, and possibly have an adverse effect on other developing organs[20,21]. In Sweden, anti-VEGF injection is currently used primarily when laser treatment has been unsuccessful.
In some middle-income settings, anti-VEGF injections have become first-line treatment, since laser equipment and laser-trained ophthalmologists are not always available.

Sequelae of retinopathy of prematurity

In high-income settings such as Sweden, only a few percent of infants who are screened for ROP become blind (visual acuity <0.1) or severely visually impaired (visual acuity <0.3) due to ROP[22]. However, in middle-income settings, the risk of blindness or visual deficit due to ROP is higher. Gilbert et al. reports that in middle income settings, such as in Argentina, Cuba and the Czech Republic with fewer high level NICUs up to 60% of childhood blindness may be due to ROP. While the quality of neonatal care is improving, not all NICUs have ROP screening programs, adequate screening equipment, and ophthalmologists who are trained to detect and treat ROP[23]. Hence, many infants in middle-income settings become blind without ever being examined by an ophthalmologist[5]. Zepeda-Romero et al. reported that in Guadalajara city, Mexico, only 50% of the NICU's had a regular ROP program. In schools for the visually impaired in Guadalajara city more than 40% of the children (under the age of 5 years) were blind due to ROP. The majority of these infants had not been treated for ROP[24].

Even though severe ROP can be treated successfully and most blindness due to ROP and preterm birth can be prevented, ROP can have additional long-term eye sequelae. Refractive errors, anisometropia, subnormal visual acuity, and strabismus more frequently affect preterm infants with ROP, especially if ROP treatment has been performed. Infants born preterm without ROP are also at higher risk of developing these ophthalmological conditions than infants born at full term[25-28]. Preterm infants who have other neurological complications, such as intraventricular hemorrhage (IVH), cerebral palsy, and/or mental retardation, also have an additional increased risk of ophthalmological problems, including visual perception deficiencies[27,29,30].

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Severe ROP has also been associated with poor neurodevelopmental functional outcome, i.e. motor and cognitive impairment and severe hearing loss[31,32]. Systemic complications such as increased blood pressure have also been associated with severe ROP[33].

Most pediatric ophthalmologists agree that long-term follow-up is valuable for preterm infants. However, the optimal follow-up period remains a matter of debate. After ROP treatment, individualized follow-up has been suggested. In severe cases life-long follow-up may be warranted due to the increased risk of late sequelae such as retinal detachment[34-36.] For infants that not have been treated for ROP, some authors propose performing a first follow-up when the child is 2.5 years of age, while others claim that a follow-up at 4–5 years of age is sufficient to detect ophthalmological problems[27,37.] Most authors agree that special attention should be paid to infants with neurological complications.

Screening for severe retinopathy of prematurity

In most cases, blindness caused by ROP is preventable. It is therefore crucial to identify infants who are at risk of ROP. GA and birth weight (BW) are well-known major risk factors for ROP that reflect the infants' degree of immaturity[38,39]. In 1988, the first ROP screening guidelines were presented in the study "Multicentre trial of cryotherapy for retinopathy of prematurity"[40]. These guidelines recommended that preterm infants with a BW <1251 g undergo ROP screening. In subsequent decades, these guidelines were adapted to the changing characteristics of the premature population, since advances in medicine led to higher survival of infants at lower GA, and more mature infants in high-income settings are less affected by ROP[3]. However, larger and more mature infants in low-income and middle-income settings can still develop ROP and require treatment; hence, ROP screening guidelines have to be adapted to the reality of quality of local medical care[23].

Most of the commonly used ROP screening guidelines are based on GA and/or BW. Currently, in Sweden as in comparable high-income settings/countries, infants born at GA <31 weeks are screened for ROP. ROP screening starts at 5 weeks of age but should be conducted no earlier than at a postmenstrual age (PMA) 31 weeks (Figure 4).

The ROP eye examination is usually initially performed weekly or biweekly until the retina is fully vascularized, which is usually at ~40 weeks PMA. If ROP is detected, the frequency of eye examinations is determined on an individualized basis. An extremely preterm infant that develops severe ROP may undergo up to 30 eye examinations until the ROP is resolved[41].

The eye examinations themselves can be stressful as well as painful for the fragile preterm infant[42]. Elevated blood pressure, an increased need for oxygen supplementation, and increased heart rate are general signs of distress that are seen during or/ and after ROP screening examinations[43-45].

Figure 4. Mio is four weeks old, and soon it will be time for his first ROP screening examination. ©Stina Fahlén

Several interventions during the eye examination can have adverse effects on the infant. The eye examination is performed through dilated pupils, and the mydriatic drops that are given before the examination can cause oxygen saturation problems and gastrointestinal side effects[45,46]. Manipulation of the eye during the examination may also cause distress when the ophthalmologist inserts an eyelid speculum or uses cotton-tipped applicators, or fingers to separate the eyelids (Figure 5)[43].

Figure 5. The retinopathy of prematurity (ROP) screening examination. ©Ann Hellström.

The administration of sucrose, a pacifier, and local anesthetics during the ROP examination can reduce the infant's distress and enhance recovery after ROP screening[47,48].

The need for ROP screening is determined according to WHO's established screening criteria[49]. The disease is well-defined, the natural course of the disease is relatively well-known, and there are facilities available for diagnosis and treatment that are acceptable to the population being screened. However, improving the specificity of ROP screening of preterm infants is desirable since ~90–95% of infants screened for ROP do not develop ROP that requires treatment.

Risk factors for retinopathy of prematurity

Many risk factors besides GA and BW have been identified since ROP was first described in Boston, Massachusetts (USA) in 1942 by T. Terry[50]. ROP is a multifactorial disease and additional risk factors may be: oxygen supplementation, multiple births, race, gender, prolonged mechanical ventilation, blood transfusion, steroids, hyperglycemia, renal insufficiency, sepsis, poor postnatal weight gain, poor nutritional intake, growth factors, bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA) and intaventricular hemorrhage (IVH) (Figure 6)[51-64].
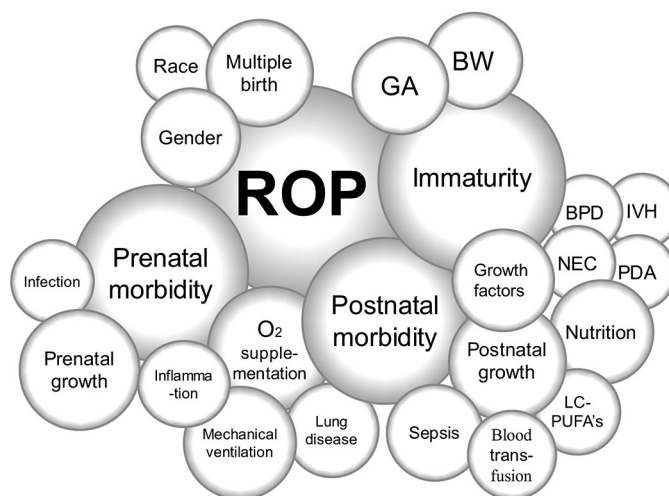

Figure 6. Retinopathy of prematurity (ROP) is a multifactorial disease.

Oxygen supplementation

Unrestricted oxygen supplementation has been a known risk factor for ROP since the 1950s[64]. However, guidelines concerning optimal oxygen saturation have not been definitively determined. Furthermore, optimal saturation targets seem to vary in different phases of ROP. In phase I (the first weeks of life), low oxygen saturation decreases the risk of severe ROP. In contrast, in phase II (after ~31 weeks PMA), high oxygen saturation seems to decrease the risk[63,65]. Moreover, introducing lower

oxygen saturation targets might be problematic since increased mortality rates in preterm infants have been associated with lower saturation targets[66]. The need for respiratory support, as quantified by the number of days on mechanical ventilation, has also been found to be a risk factor for severe ROP[6,67,68].

Prenatal and neonatal infection and inflammation
It has been suggested that peri- and postnatal infections that elicit an inflammatory response in the preterm infant can predispose the retina to severe ROP[69]. One hypothesis is that circulating inflammatory cytokines affect the retina. Cytokines have the ability to modulate angiogenesis: they can have both anti- and pro-angiogenic activity as well as both anti- and pro-inflammatory activity. Coordinated temporal and spatial cytokine expression appears to be necessary and important for normal development of the retina[70].
Prenatal infection, e.g. chorioamnionitis and elevation/dysregulation of inflammation-related proteins such as cytokines, is associated with preterm birth as well as with postnatal morbidities such as IVH, BPD, NEC, and ROP[70-73]. Neo- and postnatal sepsis, candida sepsis, and general sepsis also increase the risk of ROP[74-76].

Gender
When ROP was first recognized in the 1940s, male infants were reported to be more frequently affected by the disease[77]. Some subsequent studies have confirmed these results[61,62,78]. It is not known how gender affects the development of ROP, but male fetuses have generally been found to be more vulnerable than female fetuses. Male gender is associated with an increased risk of preterm birth and neonatal mortality and morbidity[79]. In particular, male infants who are born extremely preterm have an increased risk of visual impairment compared to female infants[80].

Growth restriction at birth
Whether growth restriction at birth is a risk factor for severe ROP remains controversial. In some studies, growth restriction at birth was found to be a risk factor for ROP[78,81-83], while other studies found no such pattern[6,59,84,85]. There is no consensus regarding the definition of growth restriction or the reference standard that should be used when calculating growth restriction.

Definitions of growth restriction at birth
As an estimation of whether an infant is growth restricted at birth, infants are commonly classified as "small for gestational age" (SGA) or "appropriate for gestational age". In Sweden, SGA is usually defined as BW, relative to GA and according to gender, that is 2.0 standard deviation scores (SDS) below normal. Another definition of SGA is BW below the 10th percentile, which approximately corresponds to 2 SDS below the relative GA mean. The term "severe growth restriction" is also used in some studies and is commonly defined as BW below the 3rd percentile.
Growth restriction at birth can also be described as divergence in birth weight standard deviation score (BWSDS), which allows for a continuum description of growth restriction.

Growth charts

Different growth charts are used in different places around the world to estimate a preterm infant's growth deficit at birth and to estimate an infant's postnatal growth. Some growth charts aim to describe undisturbed intrauterine growth and are based on longitudinal fetal ultrasound weight estimates[86,87]. Other growth charts are based on live and still births,[88] and others are based on live birth preterm infants[89,90]. The use of different growth charts leads to inconsistencies in the descriptions of infant characteristics in different study cohorts. Using growth charts that are based on live and still births, such as Kramer's growth chart,[88] minimizes the birth weight deficit of the infants in the study population, while using a growth chart based on fetal ultrasound, such as Marsal's growth chart,[86] emphasizes the birth weight deficits of preterm infants. After birth, neonatal care generally aims to mimic intrauterine growth[91].

Poor postnatal growth

In 1956, Hellström et al. reported a correlation between poor postnatal weight gain, nutritional status, and the development of retinal neovascularization in oxygen-exposed mouse pups[92]. Poor postnatal weight gain emerged as a risk factor for severe ROP in preterm infants around the beginning of the new millennium. Both Wallace et al. and Fortes Filho et al. have reported that weight gain measured at 6 weeks after birth could be used as a predictor for severe ROP[93,94]. Several other studies confirmed these results. Thus adequate weight gain during the first weeks of life may prevent the development of ROP, a disease that appears several weeks later (Figure 7)[67,94-96].
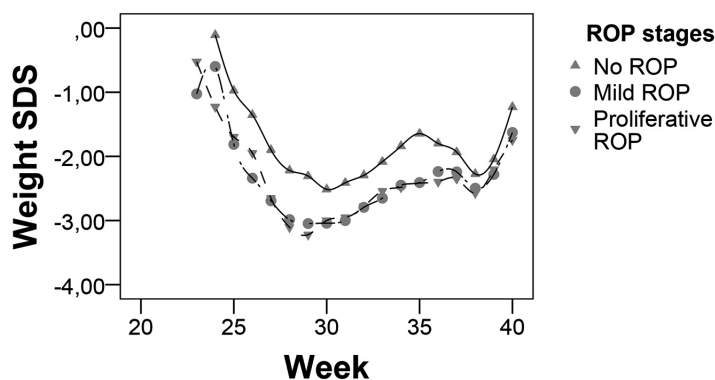
Figure 7. Longitudinal mean weight standard deviation (WSDS) from the historical norm at birth for 131 Swedish infants from week 23 to 40 GA for infants with no ROP (stage 0) (n=68), mild ROP (stages 1 and 2) (n=40) and proliferative ROP (stage 3 and above) (n=23)[97,98]. (Adapted from A Hellström, New insights into the development of retinopathy of prematurity – importance of early weight gain. Acta Paediatr. 2010 Apr;99(4):502-8. By permission of Wiley production.)

Estimating growth in preterm infants

The growth of preterm infants is generally assessed by measuring the infant's weight, length, and head circumference (Figure 8).
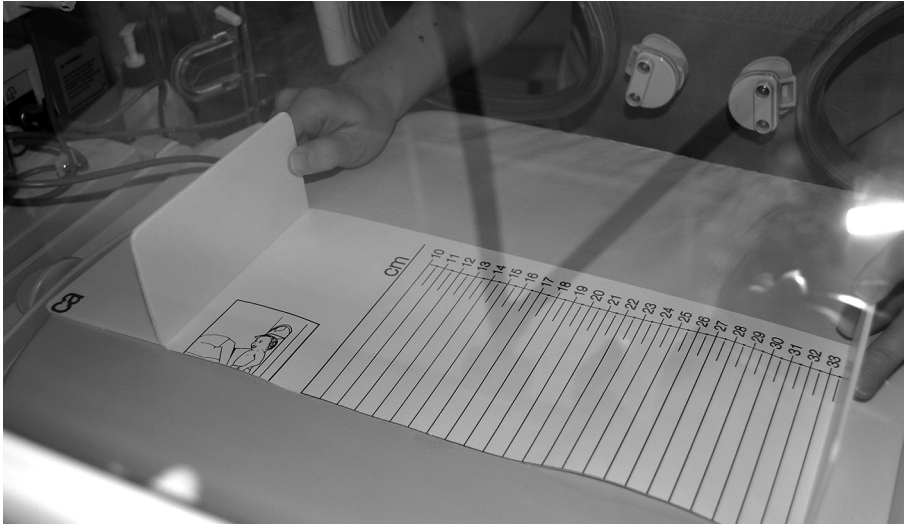

Figure 8. Flexible measuring tape inside an incubator. ©Elisabeth Stoltz Sjöström

Although measuring infant length and head circumference can be challenging in a preterm infant, weight measurements are usually preformed routinely—either weekly or daily at the neonatal ward—as an indicator of overall growth.

In term infants, weight loss after birth that is less than 10% of the BW is considered the normal consequence of post-birth changes in cellular fluid compartments[99.] The time at which the infant weighs the least, termed the nadir, usually occurs at postnatal day 3 in term infants. Term infants often regain their BW by postnatal day 5–10. However, postnatal growth deficit is a common problem in preterm infants[100], who often show greater and more prolonged initial postnatal weight loss[101].

If his or her course is favorable, a preterm infant is hospitalized until PMA ~36–38 weeks. At discharge, most preterm infants have not achieved the median birth weight of a reference fetus of the same PMA[102]. In the Extremely Preterm Infants in Sweden Study (EXPRESS) cohort, which included infants born at GA <27 weeks in Sweden during 2004–2007, 16% of the infants were classified as growth restricted at birth (BW <-2.0 SDS), while at the time of discharge at PMA 36 weeks, the percent of infants defined as growth restricted had increased to 44%[6].

When estimating infant growth using weight measurements, the physician needs to assess whether the infant's weight reflects their overall growth. This is because the measured weight can be a false indicator of satisfactory postnatal growth. Excessive edema, hydrocephalus, and medical equipment attached to the infant can cause non-physiological weight gain that does not reflect actual growth.

Low levels of growth factor IGF-I

Low levels of postnatal IGF-I have been associated with the development of severe ROP and poor postnatal weight gain[51,96,103]. From GA ~29 weeks, the serum levels of IGF-I in the fetus increase two- to three-fold. Fetal IGF-I levels correlate with both fetal size and GA[104]. After the maternal-fetal interaction is interrupted by birth, postnatal levels of IGF-I in the newborn infant decrease considerably[105]. While term infants restore their IGF-I levels within the first few postnatal weeks, preterm infants are poorly equipped to maintain the in-utero IGF-I levels[106]. IGF-I is mainly synthesized in the liver, and serum levels reflect the multifactorial influences of, for example, nutritional status, infection status, and endocrine conditions[107].

Low postnatal levels of IGF-I in preterm infants have been associated with overall growth deficiency and with vascular growth retardation[108]. IGF-I mediates VEGF vessel growth[109]; consequently, IGF-I is considered an important actor in phase I and phase II of ROP.

Poor nutritional intake

Poor nutritional intake during the first weeks of life was identified recently as a risk factor for severe ROP. VanderVeen at al. demonstrated that infants with the lowest total energy intake during the first four weeks of life have an increased risk of severe ROP[52]. Increased protein and energy intake during the first weeks of life are also associated with a favorable neurodevelopmental outcome in preterm infants[110].

Optimizing nutritional intake

Nutritional deficits and poor postnatal growth are major problems in preterm infants[111,112]. The metabolic rates of preterm infants are higher than in age-matched fetuses and, consequently, their nutrient and energy requirements are extremely high[113]. Evidence-based recommendations for preterm infants in terms of energy requirements remain largely incomplete, and the standard goal of mimicking intrauterine growth is difficult to accomplish. Furthermore, in the intrauterine environment, the placenta, amnion, and amniotic fluids provide the fetus not only with nutrients, but also with growth factors such as IGF-I, hormones, and probably other as-yet unidentified factors. It has been suggested that ~45% of the variation in postnatal growth is related to energy intake[112].

During the first weeks of a preterm infant's life, nutrients are usually supplied by parenteral as well as enteral routes in an attempt to optimize the nutritional supply (Figure 9).

Figure 9. Mio is growing satisfactory; he is now 6 weeks old. ©Stina Fahlén

It is recommended that enteral feeding should be started as soon as possible after birth, and human milk is the preferable standard enteral source[114]. Early intake of human milk stimulates the maturation of the intestinal system and reduces the risk of NEC[115,116]. Nutritional intake is usually calculated from parenteral and enteral sources as intake of energy (kcal/kg/d) and as intake of macronutrients (protein, fat, and carbohydrates expressed as g/kg/d). The estimated requirements for energy intake differs depending on whether parenteral or enteral sources are used, recommendations for energy intake for growing, extremely preterm infants (after the first five days of life) are as follows: 110–130 kcal/kg/d[117] for enteral nutrition and 105–115 kcal/kg/d[118] or 110–120 kcal/kg/d[119] for parenteral nutrition. However, adequate nutritional intake are seldom achieved in extremely preterm infants[120].

Omega-3 long-chain polyunsaturated fatty acids
Fatty acids, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) that are omega-3 long-chain polyunsaturated fatty acids (LCPUFAs), are essential for the development of neurons and blood vessels in the brain and retina[121]. During the third trimester, there is a major selective transfer of LCPUFAs from the mother to the fetus concomitant with the rapid development of neurons in the brain and the retina[122,123]. When they are born preterm, infants lose this essential transmission of LCPUFAs, and the preterm infants are unable to synthesize enough of these fatty acids themselves[124]. Thus it is crucial that lipid supplementation is begun during the preterm infant's first days of life. An early lipid supply is associated with improved neurological outcome[125].

Animal studies have indicated that the quality of the fatty acids may be important in ROP development. A lipid diet that is rich in omega-3 LCPUFAs, e.g. DHA and EPA, reduces the risk of ROP in mouse pups and in humans[126,127].

The parental nutrition that is most commonly used today contains mainly omega-6 LCPUFAs and almost no omega-3 LCPUFAs. Pawlik et al. reported the beneficial effects of omega-3 LCPUFAs on ROP and those results are supported by data from an ongoing study in Sweden (EUCTR2008-000046-31-SE). Specifically, the preliminary data suggest a substantial reduction of severe ROP in infants that were given fortified omega-3 supplementation, and these results are in agreement with similar studies.[127]

Preterm morbidities associated with retinopathy of prematurity
Several comorbidities that affect the preterm infant have been found to correlate with ROP, such as BPD, NEC, PDA and IVH[55-57].

Bronchopulmonary dysplasia
BPD is characterized by distal lung growth and maturation failure, such as impaired alveolarization and impaired vascular growth[128]. The disease is considered to have a multifactorial etiology that involves both oxygen toxicity and pre- and postnatal inflammation processes[129,130]. BPD is commonly defined as the need for supplemental oxygen 28 days after birth or at PMA 36 weeks; this need reflects the severity of the disease[131]. Systemic corticosteroids are currently the most common treatment for BPD, although this is controversial since systemic corticosteroids increase mortality in preterm infants and have a negative effect on early growth and neurodevelopment[131].

Necrotizing enterocolitis
NEC involves inflammation and a bacterial invasion of the bowel walls that usually occurs within the first weeks of life. Like BPD, NEC also has a multifactorial etiology. The symptoms of NEC include both abdominal and nonspecific systemic signs. The abdominal signs may include a distended abdomen, an absence of bowel sounds, and blood in the stool. The systemic signs of NEC can include increased apnea, bradycardia, irritability, and lethargy[132]. In the worst-case scenario, NEC can cause bowel necrosis. NEC is a severe morbidity and one of the leading causes of mortality in preterm infants. Approximately 20–30% of infants with NEC die despite treatment[133]. The treatment depends on the stage of the disease; in mild cases, nasogastric decompression, parenteral feeding, and broad-spectrum antibiotics are recommended, while in severe cases of NEC, surgical intervention is warranted. Infants who survive NEC have additional gastrointestinal complications as well as an increased risk of neurological impairment[134].

Patent ductus arteriosus
PDA is a condition in which the fetal ductus arteriosus vessel, which connects the main pulmonary artery to the descending aorta, does not close after birth (it usually closes within the first 24–28 hours in infants born at term). PDA is most frequently

found in the most immature infants and it can cause circulatory and saturation failure[6,135]. It can be treated effectively either by surgical closure or by pharmacological treatment, depending on the local guidelines.

Intraventricular hemorrhage

The etiology of IVH in neonates is multifactorial but is considered to be caused primarily by instabilities in cerebral blood flow[136]. IVH usually manifests within the first 72 hours of life, and extremely preterm infants are the most affected[137]. Their immature brains are especially sensitive to changes in cerebral perfusion, hypoxia, and oxidative stress, leading to cell death and bleeding[138]. IVH is classified as grade 1 to 4, with IVH grades 3–4 considered severe IVH[139]. In preterm infants, severe IVH impacts mortality rates and short- and long-term morbidity. Hydrocephalus, cerebral palsy, and neurodevelopment disabilities have been reported in affected infants[140]. Currently there is no way of stopping the bleeding associated with IVH. If the bleeding causes hydrocephalus a ventriculoperitoneal shunt may be warranted[141].

Novel Approaches to predicting severe retinopathy of prematurity

Most ROP screening guidelines are based on GA and/or BW and do not take into account postnatal risk factors for severe ROP. Established ROP screening guidelines result in repeated examinations of very fragile infants. Notably, 90–95% of these infants will never need treatment for ROP, and the examinations are both painful and stressful. To reduce the burden of these examinations on the infants, identification of those most at risk for sight-threatening ROP must be improved.

Biomarkers as predictors for retinopathy of prematurity

Biomarkers are measurable indicators of some biological state or condition. Biomarkers are often isolated from serum, urine, or other fluids whose detection indicates the presence or severity of a particular disease. Some biomarkers have been correlated with ROP. For example, biomarkers in circulation associated with oxidative stress, inflammation, glucoses metabolism and also growth factors[103,109,142-146]. However, the value of these biomarkers is yet to be defined as they have not been validated in clinical practice. Postnatal weight measurement is so far the only biomarker that has been found to be applicable in ROP screening models.

WINROP

The finding that low levels of serum IGF-I and poor postnatal weight gain correlate with ROP prompted the development of a web-based surveillance system for predicting severe ROP. This algorithm is termed WINROP, which stands for Weight, IGF-I, Neonatal, Retinopathy Of Prematurity. WINROP was developed based on a study of Swedish preterm infants, which was later validated, that reported that postnatal serum levels of IGF-I and weekly weight measurements could predict whether an infant was at high risk of developing severe ROP that required treatment[51,97]. After further development of the WINROP algorithm, weekly weight measurements

without IGF-I measurement were found to be equally valuable for making these predictions. Accordingly, serum IGF-I measurement was removed from WINROP[96]. By recording the infant's BW and GA along with weekly weight measurements, WINROP accumulates and calculates the infant's risk of developing severe ROP that requires treatment. The WINROP system has an alarm function that signals if an infant is at high risk of developing ROP requiring treatment. Even if WINROP does not give this signal, weekly weight measurements must be performed until the infant is at PMA 35–36 weeks. Not until then can the infant be completely free of risk as evaluated by WINROP.

WINROP has been validated in several Swedish cohorts as well as in cohorts in other countries such as the United States, Mexico, Brazil, China, Korea, and the United Kingdom[98,147-152]. The largest study to date included 1700 infants in a multicenter cohort in North America. The sensitivity and specificity of WINROP varies slightly between the cohorts; this is proposed to be due to the characteristics of the infants that are born preterm. Notably, the characteristics of the preterm population can differ in different countries/hospitals according to the quality of pre- and neonatal care. It has been suggested that WINROP could be more useful if it was adapted to the characteristics of different populations[148].

WINROP is meant to be a supplement rather than a substitute to established ROP screening practices. The aim of WINROP is to safely minimize the number of ROP screening examinations in infants at low risk of ROP requiring treatment and to alert physicians to pay special attention to infants who are at high risk.

To our knowledge, WINROP (www.winrop.com) is the first and only validated web-based surveillance system that estimates the preterm infant's risk of developing ROP requiring treatment.

ROP score

The "ROP score" of Eckert et al. involves recording BW, GA, blood transfusion data, and oxygen therapy data in the first 6 weeks of life and weight at 6 weeks of life in an Excel sheet that calculates a ROP score. Cut-off points based on the ROP score then predict the infant's risk of developing any ROP and severe ROP. In a Brazilian cohort (n=474) the ROP score was found to have a sensitivity of 98% and a specificity of 56%[153].

CHOP ROP score

Another new model to predict ROP type 1 is the Children's Hospital of Philadelphia (CHOP) ROP model proposed by Binenbaum et al.[154]. In the CHOP ROP model, BW, GA and mean daily weight gain data is calculated on a weekly basis. A normogram or a hand calculator is required to use the CHOP ROP model. The CHOP ROP model was found to have a sensitivity of 98% and a specificity of 53% in a cohort from Philadelphia (USA) (n=524).

CRIB score

The Clinical risk index for babies (CRIB) score estimates illness severity during the first 12 hours after birth by 6 variables: BW, GA, congenital malformation,

maximum base excess and maximum and minimum appropriate FiO_2 (fraction of inspired oxygen)[155]. Yang et al. investigated high CRIB score in correlation to ROP warranting treatment. They found that a high CRIB score was an independently significant predictor for ROP warranting treatment as well as in combination with male gender and nonblack race when GA and BW were excluded[61].

Aim

General aim and hypothesis
The general aim of the studies performed in my thesis was to evaluate growth and nutrition variables as predictors of severe ROP in preterm infants. The primary focus has been to further investigate whether the peri- and postnatal weight deficit is a robust and useful predictor for severe ROP.

The specific aims were as follows:

• To validate the WINROP algorithm in an extremely preterm nation-based cohort (Paper I).

• To investigate if the infant's weight at first detection of ROP correlated with the risk of developing severe ROP (Paper II).

• To investigate birth weight deficit as a risk factor for severe ROP (Paper III).

• To evaluate the effects of energy and macronutrient intake during the first weeks of life on the risk for severe ROP (Paper IV).

Patients and Methods

Study populations
Papers I, III, and IV studied the EXPRESS cohort (Paper III studied additional cohorts as well; see below). EXPRESS is a retrospective nation-based study that included all infants born at GA <27 weeks in Sweden between 2004 and 2007 (n=707 live births)[4].

Paper II studied infants born at GA< 32 weeks in the Gothenburg region in 2011–2012. All infants were born and were screened for ROP at the Sahlgrenska University Hospital (n=194).

Paper III included infants from five WINROP studies that were performed previously. The cohorts were from the following studies: EXPRESS (n=426), North America (n=1772)[150], Boston (n=338)[151], Lund (n=52)[98], and Gothenburg (n=353)[96]. All infants were born at GA <32 weeks, and all were screened for ROP.

Data collection
Birth characteristics
The infants' BW, GA, and gender data were retrieved retrospectively from hospital records in all studies. For infants in the EXPRESS cohort, data related to neonatal morbidity, such as days of mechanical ventilation, PDA, IVH, antibiotic treatment, and steroid treatment, as well as the CRIB score, were retrieved from the original EXPRESS studies[4,6].

Weekly weight measurements
Weekly weight measurements were retrospectively retrieved from hospital records or after discharge from records at the local children's healthcare center. Since WIN-ROP needs weekly weight measurement data for each PMA week, the aim was to retrieve one weight measurement each PMA week and, preferably, to have a seven-day interval between measurements. Weekly weight measurements were retrieved for all infants until PMA 32–40 weeks, depending on the study design.

ROP screening
ROP screening was performed in all cohorts according to the then current national guidelines and consisted of dilated ocular fundus examinations. All infants were examined until complete retinal vascularization or until spontaneous or post-treatment regression of ROP. The revised International Classification of Retinopathy of Prematurity was used for classifying ROP, and the recommendations of the Early Treatment for Retinopathy of Prematurity Cooperative Group were followed for ROP treatment[15,34.] Depending on the study design, the following ROP variables were retrieved: the number of ROP eye examinations, the date at which ROP was

first detected, the maximal ROP stage, the affected zone in each eye, and ROP treatment, if required, including the date(s) of treatment.

Nutritional data
Nutritional data for the EXPRESS cohort were mainly obtained retrospectively from hospital records by a registered dietitian (the first author of Paper IV). Comprehensive enteral and parenteral nutritional intake data were retrieved daily for the first 28 postnatal days; thereafter, we obtained data for one day per week (i.e. for days 35, 42, etc.) until data were no longer available. Nutritional intake was assessed as energy (kcal/kg/d) and as macronutrients (protein, fat, and carbohydrates expressed as g/kg/d). Enteral nutritional sources included the mother's milk, donor human milk, and various formula and human milk fortifiers. Later on, supplements such as vitamins and minerals were also defined as enteral sources. Parenteral nutritional sources included various nutritional preparations that contained glucose, amino acids, and lipids. Intravenous fluids such as blood products, drug infusions, and flush solutions were also included.

Exclusions
Infants were excluded if the infant died before ROP screening was completed e.g. before a PMA of 40 weeks. Infants with incomplete weight data, mainly incomplete weekly weight measurements, were excluded, as were infants in whom the weight measurements were judged to inaccurately reflect physiological postnatal weight gain (e.g. when the weight also reflected accumulated fluid, as in hydrocephalus). In Paper IV, infants with missing nutritional data were excluded. For detailed information regarding excluded infants please see Papers I-IV.

Statistics
In summary, normally distributed data were presented as means and 95% confidence intervals (CIs), and data that were not normally distributed were presented as medians and ranges. Odds ratios (ORs) with 95% CIs were calculated for each risk factor in logistic regression analyses. The Hosmer-Lemeshow goodness-of-fit test was used to check the fit for logistic regression models. In logistic regression analysis, the OR and 95% CI were calculated for each risk factor, and the level of significance of a risk factor was set as p value <0.05.

Paper I: The sensitivity, specificity, and positive and negative predictive values were calculated using Vassar Stats (www.vassarstats.net). The outcome variable was ROP type 1 requiring treatment.

Paper II: BWSDS and WSDS were calculated with a Swedish gender-specific reference[86]. The Mann-Whitney U test was used for calculating differences in the medians of variables at first detection of ROP. Univariate and multivariate logistic regression analysis was used to evaluate risk factors. Pearson's correlation coefficient was used to estimate correlations between risk factors. Receiver operating characteristic

(ROC) curve and area under the curve (AUC) analysis were performed to estimate the predictive capacity of the models. The outcome variable was ROP requiring treatment.

Paper III: BWSDS was calculated using a Swedish gender-specific reference as well as using a Canadian gender-specific reference[86,88]. Using the Swedish growth reference, SGA was defined as BW <-2.0 BWSDS; using the Canadian growth reference, SGA was defined as BW <10th percentile. Severe growth restriction was defined as BW <3rd percentile using the Canadian growth reference. The Wilcoxon signed-rank test was used to compare the two BWSDS references. The Mann-Whitney U test was used for group comparisons. Correlations were assessed using the Spearman correlation coefficient (rs). By designing an interaction term (BWSDS*GA group), we assessed whether BWSDS had a different impact as a risk factor depending on the infant's GA at birth (GA group 0 if GA <26 weeks and GA group 1 if GA ≥26 weeks). Univariate and multivariate logistic regression analysis were used to evaluate risk factors. The outcome variable was ROP requiring treatment.

In **Papers II and III,** the data were analyzed using IBM SPSS Statistical software for Windows, version 20.0 (Armonk, NY, USA, IBM Corp) and SAS version 9.3 (Cary, North Carolina, USA).

Paper IV: BWSDS and WSDS were calculated using a Swedish gender-specific reference[87]. Univariate and multivariate logistic regression analysis were used to evaluate risk factors. A categorical hospital variable was included in the final analysis. The outcome variable was severe ROP defined as ROP stages 3–5.

In **Paper IV**, the data were analyzed using IBM SPSS Statistical software for Windows, version 20.1 (Chicago, IL, USA, IMB Corp) and R (version 3.01).

Ethical considerations

The studies in this thesis were carried out in compliance with the ethical principles for medical research involving human subjects outlined in the Declaration of Helsinki.

The Regional Ethical Review Board in Gothenburg, Sweden approved the studies in Papers I, II, and III (Dnr 504-09).

In Paper III, the North American studies were approved by the institutional review boards at all participating centers.

The Regional Ethical Review Board in Lund, Sweden (Dnr 138-2008) approved Papers I and IV.

Results

This thesis comprises four papers that evaluated risk factors that could be used to optimize ROP screening (Figure 10).
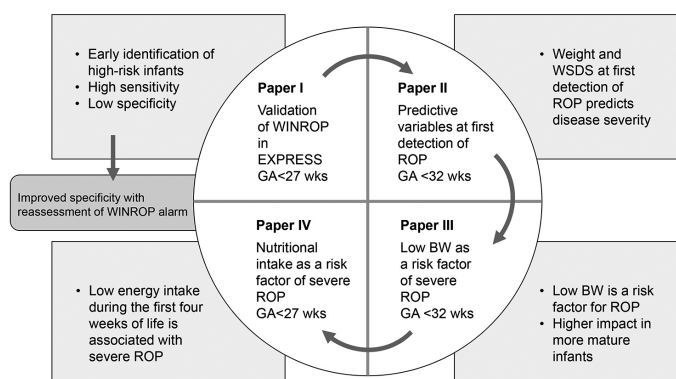

Figure 10. An overview of the main aims and results of the four studies in this thesis (Papers I–IV) and the additional results that are included in this thesis.

Infants' birth characteristics

In this thesis, a total of 3088 preterm infants were included from six study cohorts. The birth characteristics of the infants in the different study cohorts are presented in Table 1. The birth characteristics of the EXPRESS cohort are presented as in Paper III. There were minor differences in the characteristics of the EXPRESS cohort included in Papers I (Table 1) and IV due to differences in study design.

Table 1. The birth characteristics of the preterm study infants.

Cohort	Infants, n	GA, weeks+days	BW, grams	BWSDS*	Male gender
EXPRESS (Paper III)	426	25+4/7 (22+1/7 to 26+6/7)	780 (361–1315)	-0.68 (-5.18 to 3.88)	54.0%
Gothenburg (Paper II)	147	29+1/7 (22+6/7 to 31+6/7)	1185 (440–2840)	-1.04 (-4.55 to 5.20)	51.7%
North American (Paper III)	1772	28+0/7 (22+5/7 to 31+6/7)	1010 (378–2240)	-1.34 (-6.62 to 3.35)	52.5%
Boston (Paper III)	338	28+5/7 (23+1/7 to 31+6/7)	1050 (450–2400)	-1.31 (-5.18 to 4.47)	54.7%
Lund (Paper III)	52	26+1/7 (23+0/7 to 30+5/7)	850 (448–1716)	-0.77 (-5.00 to 0.89)	51.9%
Gothenburg (Paper III)	353	29+4/7 (23+3/7 to 31+6/7)	1290 (425–2210)	-1.04 (-5.59 to 2.50)	59.2%

Values are reported as median (range) as appropriate
*Swedish reference (Marsal K, 1996)86

Frequency of retinopathy of prematurity

The frequency of ROP varied as expected according to GA, with the most immature infants having the highest risk of developing any ROP and severe ROP. The frequency of ROP according to GA is presented in Table 2, which shows data from all infants included in the studies in this thesis (n=3088).

Table 2. The frequency of retinopathy of prematurity (ROP) and ROP treatment in preterm infants in the study cohorts.

GA, weeks	No ROP	ROP stages 1–2 (mild ROP)	ROP stages 3–5 (severe ROP)	ROP treatment
22 w (n=6)	—	16.7%	83.3%	83.3%
23 w (n=125)	6.4%	36.8%	56.8%	45.6%
24 w (n=304)	5.9%	42.5%	51.6%	34.5%
25 w (n=407)	17.0%	53.3%	29.7%	18.4%
26 w (n=445)	33.5%	49.7%	16.8%	8.3%
27 w (n=294)	40.5%	49.7%	9.8%	7.1%
28 w (n=364)	62.1%	34.3%	3.6%	1.4%
29 w (n=374)	72.8%	25.9%	1.3%	1.1%
30 w (n=428)	89.7%	9.6%	0.7%	0.2%
31 w (n=341)	93.8%	6.2%	—	—
Total (n=3088)	**50.7%**	**33.8%**	**15.5%**	**10.0%**

The most immature infants constitute a small part of the population of infants screened for ROP, although a large proportion of them develop severe ROP. Figure 11 show the ROP frequency in preterm infants as percentages.
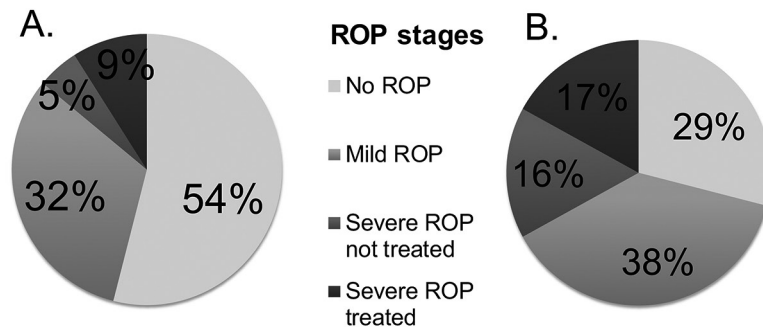

Figure 11. A. The percentage of preterm infants according to ROP development (n=2662). The EXPRESS cohort was excluded from this analysis. B. The percentage of preterm infants according to ROP development in the EXPRESS cohort (n=426).

Figure 12 shows the number of infants with ROP according to GA.
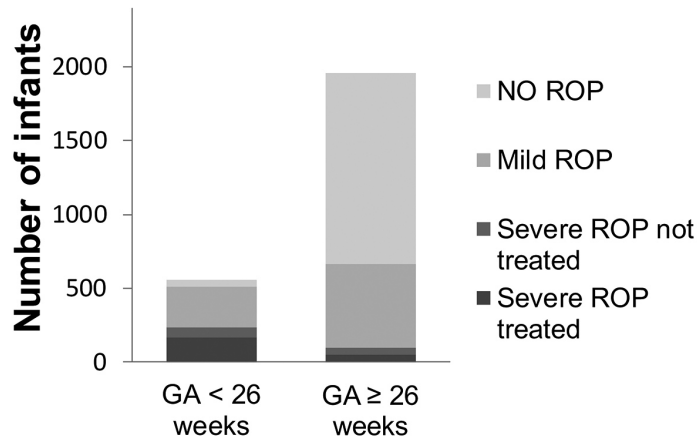

Figure 12. The number of infants born at GA <26 weeks and GA ≥26 weeks in relation to ROP development (n=2662). The EXPRESS cohort was excluded from this analysis.

Validation of WINROP in the EXPRESS cohort

In the EXPRESS cohort, we found that the sensitivity of WINROP for predicting ROP type 1 was high, 95.7%, but the specificity was low, 23.9%. WINROP signaled an alarm in 319 (78%) of the extremely preterm infants; however, only 47 (12%) of the infants in the cohort developed ROP type 1 requiring treatment (Table 2, Paper I). In addition to the 47 infants that developed ROP type 1, an additional 19 infants who did not fulfill established treatment criteria received treatment (Table 1, Paper I). Two infants had ROP type 1 that was not predicted by WINROP; both were born at GA 25 weeks and had a severe clinical postnatal course (Table 3, Paper I).

WINROP signaled early with an alarm at a median of 3 weeks after birth. The alarm was signaled several weeks to months before treatment, with signals a median of 9 weeks before the first treatment. The median PMA for receiving the first treatment was 36 weeks in the EXPRESS cohort (Figure 3, Paper I). In the EXPRESS cohort, a mean of 11 eye examinations were performed per infant (Paper I).

Reassessment of the WINROP alarm

With the intention of improving the specificity of WINROP for extremely preterm infants, we retrospectively analyzed the infants' weight at the time of WINROP alarm. Our analysis included the EXPRESS cohort (n=407) as well as GA-matched extremely preterm infants from the North American cohort (n=566). We analyzed the weight at time of the WINROP alarm in relation to GA at birth and PMA at alarm. Many of the extremely preterm infants had poor postnatal weight development; thus, many had a WINROP alarm. However, we were able to establish a "safe" alarm weight. If the infant had gained enough weight at the time of the alarm, the infant did not develop ROP type 1. Infants born at GA 26 weeks had a lower

"safe" weight at the time of the alarm compared to infants born at GA of 23–25 weeks, leading to two subgroups of "safe" alarm weight limits (Figure 13).
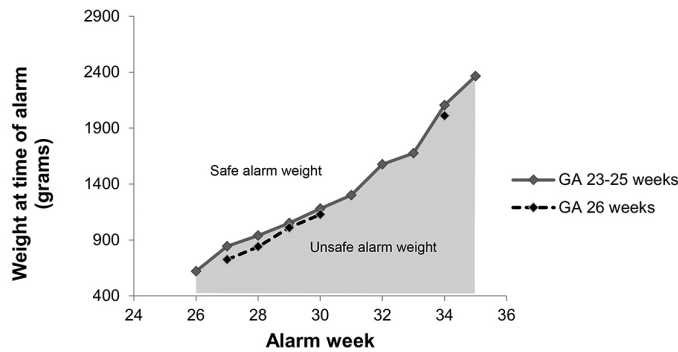

Figure 13. WINROP alarm weight cut-offs (n=973). Infants with an alarm weight in the "safe" zone did not require ROP treatment, while infants in the "unsafe" zone did.

Using the constructed alarm weight cut-offs, we performed an inter-sample reassessment of the WINROP alarm in the same cohorts (i.e. the EXPRESS and North American cohorts). Infants with alarm weight below the cut-off value remained at risk, and infants with alarm weight above the cut-off were considered to have no alarm.

In the EXPRESS cohort, 12.5% of the infants that previously received a WINROP alarm were now reassessed as having no alarm. The specificity of WINROP in EXPRESS improved from 23.9% to 35.0%. In the North American cohort, 15.4% were reassessed as having no alarm, and the specificity increased from 8.5% to 26.6% . The sensitivity was not affected in either cohort (unpublished data).

The majority of infants who were born extremely preterm that could be reassessed as having no WINROP alarm were born at GA 26 weeks and had an early alarm (Figure 14).
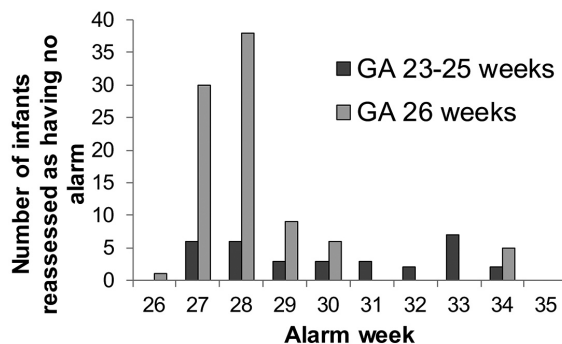

Figure 14. The number of preterm infants reassessed as having no WINROP alarm according to alarm week. The EXPRESS and North American cohorts were included in the analysis (n=973).

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Weight at first detection of retinopathy of prematurity as a risk factor

In Paper II, we found another possible weight cut-off for predicting ROP disease. Specifically, we found that the infants' weight at the time of first detection of ROP could be used as a predictor for the progression of ROP.

In the Gothenburg cohort (n=147), we found that infants that were later treated for ROP had a lower median weight (1540 g vs. 1995 g; p<0.001) and lower median WSDS (-1.18 vs. -2.19; p<0.001) at the time of first detection of ROP compared to infants that were not treated (Table 1 and Figure 2, Paper II).
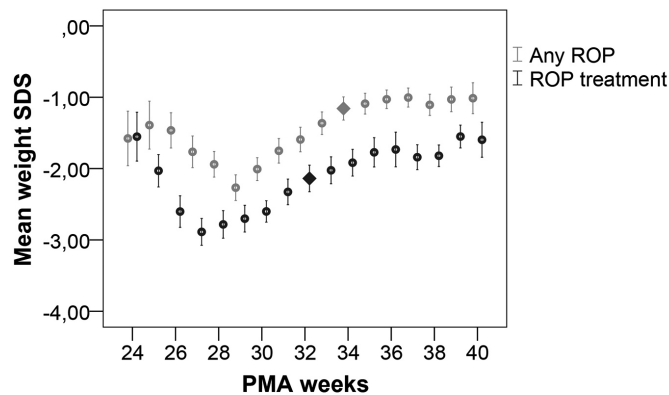

Figure 15. Weight development in the weight standard deviation score (WSDS) in infants who developed any ROP or who required ROP treatment. Diamonds represent the time at first detection of ROP. The error bars represent ± 1 SE.

When performing stepwise backward multivariate logistic regression analysis with the variables GA at birth, BW, PMA at first detection of ROP, weight at first detection of ROP, and WSDS at first detection of ROP, we found that the best combination of risk factors was GA and WSDS at first detection of ROP, which correctly classified 69% of infants requiring treatment. A received operator ROC curve that included GA at birth and WSDS at first detection of ROP showed an AUC of 0.93 (Figure 3, Paper II).

In Paper II, we again found indications that weight cut-offs could be useful for predicting the need for ROP treatment. No infant with weight >2000 g or a WSDS above -1.13 SDS at first detection of ROP was later treated for ROP.

Birth weight as a risk factor

In Paper III, we analyzed BW relative to GA and gender as a risk factor for ROP treatment in 2941 infants. We found that depending on the infants' GA, low BW could be a risk factor for ROP requiring treatment.

We calculated the BWSDS using two different growth references, a Canadian and a Swedish growth reference, for each GA group: GA 22–23 weeks, GA 24–25 weeks, GA 26–27 weeks, GA 28–29 weeks, and GA 30–31 weeks. We found significant

differences in the BWSDS values using the two growth references (Wilcoxon signed-rank test, p<0.001) except in the group GA 22–23 weeks. With both growth references, there was a negative correlation between BWSDS and GA at birth i.e. growth restriction at birth was more common in infants born after longer gestation (Figure 16).
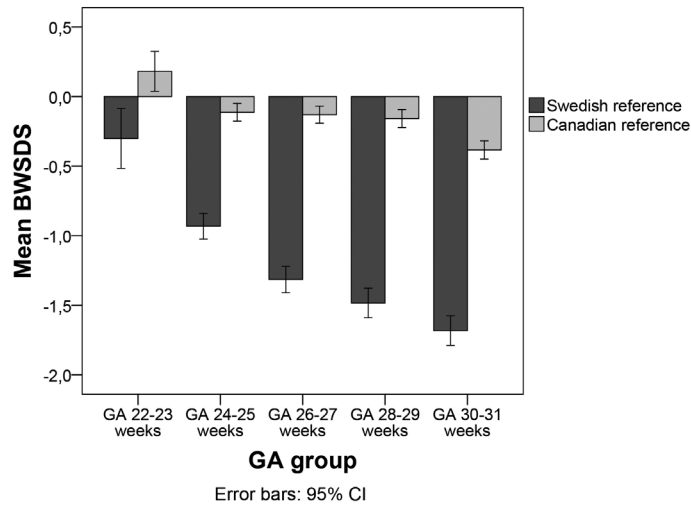

Figure 16. Mean (SD) birth weight standard deviation score (BWSDS) per gestational age (GA) group calculated with a Canadian and a Swedish growth reference.

In univariate logistic regression, all variables that reflected growth restriction at birth were highly significant as risk factors for later ROP treatment for infants born at GA ≥26 weeks. These variables included low BWSDS and SGA (both growth references) as well as severe growth restriction (Canadian reference)(Table 2, Paper III).

In multivariate logistic regression analysis, including an interaction term (which allowed different associations with BWSDS for infants born at GA <26 weeks or GA ≥26 weeks), we found that independent of which growth reference used, low BWSDS was a risk factor for later ROP treatment for all infants (Table 3, Paper III). Hence, in infants born with a GA <26 weeks, the odds of requiring treatment were reduced by 20–28% for every additional 1 SDS increase in BWSDS. For infants born at GA ≥26 weeks, there was a major reduction of 44–59% for every additional 1 SD increase in BWSDS (Figure 3, Paper III) (Table 3).

Table 3. Example of how the odds for ROP treatment differ depending on the infant's birth weight standard deviation score (BWSDS) and gestational age (GA).

	Female infant GA 24 wks+0 day 31% prevalence of ROP treatment* (OR=0.80)		Female infant GA 26 wks+0 day 7% prevalence of ROP treatment* (OR=0.56)	
BWSDS (Swedish Reference)	BW (g)	Odds for ROP treatment*	BW (g)	Odds for ROP treatment*
2.0	825	20%	1110	2%
1.0	750	25%	1040	4%
0.0	670	31%	920	7%
-1.0	610	39%	820	12%
-2.0	510	48%	720	22%

*Prevalence of ROP, Holmström et al., SWEDROP

Nutritional intake as a risk factor

In the EXPRESS cohort, there were 498 surviving infants with complete nutritional data. The macronutrient intake during the first 28 days of life was low (mean±SD): 102±14 kcal/kg/d of energy, 3.0±0.4 g/kg/d of protein, 11.2±1.1 g/kg/d of carbohydrates, and 4.8±1.2 g/kg/d of fat. The mean energy intake during the first four weeks in infants developing severe ROP was 97±13.5 kcal/kg/d compared to 108±13.5 kcal/kg/d in infants not developing ROP (Table 1, Paper IV) (Figure 17).

In logistic regression analyses that were adjusted for GA and BW, we found that low intake of energy, fat, and carbohydrates during the first 28 days of life were associated with an increased risk of developing severe ROP (Table 2, Paper IV).
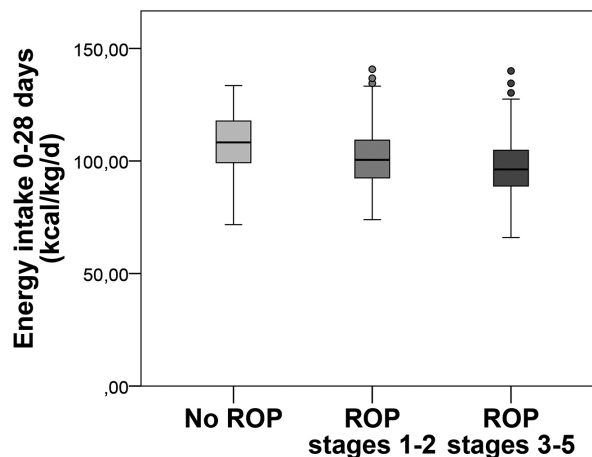

Figure 17. Energy intake during the first four weeks of life in correlation to ROP disease (EXPRESS n=498).

When estimating the nutritional intake in multiple logistic regression analyses that included recognized risk factors such as GA, BW, postnatal weight change, CRIB score, duration of mechanical ventilation, blood transfusions, proportions of enteral fluids, steroid and antibiotics treatment, PDA, and IVH, we found that energy intake remained as an independent risk factor for severe ROP together with BW, duration of mechanical ventilation, and amount of blood transfusions (Table 4, Paper IV).

Table 4. Significant risk factors for severe retinopathy of prematurity (ROP) in multivariate logistic regression analysis (n=498).

	OR	95% CI
Energy intake[1], 0–28 days	0.76**	0.65–0.90
Blood transfusions[2], 0–28 days	1.17*	1.02–1.33
Mechanical ventilation[3], days	1.03*	1.00–1.06
Body weight[4], g	0.75***	0.65–0.87

[1]10 kcal/kg/day increments
[2]10 ml/kg per week increments
[3]One-day increments
[4]100 g increments
*p<0.05, **p<0.01, ***p<0.001

Consequently, every increase in energy intake of 10 kcal/kg/day during the first 28 days of life could lower the odds of developing severe ROP by 24%.

Discussion

Major findings

The studies in this thesis all contribute to research that aims to identify novel risk factors for ROP. With accessibility to web-based programs such as WINROP, the possibility of more individualized ROP screening is not only promising, it is already available. Using WINROP, we confirmed that poor postnatal weight gain is a major risk factor for severe ROP, although WINROP can be refined further for extremely preterm infants to increase the specificity (e.g. reassessment of the WINROP alarm). Our results further indicate that estimations of risk for severe ROP can be improved by including BWSDS and weight or weight SDS at first detection of ROP. Better risk estimates provide a basis for reducing the number of eye examinations in infants with low risk for ROP while increasing vigilance for infants who are at high risk for ROP.

Our results also indicate that low energy intake during the first four weeks of life is an independent risk factor for severe ROP. Adequate provision of energy intake during the first four weeks of life may be an effective method to reduce the risk of severe ROP in extremely preterm infants.

WINROP identifies high-risk infants in the EXPRESS cohort

When validating the WINROP algorithm in the EXPRESS cohort, we found that, as in previous WINROP studies, the WINROP alarm signal was early, i.e. at a median of 3 weeks after birth. As expected, WINROP also signaled an alarm several weeks to months before the time of treatment, with a median time of 9 weeks from alarm to treatment. Identifying infants at high risk of severe ROP several weeks before the onset of ROP possibly provides the neonatologist with an opportunity to investigate postnatal preventive strategies. In particular, increased early nutrition may modify the course of ROP, as we proposed in Paper IV.

The sensitivity of WINROP in the EXPRESS cohort was high—95.7%—and was comparable to that found in previous WINROP studies. However, the specificity was 23.9%, which is lower than in previous studies[98,147,150-152]. The low specificity in the EXPRESS cohort prompted us to look for additional variables to identify infants at risk of developing ROP requiring treatment (ROP type 1). Many or even most of the extremely preterm infants had poor postnatal weight development, and, not surprisingly, 78% had a WINROP alarm. When analyzing infant weight at the time of the alarm in relation to GA and alarm week, we found that there appeared to be a "safe weight" at the time of the WINROP alarm. That is, if an infant's weight at the time of the WINROP alarm was above this safe weight cut-off, the infant would not develop ROP requiring treatment. When we conducted an inter-sample reassessment of the WINROP alarm using these alarm weight cut-offs, the specificity of WINROP in the EXPRESS cohort improved from 23.9% to 35.0% (unpublished data). This is a promising sign that WINROP can be improved and developed further, and we plan to validate the safe alarm weight cut-off in other cohorts as well.

The mean number of eye examinations per infant in the EXPRESS cohort was 11. Infants without a WINROP alarm and ROP had a mean of 6 eye examinations. In EXPRESS, we found that if an infant had not received a WINROP alarm, they could safely be screened for ROP on three occasions: PMA 31 weeks, PMA 33 weeks, and PMA 36 weeks. Such screening would ensure that no infants developing ROP type 1 would have been missed. This proposed screening schedule would have reduced the number of screening examinations by 50% for an infant with no ROP and no WINROP alarm, a reduction that would not only reduce medical costs but which would be substantially beneficial for that particular infant's well-being.

In the EXPRESS cohort, 47 infants developed ROP type 1 that fulfilled the treatment criteria, but 66 infants were treated. This reflects a well-known clinical problem: A diagnosis of ROP type 1 is challenging for the ROP screening ophthalmologist[156]. Many extremely preterm infants will develop proliferative severe ROP, but for some, the disease will resolve spontaneously without any treatment. It is difficult to distinguish these infants since the treatment criteria rely heavily on the diagnosis of plus-disease, which is based on subjective judgment and shows great variability between clinicians. In settings where WINROP is used routinely, as in the city of Gothenburg, the clinical experience is that if an infant has not received a WINROP alarm, the chance that the ROP disease will resolve spontaneously is great. This is reassuring for the ophthalmologist and means that overtreatment can be reduced.

In the EXPRESS cohort, two infants that developed ROP type 1 and received treatment for ROP were not detected by the WINROP system. Notably, both of these two infants had severe clinical courses. Thus, clinical judgment regarding the infants' risk of developing ROP requiring treatment should always overrule the WINROP outcome. The accuracy of weight measurements in critically ill infants should also be questioned, because in some cases, the measured weights might not reflect the infants' growth. Weight measurements that do not correspond to the infants' actual growth could be caused by, e.g., edema or by the medical equipment attached to a critically ill infant.

It should be pointed out that the majority of infants screened for ROP are not extremely preterm. In fact, only about 5% of preterm infants born worldwide are classified as extremely preterm[5]. However, since extremely preterm infants have a high risks of developing severe ROP, these infants tend to generate a substantial workload in ROP screening processes. Furthermore, extremely preterm infants are the most vulnerable, and in ROP screening, they often suffer from the side effects of mydriatic eye drops and from the discomfort of the examination itself; thus, reducing the number of eye examinations would be especially beneficial for the extremely preterm infant.

Variables at first detection of retinopathy of prematurity are useful predictors

For the first time, to our knowledge, we established in Paper II that infants that were later treated for ROP had a significantly lower median weight (1540 g vs. 1995 g; p<0.001) and lower median WSDS (-1.18 vs. -2.19; p<0.001) at the time of first detection of ROP compared to infants that did not develop ROP requiring treatment.

We also confirmed previously reported findings that a low PMA at first detection of ROP is a risk factor for the development of severe ROP[39].

Low weight and low WSDS at first detection of ROP are variables that could reflect low GA and/or poor pre- or postnatal growth. Accordingly, they could help predict an infant's ability to handle the emerging ROP. Measuring the infants' weight at first detection of ROP could be a simple way to assess poor weight gain, especially in clinical settings where complementary screening tools for poor postnatal weight gain such as WINROP are not available.

In Paper II, we also found that no infant weighing >2000 g or with a WSDS above -1.13 SDS at first detection of ROP was treated later on. We intend to further evaluate whether these weight limits at first detection of ROP are applicable in other cohorts.

Low birth weight is a risk factor for retinopathy of prematurity that depends on gestational age at birth

In Paper III, we established that low birth weight is a risk factor for severe ROP in preterm infants and that the impact of the growth deficit at birth is dependent on the GA at birth. Low birth weight as a risk factor for ROP has been assessed previously in many studies, but the results have been contradictory[6,59,78,81-85]. The results presented in Paper III help clarify these contradictory results.

To try to determine the possible reasons for the inconsistences in the literature, we calculated the birth weight deficit using two different growth charts. In addition, we defined prenatal growth restriction several ways, plus we divided the infants into pair-wise GA groups to correct for differences in results that were due to the infants' degree of immaturity. Our results showed that low birth weight, regardless of the growth reference, is a risk factor for severe ROP for preterm infants; however, the impact of growth deficit is dependent on GA at birth. Consequently, Paper III helps explain previous inconsistences.

We detected a shift in the impact of low BWSDS at around GA 26 weeks: For infants born at GA <26 weeks, the risk of requiring treatment for ROP was reduced by 20–28% (depending on the growth reference used) for every 1 SD increase in BWSDS. For infants born at GA ≥26 weeks, there was a 44–59% reduction for every 1 SD increase in BWSDS.

In clinical practice, this would suggest that a growth-restricted newborn (BW -2 SDS) born at GA 26 weeks has a risk of developing severe ROP that is equal to that of a well-nourished infant (BW 2 SDS) born at GA 24 weeks. Currently, BWSDS is rarely calculated or available at the start of an infant's ROP screenings. Thus, calculating BWSDS could be another simple procedure that is part of a new approach to individualize ROP screening.

We recommend the use of the BW deficit as calculated in BWSDS rather than the use of strict cut-offs like SGA. Calculating a deficit in BWSDS offers a continuum estimation of growth deficit as a risk factor. In our study, we obtained different results in terms of whether or not SGA was a risk factor for severe ROP depending on the definition of SGA and depending on the growth reference we used. This is unfortunate and makes the use of SGA less definitive.

The impact of nutrition on retinopathy of prematurity

When evaluating nutritional intake in the EXPRESS cohort (Paper IV), we found that the mean energy intake during the first four weeks of life was 102 kcal/kg/day, which was distinctly lower than the lowest estimated requirement of 105–110 kcal/kg/day for preterm infants[118]. Low intake of energy, fat, and carbohydrates correlated with an increased risk of severe ROP. Our results indicate that an increase in energy intake of just 10 kcal/kg/day during the first four weeks of life could reduce the risk of developing severe ROP by 24% (OR=0.76). Similar findings were also reported in a study by VanderVeen et al.[52] However, under-nutrition might be due to feeding difficulties and to a reduced ability to assimilate nutrients, making prevention via nutrition somewhat difficult. Though challenging, achieving required energy and nutrient intake from parenteral and enteral nutrition may be feasible through the use of concentrated parenteral nutrition solutions, early fortification of breast milk and daily monitoring of growth and calculated nutrient intakes.[157]

Strengths and limitations of the studies

Strengths

A major strength of this thesis was the large study cohorts. The EXPRESS cohort was nation-based and only included extremely preterm infants. Thus there was a substantial number of very low GA infants, which makes the results robust. Weekly weight measurements were performed throughout the neonatal period of all infants, making the longitudinal weight data reliable. Careful validation of data was performed using check routines for correctness of data input to the systems. Check routines also included an additional validation by a second statistician.

Limitations

One limitation of all of the included studies is that they were retrospective chart reviews. In prospective studies where a research protocol has been developed the risk of missing data is considered to be minor in comparison to retrospective studies. In our studies some infants had missing data or did not complete their study course and therefore were excluded. Infants were mainly excluded due to missing weekly weight measurements. The number of infants that were excluded differed depending on the study design. Unfortunately, we can only speculate about the causes for the missing weight measurements, but severe morbidity is often a reason that weight measurements are not performed routinely. For example, in Paper I we found that the excluded infants had lower GA as well as lower BW compared to infants with complete weekly weight measurements.

Another limitation of the studies is that when evaluating weight at birth and postnatal growth as risk factors for severe ROP, other established risk factors and comorbidities were not considered.

Conclusions

The work in this thesis identified several new growth variables of preterm infants that could be used as predictors of ROP as part of a new approach to individualized ROP screening. By calculating an infant's BWSDS, which is rarely done on a routine basis, we could determine an additional risk factor at birth. The web-based surveillance system WINROP, which identifies infants with poor postnatal weight gain, can identify infants at high risk of ROP, usually within the first few weeks of life. By calculating an infant's WSDS when ROP is first detected, we hope to get some information about the future course of the ROP (Figure 18). Early identification of high-risk infants might provide neonatologists and ophthalmologists with an indication that special attention is warranted.

By using a new approach to ROP screening and by developing new surveillance systems that complement established ROP-screening guidelines, we can significantly improve the identification of infants who are at high risk for severe ROP, thus sparing fragile infants unnecessary painful examinations and additional suffering.
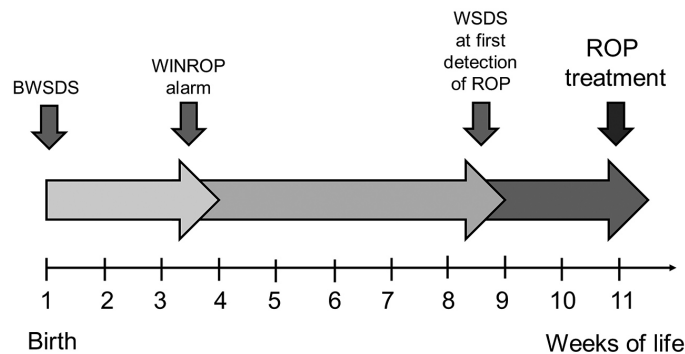

Figure 18. New approaches to identifying an infant who needs treatment for retinopathy of prematurity (ROP) are exemplified by monitoring the changes in weight over time of a preterm infant.

Future perspectives

In the near future, we aim to further refine the WINROP surveillance system based on the findings in this thesis. Our findings indicate that we can significantly improve the specificity of WINROP, especially for extremely preterm infants, by further assessing infant weight when there is a WINROP alarm. We also aim to validate and to further assess whether weight cut-offs at first detection of ROP can be used safely as predictors of the course of ROP.

WINROP signals with an early alarm, and this may allow time to initiate new preventive interventions. We intend to evaluate whether increased nutrition after a WINROP alarm can prevent the development of ROP.

Since ~2010, most NICUs have made changes in their nutritional practices: nutritional intake have been calculated on more of a routine basis, more concentrated parenteral solutions have been used, and there has been earlier fortification of human milk. All of these changes are intended to optimize the nutritional intake of preterm infants. We plan to compare nutritional intake, growth, and ROP outcome in the EXPRESS cohort with those of a more recent cohort of preterm infants (Umeå and Stockholm 2009–2012).

The WINROP database gives us access to longitudinal weight data from over 3000 infants, and we plan to further analyze these data. To our knowledge, no one has previously reported differences in growth patterns in relation to ROP according to infant GA. If poor postnatal weight gain as a risk factor for ROP differs according to GA, this information could be useful for further individualizing ROP screening. Finally, we have just begun to analyze preliminary data in a follow-up study of the EXPRESS infants and their vision outcomes when they are at school age, i.e. 6.5 years old. The preliminary data indicate that extremely preterm infants have significantly more ophthalmologic abnormalities than age-matched controls.

Figure 19. Mio: "looking towards the future". ©Stina Fahlén

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