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# Topics on Critical Issues in Neonatal Care

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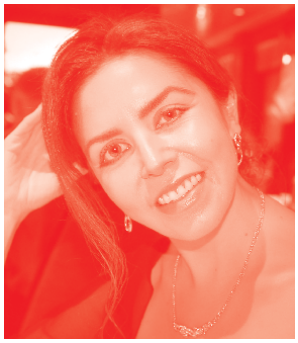

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# Meet the editor

R. Mauricio Barría, DrPH, is a principal investigator and associate professor at the Faculty of Medicine, Universidad Austral de Chile. He was trained as an epidemiologist and received his MSc in Clinical Epidemiology from Universidad de la Frontera, Chile, and his DrPH from Universidad de Chile. His research interests include maternal-child health, neonatal care, and environmental health. He is skilled in epidemiological study design with a special interest in cohort studies and clinical trials. From 2010 until 2017 Dr. Barría was director of the Evidence-Based Health Office. He is currently the director of the Institute of Nursing, Faculty of Medicine, Universidad Austral de Chile.

# Contents

# Preface

Neonatal care requires updated knowledge support to provide relevant care in each context and health situation. As in other areas of health sciences, neonatology has shown important advances, and despite the techno-scientific progress, there are problems faced by newborns that are always present in neonatal units. Consequently, it is necessary to review the advances in prevalent neonatal health problems and include new approaches for the health care of this risk group.

In this sense, this book, with both a preventive and therapeutic perspective, includes six chapters that discuss crucial topics in neonatal health care. It is a useful resource for healthcare professionals who treat premature and high-risk newborns.

Chapter 1, "Antenatal Corticosteroids and Magnesium Sulfate in Twin Pregnancy for the Prevention of Neonatal Morbidity", discusses the use of corticosteroids and magnesium sulfate as measures to prevent unfavorable outcomes for newborns from a twin pregnancy. Given that the determination of the use of corticosteroids in multiple pregnancies remains controversial due to the scarcity of studies in this group, this chapter analyzes the effectiveness of the use of corticosteroids in twin pregnancies in early and late preterm, evaluating their results in respiratory morbidity and metabolic aspects of the newborn.

Chapter 2, "Breastfeeding and the Influence of the Breast Milk Microbiota on Infant Health", presents a description of the value of breast milk as a continuous source of commensal and beneficial bacteria, highlighting its role in the initiation, development, and composition of the intestinal microbiota of the newborn, thanks to its pre and probiotic components.

Chapter 3, "Neonatal Anemia", provides an updated review of neonatal anemia and describes its pathophysiology in term and preterm infants. It also presents a detailed discussion of traditional and innovative laboratory tests for the diagnosis and evaluation of this condition in NICUs.

Chapter 4, "Prolonged Jaundice in Newborn", highlights the importance of determining whether prolonged jaundice is of a benign cause or due to a significant disease. To this end, the chapter discusses the use of different tests and the elements that should be taken into account in an approach to the etiology of the disease.

Chapter 5, "Retinopathy of Prematurity: A NICU-based Approach", offers a comprehensive review of this problem, with an emphasis on screening, but also develops elements on prevention and current treatments.

Finally, Chapter 6, "Reducing Toxic Phthalate Exposures in Premature Infants", develops an interesting topic from an epidemiological approach to highlight the importance of the exposure of premature infants to phthalates and their sources of exposure. In this way, this chapter, in addition to analyzing the exposure to phthalates in premature babies, proposes some practical solutions that can be

implemented in daily practice and some suggestions to the manufacturers of different supplies and devices used in neonatal care.

As described, this book includes different topics of interest for all those students and health professionals who are dedicated to neonatal health care, hoping it will be a contribution to their daily work.

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Chapter 1

# Antenatal Corticosteroids and Magnesium Sulfate in Twin Pregnancy for the Prevention of Neonatal Morbidity

*Julio Elito Jr and Micheli Goldani Shuai*

## Abstract

The use of corticosteroids is one of the most important therapies used in prenatal care to improve the outcomes of the newborn by reducing the rates of respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis and contribute to the survival of extreme preterm infants. In addition to steroids, the use of magnesium sulfate protects the newborn from cerebral palsy in cases of extreme preterm births. All of these conditions increase perinatal morbidity/mortality and are related to potentially serious illness in the newborn requiring care in neonatal intensive units. The use of corticosteroids and magnesium sulfate are measured to prevent unfavorable outcomes of premature newborns admitted to a neonatal intensive care unit. The incidence of twin pregnancy is only 3% of all live births, however, it accounts for 15% of extreme preterm births less than 32 weeks. Women with multiple pregnancies are six times more likely to terminate the pregnancy before term compared to single pregnancies. The determination of the use of corticosteroids in multiple pregnancies remains conflicting due to the scarcity of studies related to this group. Therefore, this chapter aims to evaluate the effectiveness of the use of corticosteroids in twin pregnancies in early and late preterm, evaluating its outcome in respiratory morbidity and metabolic aspects of the newborn.

**Keywords:** antenatal corticosteroids, magnesium sulfate, multiple gestations, twins, neonatal morbidity

## 1. Introduction

Multiple pregnancies occur in 1 to 3% of live births, with special attention in the obstetric sphere due to its peculiarities, as it increases the risk of practically all prenatal pathologies. It represents about 12% of cases of extreme prematurity (less than 32 weeks), 25% of newborns weighing less than 1500 g, and also increases the risk of death before the first year of life by seven times [1].

Prematurity is responsible for unfavorable outcomes of the newborn that can evolve with respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis. All of these conditions increase perinatal morbidity/mortality and are related to potentially serious illness in the newborn requiring care in neonatal intensive units.

Prematurity represents the largest cause of perinatal mortality in the world, being a major focus of attention in the main research in recent years. In a twin pregnancy, it is 3 to 4 times greater, representing 12% of preterm infants. Throughout the literature, it was understood that as the number of fetuses increases, the duration of pregnancy decreases; approximately 50% of twins are born before or at week 36 and 50% of triplets or from pregnancies with more than three fetuses, before 32 weeks; and the mean gestational age at birth for twins is 36 weeks, for triplets 32 to 33, and for quadruplets approximately 31 [2].

The causes that determine preterm labor in multiple pregnancies are multifactorial [1].

Due to these facts, a twin pregnancy is said to be of high risk, therefore, the obstetrician must be fully updated to conduct challenging prenatal care and allow the best possible results for the maternal-fetal binomial to be obtained.

## 2. Corticosteroids

Even with the improvement in the survival of preterm newborns, mainly resulting from neonatal intensive care. Preterm birth remains an important public health problem and a leading cause of perinatal mortality and severe short- and long-term morbidities (blindness, deafness, developmental delay and/or other neurological impairments).

The use of corticosteroids is one of the most important therapies used in prenatal care to improve the outcomes of mortality and morbidity in the newborn [3].

The use of corticosteroids in a single course is done in situations of risk for premature birth between 24 and 34 weeks, including multiple pregnancies, premature rupture of ovular membranes and can also be used in fetuses with the gestational age of 23 weeks, with eminence for delivery within 7 days [4].

Studies initiated by Liggins in 1969 analyzed the effect of steroids on the lungs of fetuses of sheep and found that there was a discrepancy in the lungs of full-term offspring compared to premature offspring. With this experience, several clinical trials were carried out on its effect [5].

The first structured review addressing the use of this drug in premature fetuses was published by Crowley et al. in 1990. Later, in 1995, the author published a meta-analysis review citing 15 randomized clinical trials carried out between 1972 and 1994 and concluded that corticosteroids reduce in approximately 50% the risk of respiratory distress syndrome in fetuses born prematurely, when birth occurs after 24 hours to 7 days of the first dose of the drug [5, 6].

It is a practice-based on scientific evidence adopted for 20 years, which aims to reduce the incidence of neonatal death, respiratory distress syndrome, intraventricular hemorrhage or necrotizing enterocolitis and contribute to the survival of extremely preterm infants, having effects such as: cardiovascular adaptation, increased blood pressure, improved renal function and improved skin keratinization [7–10].

Corticosteroids, unlike other forms of steroids, are easily transported across the placenta. They act on the airways in various ways, such as fetal lung maturation through thinning of the alveolar septa and alveolar differentiation with type 2 pneumocyte induction, which stimulates the production of surfactants [11].

In the fetus, cortisol plays an important role in development as it promotes the maturation of major organs in late pregnancy, including the respiratory system, kidney, gastrointestinal tract and brain. Fetal adrenal cortisol levels increase dramatically in the last six weeks before term completion and play a crucial role in later stages of organogenesis [12, 13].

The most important fetal organ in terms of survival at birth is the lung, where glucocorticoids act in the mesenchymal tissue to reduce the distances from blood vessels to the airways to allow for proper gas exchange. Glucocorticoids, in addition to promoting the production of surfactants, mature the pulmonary mechanisms for the elimination of fluid from the lung's airways at birth [12].

After birth, dexamethasone has important anti-inflammatory effects in the neonatal lung, including direct suppression of proinflammatory cytokine gene expression, inhibition of cytokine production in lymphocytes and macrophages, and leads to apoptotic cell death of lymphocytes to further promote immune suppression [14].

The use of corticosteroids provides benefits by improving short-term respiratory morbidity. Premature newborns, when they require care, usually need to be admitted to an intensive care unit, with good recovery in most cases.

Despite these benefits, the use of corticosteroids can cause harm, including neonatal hypoglycemia. It has been shown that antenatal betamethasone results in elevated betamethasone concentrations and decreased cord blood cortisol concentrations at birth, leading to fetal hypothalamic pituitary adrenal axis suppression, persisting for up to seven days after birth. This practice results in high levels of blood glucose and C-peptide at birth and consequent hypoglycemia in the early neonatal period, which although appearing to be self-limited, has been reported to be associated with poor neurological outcomes [15, 16].

Seckl et al. in experimental work with animals reported that there may be adverse consequences in prenatal exposure to excess corticosteroids, as well as being a trigger for some diseases in adult life, such as cardiovascular diseases (dyslipidemia and hypertension), type 2 diabetes and impaired glucose tolerance [17–19]. Clark et al. and Edwards et al. reported the same result in their studies. Kari et al. observed delay in neurological development in childhood, but with a small sample size (n = 82) [20–23].

Based on scientific reviews to date, the use of steroids has become the mainstay of prophylactic treatment in fetuses at risk of premature birth in singletons. However, there are still some outstanding issues regarding the use of medication, such as the increase in the rate of stillbirths in women with hypertensive syndromes. In addition, its use in parturients diagnosed with premature rupture of ovular membranes may increase the risk of neonatal infection [24, 25].

There are still insufficient data on childhood follow-up in fetuses who received corticosteroids during intrauterine life. There are only two studies that have followed steroid-treated fetuses into adulthood and found no differences in intellectual impairment or school performance [22, 26, 27].

Prophylactic corticosteroids use in multiple-dose protocols has not shown benefit in respiratory distress syndrome, in addition to having adverse effects on fetuses. Therefore, betamethasone is administered intramuscularly, in a daily dose of 12 mg, for two consecutive days or dexamethasone intramuscularly four 6 mg doses every 12 hours, in just one cycle and, exceptionally, two cycles [4].

### 2.1 Corticosteroids in multiple pregnancy

In dichorionic pregnancies the term is 38 weeks, in uncomplicated diamniotic monochorionic pregnancies it is 36 weeks and in monoamniotic it is 32 weeks. As the term in twins is earlier, there is a greater risk of perinatal morbidity and mortality, especially in monochorionic, because the delivery is in late prematurity [28].

Routine use of corticosteroids in the twin is not recommended except in risky conditions for discontinuation before 34 weeks. There is still doubt in the literature regarding the dose, since the pharmacokinetics after administration of

corticosteroids in twins is different in single pregnancies, as the recommended dose is the same for both [29]. Because of this, despite the short-term benefit, many questions remain unanswered in this population: such as the long-term effect of the drug, its pharmacokinetics, its real involvement in neurodevelopment and future metabolic consequences.

In the study by Hashimoto et al., evaluated in their retrospective cohort study consisting of 652 premature twins and 1705 premature single fetuses who were born weighing 500 to 1500 g (from 1991 to 1999), they concluded that the effect of corticosteroids in the prevention of neonatal mortality in twin pregnancies is similar to that observed in single pregnancies [30].

Blickstein and colleagues reported that a complete course of corticosteroids significantly reduced the incidence of respiratory distress syndrome in single children as well as in twins and triplets [31].

Melamed et al. administered a complete course of corticosteroids during prenatal care to twin pregnancies with a gestational age between 24 weeks to 33 weeks and 6 days. The outcome was a 58% reduction in neonatal mortality, decreased respiratory morbidity and severe neurological damage. The same results were observed in singleton pregnancies. The study was carried out in a tertiary neonatology center in Canada (Canadian Neonatal Network), from 2010 to 2014, with 9466 participants, consisting of 2516 mothers of twins and 6950 of singletons [32].

### 2.2 Corticosteroids in late prematurity

The effectiveness of the use of corticosteroids in multiple pregnancy was only approached retrospectively in 1996. Some difficulties were raised regarding its benefit in twin pregnancies with gestational age after 34 weeks in late prematurity [33, 34].

It has recently been biologically proven that administration of corticosteroids after 34 weeks of gestation reduces respiratory morbidity rates up to 72 hours after birth [33].

Three randomized clinical trials were published by Feitosa et al., Kamath et al. and Ramadam et al. confirming the beneficial effect in reducing transient tachypnea in newborns who used the full cycle of steroids during single gestation in late prematurity [35–37].

In 2016, a large randomized double-blind multicenter trial called Antenatal Betamethasone for Woman at Risk for Late Preterm Delivery (APLS) was published. Seventeen universities from the Eunice National Institute for Child and Human Development (NICHD) Fetal Medicine Units Network in Kennedy Shriver were included. Participants were assigned to receive two injections of betamethasone 12 mg in 24 hours.

A total of 2827 pregnant women with a gestational age of 34 weeks to 36 weeks +5 days were eligible, totaling 1427 women in the betamethasone group and 1400 in the placebo group. It resulted in significantly lower rates of severe respiratory complications in the betamethasone group compared to the placebo group (8.1% and 12.1%) and rates of various disorders were significantly lower in the betamethasone group than in the placebo group, they are: transient newborn tachypnea (6.7% and 9.9%), bronchopulmonary dysplasia (0.1% and 0.6%), transient newborn tachypnea or apnea (13.9% and 17.8%), resuscitation at birth (14.5% and 18.7%) and use of surfactant (1.8% and 3.1%). Apnea and pneumonia rates were similar in both groups [38].

The administration of corticosteroids beyond 34 weeks' gestation has been the subject of many discussions around the world, as they are also at risk of significant morbidities, with an emphasis on respiratory morbidity.

However, there is no international consensus regarding the use of corticosteroids above 34 weeks due to the scarcity of trials and meta-analyses, as the long-term effects are still unknown.

## 3. Magnesium sulfate as fetal neuroprotection

Premature birth is increasing in most developed and developing countries. Overall, preterm birth is the leading cause of perinatal mortality and cerebral palsy remains one of the main long-term morbidities associated with preterm birth.

Cerebral palsy (CP) is the most common neurological impairment related to prematurity. The CP rate is closely linked to the prematurity rate; the more premature, the greater the risk of CP. The incidence of cerebral palsy is 14.6% between 22 and 27 weeks' gestation, 6.2% between 28 and 31 weeks' gestation, 0.7% between 32 and 36 weeks' gestation, and 0.1% at the term of pregnancy [39].

Due to its association with preterm birth, multiple pregnancies also contribute to an increased risk of cerebral palsy, which increases 8-fold in double pregnancy and 47-fold in triple pregnancy [40].

It is a complex disease characterized by motor and/or postural dysfunction, has a permanent and non-progressive character, and can be recognized in the early stages of life. The four main types of cerebral palsy are spastic (increased muscle tone), dyskinetic (uncontrolled or slow movements), ataxic (difficulties with balance and depth perception), and mixed. Most cases of cerebral palsy (85–90%) are congenital and the most common type is spastic, which affects approximately 80% of individuals affected by the disease [41].

As there is no cure for cerebral palsy, among the various drugs used in an attempt to protect premature newborns from neurological complications, magnesium sulfate (MgSO4) has been the subject of a growing number of studies over the last decade.

The history of MgSO4 for neuroprotection began with the observation that the CP rate in pre-eclampsia was markedly lower compared to the gestational age in normotensive pregnancies and the subsequent decrease in neonatal neurological morbidity rates was initially suggested by some observational studies of the decade of 1990. The results of these studies supported the publication of systematic reviews and, also, opinion articles and guidelines by respected international scientific entities. Despite several large studies, with mostly positive results, the debate about routine MgSO4 continues, not only about dosage and administration, but also about safety aspects [42].

There are three large studies: ACTOMgSO4 (2003), PREMAG (2007) and BEAM (2008) that agreed that prenatal use of MgSO4 reduces the rate of cerebral palsy in newborns.

In 2003, Crowther et al. published the results of the ACTOMgSO4 study. A total of 1062 Australian and New Zealand women (1255 children) with less than 30 weeks' gestation and expected to give birth within 24 hours were randomized. The initial dose of MgSO4 was 4 g IV in 20 minutes, followed by 1 g/hour IV for a maximum of 24 hours. As a conclusion of the study, the administration of MgSO4 before preterm delivery improved important pediatric outcomes without causing serious adverse effects [43].

In the work carried out by Marret et al. (PREMAG (2007)), 573 French women (688 children) with less than 33 weeks of pregnancy and with expected or planned delivery within 24 hours were included. A single IV dose of MgSO4 (4 g) or placebo was administered in 30 minutes. The main outcome of the study was to assess the existence of damage to the newborn's cerebral white matter, whose diagnosis was made by cranial ultrasonography in the neonatal period. Data on the follow-up of surviving children up to two years of corrected age were described. That without

statistical significance, there was protection against the combined outcome of cerebral palsy or death with the use of MgSO4 (RR 0.65; 95%CI 0.42–1.03) [44].

No serious maternal complications were observed in the group treated with MgSO4. Finally, the multicenter study BEAM (2008) had as sample number 2241 American pregnant women (2444 children) with imminent risk of delivery between 24 and 31 weeks. Pregnant women were randomly assigned to receive MgSO4 (6 g IV followed by a 2 g/h infusion) or placebo. MgSO4 administration was interrupted in cases where delivery did not occur within 12 hours. The primary outcome was the combination of fetal death or infant death with one year of corrected age or the presence of moderate/severe cerebral palsy with two years or more of corrected age. There was no significant difference between the primary outcomes of the MgSO4 and placebo groups (11.3 versus 11.7%; RR 0.97; 95%CI 0.77–1.23). However, in a prespecified secondary analysis, the rate of moderate/severe cerebral palsy was significantly lower in the MgSO4 group (1.9 versus 3.5%; RR 0.55; 95% CI 0.32–0.95) [45].

Despite MgSO4's role in preventing CP, many physicians are still concerned about side effects for both mother and baby. Since this mode of prevention is inexpensive, easy to use, and can reduce the rate of cerebral palsy by up to almost 50% [45].

Most guidelines (RCOG and Australian) recommend the administration of MgSO4 for fetal neuroprotection of viability for less than 30 weeks. Only SOGC has an upper cutoff at 32 weeks [46–48].

The definitive mechanism of action of the use of magnesium sulfate in fetal neuroprotection remains unclear. Biochemical and histopathological findings have been implicated as a possible mechanism to explain the neuroprotective effects of MgSO4 and these mechanisms include: neuroprotective ability to prevent early apoptosis of abnormal neuronal cells, decreased neuroinflammation to increase the seizure threshold, decreased hemorrhage of the cerebellum, stimulation of local adaptation responses through vasodilation and stimulation of neurogenesis in the premature maturation of brain cells by encouraging the secretion of neurotrophic factors [49, 50].

The developing brain (in preterm fetuses) is unconditionally vulnerable to insult and its response to adapt to a hypoxic condition is much lower compared to the brain in term fetuses. Damage to the fetal brain primarily occurs in the periventricular region, so it is often called periventricular leukomalacia, and lesions in this area can lead to clinical manifestations of cerebral palsy [50–52].

Until now, most maintenance doses have been advocated at 1 g/h. However, some studies suggest that if the objective were to optimize results, the dose would potentially have to be increased to a maintenance dose of 2 g/h. At this dose, the maximum dose to be administered is 64 g as shown by statistical simulation by Brookfield et al. However, other researchers claim that a maximum dose to avoid harmful effects would be 50 g [44, 53, 54].

Prenatal MgSO4-related maternal adverse events are widely researched. Common minor side effects include flushing, headache, heat and sweating, nausea, vomiting, blurred vision, or intramuscular discomfort at the injection site. Major adverse events can progress to respiratory and cardiac arrest and even death [55].

Several recent publications have confirmed the efficacy and safety (4 g loading dose, 1 g/h maintenance) of prenatal MgSO4 for preventing cerebral palsy, and this is considered an effective dose without compromising safety [56]. Other neuroprotective agents such as melatonin are being actively researched.

## 4. Conclusion

Twin pregnancy is an important cause of prematurity. To reduce perinatal morbidity/mortality, antenatal corticosteroids are used. Between 24 and 34 weeks the

use of betamethasone in a dose of 12 mg intramuscularly two doses with an interval of 24 hours or dexametasone 6 mg intramuscularly 4 doses every 12 hours. It should be restricted to one or at most two cycles of corticosteroids.

The use of steroids in late prematurity in twin pregnancy is still controversial, however, several guidelines have suggested the use of steroids in elective preterm births such as in monochorionic pregnancies.

In addition to steroids, the use of magnesium sulfate protects the newborn from cerebral palsy.

Prenatal care for a twin pregnancy is challenging and the obstetrician must be aware of the use of corticosteroids and magnesium sulfate in cases of prematurity to reduce perinatal morbidity/mortality.

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Chapter 2

# Breastfeeding and the Influence of the Breast Milk Microbiota on Infant Health

*Fatima Chegdani, Badreddine Nouadi and Faiza Bennis*

## Abstract

Nutrition is an essential condition for physical, mental, and psycho-emotional growth for both children and adults. It is a major determinant of health and a key factor for the development of a country. Breastfeeding is a natural biological process, essential for the development of the life of the newborn at least during the first six months by ensuring a nutritional contribution adapted to the needs of the latter. Thus, breast milk is the physiological and natural food best suited to the nutrition of the newborn. It contains several various components, which are biologically optimized for the infant. Cells are not a negligible component of breast milk. Breast milk is also a continuous source of commensal and beneficial bacteria, including lactic acid bacteria and bifidobacteria. It plays an important role in the initiation, development, and composition of the newborn's gut microbiota, thanks to its pre- and probiotic components. Current knowledge highlights the interdependent links between the components of breast milk, the ontogeny of intestinal functions, the development of the mucus intestinal immune system, colonization by the intestinal microbiota, and protection against pathogens. The quality of these interactions influences the health of the newborn in the short and long term.

**Keywords:** breastfeeding, probiotic, microbiota, newborn, prematurity

## 1. Introduction

Nutrition is an essential condition for physical, mental, and psycho-emotional growth for both children and adults. It is a major determinant of health and a key factor for the development of a country. Nutritional disorders hamper economic growth and perpetuate poverty through three factors: direct losses in productivity linked to poor physical condition, losses resulting from increased health care costs and indirect losses due to poor cognitive function and school failures (Global Strategy 2003 WHO / UNICEF, Moroccan Strategy 2011–2019 / UNICEF) [1, 2]. Furthermore, nutritional disorders are not only the result of food insecurity since children living in good conditions are subject to deficiency syndromes, such as anemia, being under or overweight or even stunted growth (Report global malnutrition UNICEF, 2018) [3].

The global food strategy for infants and young children focuses on promoting appropriate infants and young children. Breastfeeding is the best way of providing ideal food for the growth and development of healthy infants.

Poor feeding practices would be particularly due to lack of information on the benefits of breastfeeding and complementary feeding practices. Therefore, breast milk is the most suitable physiological and natural food for the nutrition of the newborn [4]. Breastfeeding is therefore a real global public health problem. Indeed, the World Health Organization (WHO) recommends exclusive breastfeeding for at least 6 months, giving the infant a good start in life [2]. Breast milk plays an important role in the initiation, development and composition of the gut microbiota of the newborn, thanks to its pre- and probiotic components [5–7].

Breast milk is particularly important in cases of prematurity and pronounced low birth weight at term, as the child is at increased risk of infection, long-term health problems and death due to the immaturity and dysfunction of virtually all components of innate immunity [8]. At birth, newborns inherit part of their mother's microbiota, but there is growing evidence that breastfeeding forges this microbiota in the infant's gut [9, 10]. The establishment of the microbiota in the newborn is a critical period that has an impact on the overall state of his future health. The mode of colonization and the composition of the intestinal microbiota of the newborn vary depending on the lifestyle of the mother, the delivery route and environmental factors [11–13]. Children born prematurely have an intestinal dysbiosis that diminishes over time. It does not seem to be related to environmental factors but could be correlated to the length of gestation [14].

The aim of this chapter is to highlight the relationship between breastfeeding, the gut microbiota and the health of the newborn.

## 2. Human milk: composition and evolution

### 2.1 Human milk composition

Breastfeeding is a natural biological process, essential for the development of the life of the newborn at least during the first six months. Breastmilk provides all the calories and nutrients a child needs in the first few months of life and continues to provide half or more of the nutritional requirements in the second half of life, and up to one third in the second year. It promotes sensory and cognitive development and protects infants against infectious and chronic diseases [4, 15].

Human breast milk is a complex fluid made up of a number of diverse components, which are biologically optimized for the infant and which change dynamically between women (**Figure 1**) [16–19].

The beneficial health effects of human milk have been linked to the abundance of bioactive molecules present, including secretory antibodies, immune cells, antimicrobial proteins like lactoferrin, CD14 and lysozyme, regulatory cytokines and oligosaccharides [17]. Proteins and lipids beyond their role in the nutritional supply and essential source of energy for the newborn, have antimicrobial and immunomodulatory activities. Nucleotides are also beneficial for the development and maturation of the gastrointestinal tract, as well as for microbiota development and immune function. The immunoglobulins present in milk in the form of secretory IgA and secretory IgG provide initial immunity to the immature immune system of the newborn [17]. The indigestible oligosaccharides are not directly intended to feed the baby, but to nourish his intestinal bacteria [20]. Breast milk also includes short chain fatty acids (SCFAs) from the fermentation of indigestible foods which are a source of prebiotics for the breastfed infant [21].

In addition to the major components of water, carbohydrates, fats, proteins and micronutrients, breast milk is also a continuous source of non-bacterial cells and bacterial cells of maternal origin, which are beneficial to the newborn. Cells are not

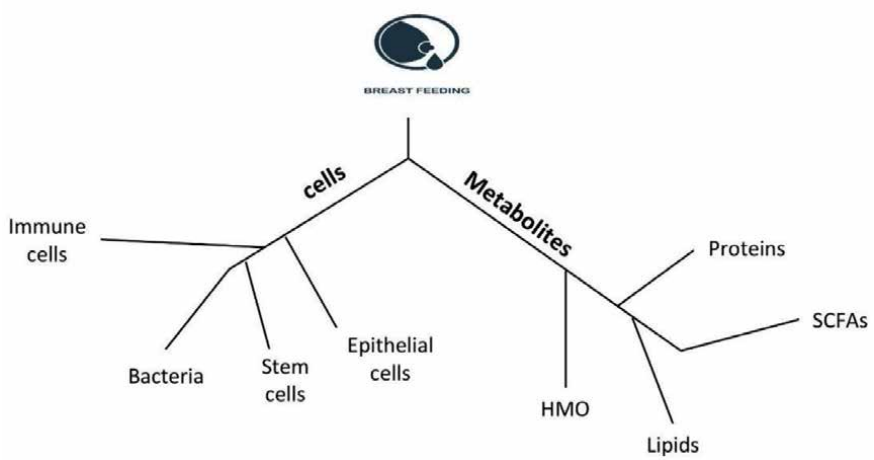


**Figure 1.**
*Schematic illustration of the different components of human breast milk.*

a negligible component of breast milk because of their impact on the health of the newborn [22]. Epithelial cells and cellular debris of glandular origin and immune cells of blood origin (macrophages, lymphocytes and polymorphonuclear cells) are present in breast milk. Recent breakthroughs have shown that breast milk is much more heterogeneous and that it also contains stem cells [22]. Ten years ago, the microbiome of breast milk was characterized in which bacteria form an ecosystem of probiotics beneficial to the newborn [23]. These probiotics are involved in the digestion of nutrients including the metabolism of oligosaccharides, although their most likely role is the modulation of immunity [24].

The predominant bifidobacteria and lactobacilli constitute the natural microbiota, originating from the gastrointestinal tract of the mother via an enteromammary cycle [5, 7, 25–30]. High throughput sequencing, by pushing back the limits imposed by the passage through microbial cultures, has allowed a more complete and faster characterization of the microbiota. In fact, metagenomics, a revolutionary approach to the genetic study of a complex sample, has made it possible to capture 90% of the diversity of the microbiota in breast milk. *Bacteroides, Blautia, Faecalibacterium, Ruminococcus, Roseburia, Subdoligranulum, Enterococcus and Escherichia-Shigella*, have been repeatedly detected in breast milk in different studies using molecular approaches based on the 16S rRNA gene [11, 29–32].

Breast milk is a natural source of nutrients, rich in various bioactive molecules. The Cells, both eukaryotic and prokaryotic, are an important component and also play a crucial role in the well-being of newborns.

## 2.2 Breast milk evolution

The major components of breast milk, namely water, micronutrients and macronutrients, change during lactation to meet the needs of the newborn, particularly in relation to the establishment of the newborn's initial immunity and the development of the intestinal epithelium and central nervous system [33–36]. This composition of breast milk is required to constantly change depending on the needs and age of the baby, the time of breastfeeding or the beginning and end of breastfeeding and the period of breastfeeding [13, 37]. Indeed, between the 3rd and 5th day, colostrum is produced, then transitional milk is produced over the following 15 days and finally, mature milk is produced approximately 3 weeks to 1 month

after the start of breastfeeding [38, 39]. This mature mother's milk from the first month, rich in prebiotics and probiotics, ensures intestinal homeostasis. In addition, breastfeeding is an interactive process where the baby's behavior can to some extent determine the composition of his food. The composition profile of breast milk is relatively stable around the world and varies only slightly depending on the lifestyle and diet of the mother [40]. SCFAs in breast milk, secondary metabolites whose role is crucial in modulating the immune system, the development of the intestinal epithelium and the intestinal barrier function of the newborn, evolve like the other components. Dai et al. [21], have shown that the concentrations of SCFAs are higher in mature milk as well as in milk from mothers of full-term infants than from mothers of preterm infants.

## 3. The newborn's gut microbiota

### 3.1 First colonization

The newborn, considered sterile at birth, presents a beginning of colonization of the gut by the bacteria in the amniotic fluid, placenta, cord blood and meconium [14, 41, 42]. The bacterial DNA detected in these different matrices comes mainly from commensal bacteria belonging to the phyla Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fusobacteria [43, 44].

Colonization at birth of the baby's digestive tract by bacteria allows an incessant dialog to be established between microorganisms and all the newborn's defenses. The immune system thus learns to recognize and differentiate between pathogenic and non-pathogenic bacteria and beneficial food proteins. However, it also learns to scale up its reactions. It is through this intensive training that a good balance between the different immune responses is achieved [45].

It is highly likely that babies are born with an initial bacterial composition from the maternal womb. This initial culture may change during pregnancy, which explains the difference in microbial diversity in newborns at birth [44]. This initial (fetal) colonization is likely to reorganize the intestinal epithelium to prepare the environment to receive the new colonizing bacteria at birth.

At birth, the genus *Streptococcus*, and particularly the species *Streptococcus thermophilus*, is among the first GIT colonizers as it has been detected in the stools and breast milk of infants [28, 46, 47]. *Streptococcus thermophilus* is able to induce differentiation of intestinal epithelial cells [48]. Indeed, work carried out on axenic rats inoculated with *Streptococcus thermophilus* has shown that this bacterial species undergoes a progressive adaptation in the gut to stabilize after 30 days. The main impact of *Streptococcus thermophilus* is to massively stimulate the glycolysis pathway, leading to lactate production in the cecum. In addition, expression studies showed overexpression of SLC16A1 and SLC5A8, monocarboxylic acid transporters, as well as p27 (kip1) a protein involved in cell cycle arrest. These results suggest that lactate could be a signal produced by *Streptococcus thermophilus* and modulating the colon epithelium development [48, 49].

### 3.2 Factors impacting the microbiota diversity

The establishment of the gut microbiota in newborns is a critical period that has an impact on the overall state of their future health. The microbiota at birth will be impacted by various environmental factors, the most important of which are mode of delivery, type of infant feeding, gestational age, infant hospitalization and infant antibiotic use. Infants born at term vaginally, at home and exclusively breastfed

appear to have healthy gut microbiota, with the highest levels of Bifidobacteria and the lowest levels of *Clostridium difficile* and *Escherichia coli* [11, 50].

Interdependencies have been demonstrated between breast milk components, ontogeny of gut function, development of the mucosal intestinal immune system, colonization by the gut microbiota and protection against pathogens [15]. Williams et al. have suggested complex microbial interactions between breastfeeding mothers and their infants and support the hypothesis that variation in the milk microbiome may influence the infant GI microbiome. In fact, the result of this work provides that infant oral, maternal oral, and milk microbial communities were predominated by Firmicutes; maternal feces by Firmicutes and Bacteroidetes; and infant feces was characterized by a relatively even distribution of Firmicutes, Bacteroidetes, and Proteobacteria. Regarding bacterial genera, the most abundant in milk and maternal and infant oral samples was *Streptococcus*, whereas *Bacteroides* were predominant in maternal and infant fecal microbiomes [51]. Moreover, the ontological prediction study carried out by *Nouadi et al.*, revealed an interconnection between the breast milk microbiota and the microbiota of the full-term newborn. The exploitation of the bioinformatics tool in this study, helped to provide a new representation and interpretation of the interactions between genes differentially expressed in the host intestine induced by the microbiota. Subsequently, giving an overview of the global patterns of gene expression in the epithelial cells of term infants. This study found that IL1β, RELA, INS, IRS1 and NFKBIA (Core Network Proteins) are the major regulators of four important biological processes. These processes induced in the first few months of a newborns' life have concerned intestinal development, effect of nutrition, and impact of other environmental exposures on the intestinal microbiota colonization. This study indicates the importance of these interactions in health homeostasis [52].

### 3.3 Role of SCFAs in intestinal development

Short-chain fatty acids, produced by fermenting anaerobic bacteria are secondary metabolites, found on the surface of the intestinal lumen; they act as signaling molecules transferring information between the microbiota and the immune system [53].

These organic acids have an aliphatic chain of 1 to 6 carbons. The most abundant SCFAs in humans are acetic acid (C2: 0), propionic acid (C3: 0) and butyric acid (C4: 0). SCFAs are the precursors of long chain fatty acids. The production of SCFAs in the gastrointestinal tract is particularly dependent on the diet and the type of host microbiota [54]. SCFAs found in breast milk are an important source of energy as well as being essential for maturing the gastrointestinal tract [55, 56].

Acetic acid represents 50–60% of SCFAs, it is produced by the genera *Bifidobacteria* and *Lactobacilli*. Acetic acid plays many roles including the synthesis of cholesterol and fatty acids, lipogenesis, insulin sensitivity and pH regulation of the intestinal lumen [57–60]. *Bacteroides*, *Firmicutes* and *Lachnospiraceae* are the main intestinal bacteria producing propionic acid. Depending on the substrate, these bacteria can also produce butyric acid. Propionic acid is involved in several biological processes, including lipid metabolism, anti-inflammatory activity, and anti-cancer activity. Butyric acid is produced by the genera *Faecalibacterium*, *Eubacterium rectale* and *Roseburia spp.* [61]. The latter is the least abundant, but exhibits significant beneficial effects on energy metabolism and intestinal homeostasis [62, 63]. Moreover, butyric acid plays a key role in the down regulation of pro-inflammatory effectors of lamina propria macrophages and determines the expression of cytokines in T cells [64, 65]. Effectively, it is involved in the activation and differentiation of a memory phenotype of CD8 + T lymphocytes [66].

## 4. Prematurity and microbiota

### 4.1 Causes of prematurity

Prematurity is a syndrome that includes all the situations associated with the occurrence of a birth before 37 weeks of amenorrhea. It is classified according to gestational age, extremely premature (less than 28 weeks), very premature (between 28 and less than 32 weeks) and moderate premature (between 32 and less than 37 full weeks of gestation) [67, 68]. Preterm birth can result in adverse effects and even short-term neonatal mortality [69, 70]. The long-term health consequences of prematurity throughout adulthood of infants is an increased risk of type 2 diabetes, obesity, hypertension, asthma, anxiety, spectrum disorders autistic, cerebral palsy, epilepsy and cognitive impairment, heart disease, chronic renal failure, lung function abnormalities and neurocognitive disorders [71–78]. These effects can have a negative impact on health care costs, education as well as quality of life [79, 80].

During pregnancy, along with hormonal, metabolic and immune changes, the different ecosystems of the mother's microbiome (vaginal, uterine, etc.) change. Indeed, the vaginal microbiota is less diverse and more stable, with an abundance of bacteria of the genus *Lactobacillus* [81]. Changes in the gut microbiota also appearing in late pregnancy may help support the increased energy requirements needed for fetal development [82]. All these observations thus suggest a real role of the microbiota in the physiology of pregnancy. The occurrence of premature rupture and preterm of membranes was preceded in one-third of cases by depletion of *Lactobacillus*, protective bacteria in the vaginal flora [83]. Dysbiosis could therefore play a role as a biomarker associated with pathology, but also be directly involved in pathophysiological processes. Indeed, vaginal dysbiosis could initiate an inflammatory cascade leading to prematurity. A modification of the vaginal microbiota, with a decrease in protective *Lactobacillus*, allows pathogens such as *Gardnerella vaginalis* or *Mycoplasma hominis* to proliferate [84] (**Figure 2**). These pathogens could then pass through the cervix and contaminate the chorio-decidual space. In the face of this aggression, an increased production of inflammatory cytokines, as a result of the immune response, would stimulate the recruitment of neutrophils, the production of prostaglandins and the synthesis of metalloproteinases [85]. This cascade of events would promote uterine contractility, shortening of the cervix, weakening of the fetal membranes and could lead to premature delivery [86]. The sequelae due to the exposure of the preterm newborn to an intrauterine inflammatory environment will accumulate with those of its developmental immaturity, which may result in early and severe neonatal disorders, such as necrotizing enterocolitis (NEC), cystic periventricular leukomalacia or bronchopulmonary dysplasia [87]. Maternal dysbiosis will influence the initial fetal culture and consequently the colonization at birth of the gut microbiota in the preterm infant.

Also, when a newborn is born prematurely, it is necessarily separated from its mother for care reasons. This maternal separation and prematurity, responsible for dysbiosis, would increase the risk of developing pathologies [88].

Environmental factors, maternal microbes and breast milk shape healthy colonization of the infant gut. The colonizing microbes influence the development of the gut and also participate in several biological processes related to growth and survival. Several factors lead to dysbiosis in the gut microbiota of newborns, increasing the risk of neonatal conditions such as necrotizing enterocolitis and long-term health problems such as asthma, hypertension and obesity.

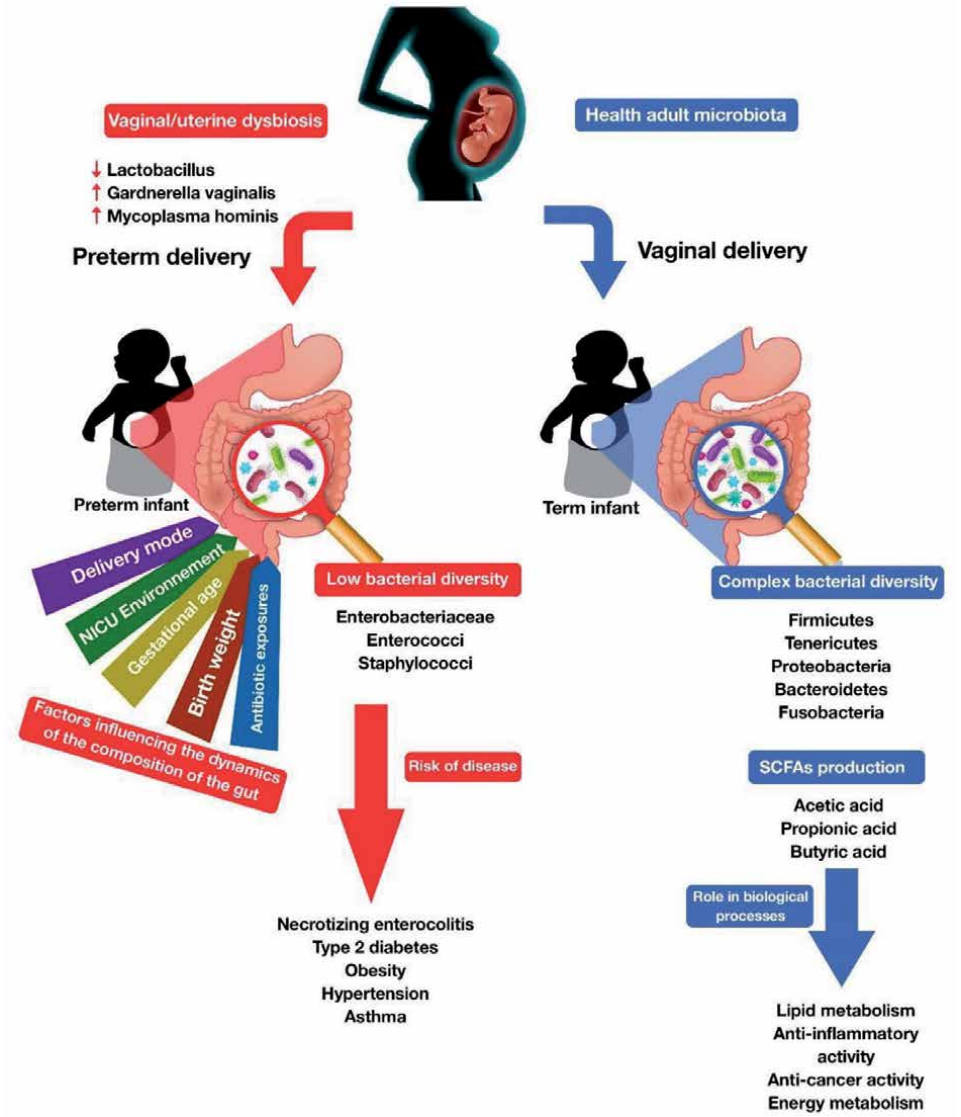


**Figure 2.**
*Factors influencing microbial intestinal homeostasis of term and preterm infants.*

### 4.2 Preterm intestinal microbiota

Premature infants may be admitted to a highly specialized area of the hospital, the Neonatal Intensive Care Unit (NICU). Prematurity and admission to the NICU are often unexpected and may occur before families have discussed the nature of feeding for their infants. Prenatal and postnatal exposure to antibiotics as well as other medical risks can affect the composition of the early gut microbiota in premature infants [89].

The immaturity of the intestine of preterm promotes pathogenic bacterial colonization due to the high permeability of the intestinal surface, which paves the way for a destructive dysbiosis. This dysbiosis, responsible for chronic inflammation and microbial translocation across the weakened intestinal barrier, is associated with life-threatening conditions of prematurity such as NEC and late-onset sepsis (LOS) [90].

The intestinal microbiota of children born prematurely has a low diversity and a reduced number of anaerobic bacteria. Two elements which would participate in the risk of developing pathologies of premature babies. In premature newborns, it was observed a significant delay in colonization compared to full-term infants as well as colonization by a smaller number of bacterial species [91].

The delay in colonization is especially marked for the anaerobic flora (*Bifidobacterium* and *Bacteroides*) while the aerobic flora (Enterobacteriaceae, Enterococci, Staphylococci) colonizes the premature baby fairly quickly [92–102]. This delay in implantation may be due to the fact that these children are more frequently born by cesarean section, are quickly separated from their mothers and placed in a highly aseptic intensive care environment and often subjected to broad-spectrum antibiotic therapy [89]. Several studies have compared the microbiota of NICU-cared preterm infants and healthy term infants after birth. A significant abundance of Staphylococcaceae has been observed in premature infants. In contrast, the abundance of Enterobacteriaceae, Streptococcaceae and Enterococcaceae is significantly lower in NICU preterm infants immediately after birth, and their level gradually increases from the 2nd week. The abundance of Bifidobacteriaceae in term newborns is greater than in preterms at birth, associated with a high concentration of acetate, a sign of good host health. However, higher levels of propionate and butyrate are noted in preterm infants, indicating less saccharolytic fermentation [103–105].

### 4.3 Breastfeeding in the intensive care unit: a way to support preterm infants

Breastfeeding in preterm infants is often initiated by administration through a nasogastric or orogastric probe. Infants switch to oral feeding when the coordination of sucking, swallowing and breathing is developed [106–108]. This oral feeding is a difficult stage for preterm infants [109], with almost 40% finding it difficult to transition to direct oral breastfeeding [110–113]. Olfactory stimulation [114–120], could remedy the difficulties of direct oral feeding and consequently reduce the risks of pathologies associated with prematurity [121, 122]. The results of several studies have shown that direct breastfeeding increases the likelihood of maintaining longer breastfeeding durations during NICU hospitalization and up to 4 months after discharge [93–95, 123–125].

In preterm infants, breast milk is associated with a significant reduction in NEC, and a reduction in other key morbidities, as well as improved neurodevelopmental outcomes [91]. These impacts have long-term benefits for the child (and mother) even after weaning. This advantage is probably due, in part, to the development of a healthy gut microbiota dominated by *Bifidobacterium* from breast milk and its bioactive components [126].

Breast milk could be a natural way to restore intestinal homeostasis newborn and meet the recommended health standards for newborn term and preterm [127]. Thus, awareness of the importance of breast milk for premature infants is essential for families whose premature birth is planned and for those whose child is in the NICU [128–132]. On the other hand, trained NICU personnel will help and encourage mothers to breastfeed more frequently during hospitalization. This breast milk and intestinal microbiota axis opens up promising research avenues for new therapies in premature babies and other high-risk infants.

## 5. Conclusion

The long-term health of the human being is programmed before birth where nutrition plays a crucial role. Indeed, the mother's lifestyle is a determining factor

in shaping her own microbiota, which will be transmitted in part to the fetus. From the moment of birth, breast milk is the optimal source of nutrition for the newborn, which will meet its nutritional and immune needs. It is recommended to be the only source of nutrition for the first six months of life and up to 2 years.

Preterm birth is a complex syndrome, the leading cause of morbidity and perinatal mortality. The immaturity of the preterm gastrointestinal tract and the initial dysbiosis are corrected by breastfeeding. Indeed, breast milk promotes an evolutionary dynamic of the intestinal microbiota and is truly a food for all newborns and particularly premature babies in intensive care. The cross-talk established between the bioactive components of milk, mainly the metabolites of the intestinal microbiota and the intestinal mucosa of the newborn, can compensate for the delay in colonization in preterm infants. This infant's breast milk-intestinal microbiota axis is a proven link between mother and baby. This symbiosis is essential for the maintenance of health and well-being.

Thus, promoting breastfeeding is the natural solution to preventing infant morbidity and mortality.

## Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

# Neonatal Anemia

*Laura M. Dionisio and Thamires A. Dzirba*

## Abstract

Neonatal anemia and iron deficiency are frequent founds in neonatal intensive care units (NICUs). The three major causes of anemia in neonates are blood loss, reduced red blood cell production, and increased degradation of the erythrocytes. Premature infants in ICUs have high levels of iron deficiency, and ascertaining the cause of anemia in this group of patients can be a challenge in clinical practice. This chapter provides an updated review of neonatal anemia. It will concern the pathophysiology of neonatal anemia in term and preterm infants and a detailed discussion of the traditional and innovative laboratory tests for diagnosis and assessment of this condition in the ICUs.

**Keywords:** anemia of prematurity, hemolysis, neonatal anemia, neonatal intensive care, physiologic anemia

## 1. Introduction

Neonatal anemia is a frequent found in neonatal intensive care units (NICUs). The diagnostic approach for neonatal anemia must establish its underlying cause for determining the proper treatment. However, due to the complexity of patients under NICUs (prematurity, infections, other diseases, ventilatory support) and the variety of laboratory tests available, determine the cause of neonatal anemia could be a challenge for clinicians.

After birth, all infants experience a gradual decrease in hemoglobin that results in varying degrees of anemia. During the first weeks of life, the hemoglobin concentration decreases significantly as a consequence of the reduced production of erythrocytes due to reduced production of erythropoietin (EPO) and also by the decreased erythrocyte lifespan during this period. Although all neonates develop the physiologic anemia, preterm infants experience a greater degree of anemia, the anemia of prematurity, which results from a combination of physiologic and pathological factors.

The iatrogenic blood loss due to phlebotomy for laboratory testing in NCIU is an important contributor for the anemia of prematurity. Because low birth-weight and premature infants usually need close monitoring, they suffer the loss of great amounts of blood.

The three major non-physiologic causes of anemia in neonates are blood loss, reduced red blood cell production, and increased degradation of the erythrocytes.

The diagnostic approach usually includes the patient history (familial, maternal, patient), physical examination and laboratory tests.

This chapter is an updated review of neonatal anemia. It concerns the pathophysiology of neonatal anemia, anemia of prematurity and non-physiological causes of anemia in NICUs patients.

We also provide a detailed discussion of the traditional and innovative laboratory tests for diagnosis and assessment of this condition in the ICUs.

## 2. Developmental erythropoiesis

To properly understand the pathophysiology aspects of neonatal anemia, is essential to comprehend how the erythroid blood cells are formed during pregnancy and the extrauterine life.

The erythropoiesis in the fetus starts in the liver, with progenitors that migrate from the yolk sac and infiltrate the liver circulation. Approximately at the sixth week of gestation, the liver becomes the main hematopoietic site, remaining its role during the second trimester of pregnancy. After the sixth month of gestation, the bone marrow is the major site of erythropoiesis, gradually replacing the liver in this function until birth [1, 2]. Fetal erythropoiesis is an extremely active process, mainly during the last 2 months of gestation, where the red blood cell (RBC) production is 3 to 5 times greater than the healthy adult ones [3].

The erythropoiesis process is regulated by cytokines and growth factors, where the erythropoietin (EPO) exerts a major role, working synergically with the other factors. EPO acts as the main regulator of erythropoiesis, leading to the differentiation, proliferation and survival of red blood cells. In the fetuses, the liver is a primary site of EPO production, which is replaced by the kidney in the extrauterine life. The synthesis of EPO and its receptor (EPO-R) is not directly regulated by hemoglobin levels, but by the systemic availability of oxygen, in a strictly regulated feedback loop [4].

During the first week of life, the hemoglobin levels of the term newborn infants start to decrease progressively for the next 6 to 8 weeks. This process occurs in both term and preterm neonates. The postnatal plasma levels of EPO exhibit a predictable pattern of change, which is inversely associated with hemoglobin levels. Also, there is a directly proportional association between the EPO levels and the reticulocyte counts [5].

## 3. Physiologic anemia of the newborn

After birth, newborn infants undergo dramatic changes in tissue oxygenation. When the lungs replace the placenta as the source of oxygen, the arterial oxygen saturation increases about two-fold, which is followed by a decrease in EPO levels, resulting in the characteristic transient decay in hemoglobin levels, referred to as the "physiologic anemia of the newborn". Furthermore, the reduction in hemoglobin levels is also accompanied by a gradual decrease in the RBC count and the mean corpuscular volume (MCV). In term neonates, the physiologic anemia occurs at 4 to 6 weeks of life, persisting until the hemoglobin levels falls to approximately 10 to 11 g/dL, thus stimulating the EPO production and release by the kidneys, as a compensatory process [6].

Full-term neonates have from 50 to 80% of hemoglobin F and 15 to 50% of hemoglobin A, yet the new erythrocytes produced after birth will contain mostly hemoglobin A, allowing better tissue oxygenation, as the last has higher oxygen affinity the hemoglobin F. Another adaptative mechanism that improves oxygenation at this phase is the increase of 2,3-diphosphoglycerate concentration inside the erythrocytes, thus improving the oxygen release from hemoglobin [5, 7].

The gradual decrease in EPO production is not described as the unique cause of the physiologic anemia of the newborn. Reduced red cell lifespan and hemodilution are also involved in this process.

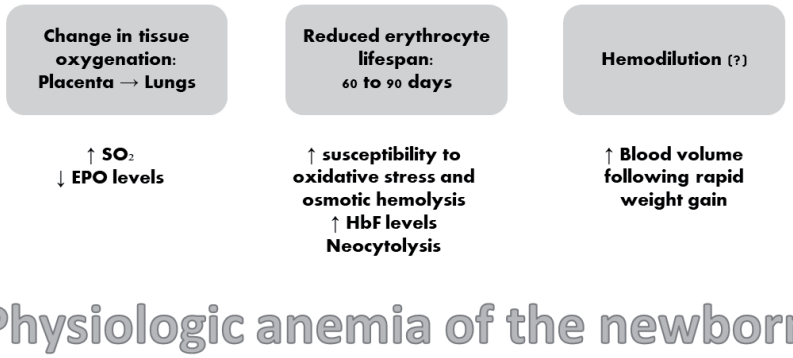


**Figure 1.**
*Causes of physiologic anemia of the newborn.*

The main causes of the physiologic anemia of the newborn are presented in **Figure 1**.

Differently from normal adult erythrocytes that have a life span of 120 days, the neonate erythrocytes life span is only 60 to 90 days [8]. This shortened survival is attributed to greater sensitivity and osmotic hemolysis and oxidative injury than the adult red blood cells. The neonatal erythrocyte has lower levels of antioxidant enzymes such as glutathione peroxidase, increasing thus their susceptibility to oxidative stress, and subsequential formation of Heinz bodies and methemoglobin, which contributes to reducing their lifespan [3, 9]. Additionally, a process named neocytolysis is also responsible for the reduced life span of the erythrocytes. It consists of the active removal of young erythrocytes generated in a relatively hypoxic condition when the individual is exposed to normoxic or hyperoxic conditions, as occurs in the change from placental to lung respiration. Furthermore, the higher concentration of hemoglobin F in the neonates is also responsible for the decreased life span of the neonatal erythrocyte, as this type of hemoglobin tends to denature and promote damage to the erythrocyte membrane from the inside [10].

The hemodilution due to the increased blood volume following the rapid weight gain in the first months of life may also contribute to the physiologic anemia, although it is not clearly understood the impact of this factor in the decreased hemoglobin levels and RBC counts [6].

## 4. Anemia in preterm infants

Although all neonates develop the physiologic anemia of the newborn, the preterm infants experience a greater degree of anemia, the anemia of prematurity, which results from a combination of physiologic and pathological factors.

The postnatal drop in hemoglobin levels occurs more rapidly in preterm infants, reaching the nadir before the term ones at 4 to 6 weeks, with approximately 7 to 8 g/dL, in contrast with hemoglobin levels of 10 to 11 g/dL at the nadir of those born in term, which generally occurs at 10 to 12 weeks of life. The hemoglobin levels are gestational age dependent, thus, the more premature neonates, and the low-birth-weight ones, usually present deeper degrees of anemia [11].

The diminished production of EPO in response to anemia in premature infants is believed to exert a key role in the pathophysiology of anemia of prematurity. This is partly explained by the fact that the liver is responsible for EPO production in intrauterine life. The switch to kidneys as the main source of EPO does not occur right after birth as in the term infants. Thus, because the development of the

preterm infant is not properly completed, after birth the liver continues to be the principal site of EPO production, which is known to be less responsive to hypoxia than the kidneys. An accelerated metabolism by clearance of EPO, which is from two to fourfold in preterm infants in comparison to adults, is also responsible for the reduced levels observed in preterm infants. Therefore, the reduced EPO levels in preterm infants are a result of both diminished production and increased metabolism [12].

The iatrogenic blood loss due to phlebotomy for laboratory testing in the intensive care units is described by many authors as the main responsible for anemia of prematurity [13–15]. The practice of intensive care neonatology requires serial laboratory testing such as blood gases, electrolytes, hemocultures, hemogram. Because small premature infants usually need close monitoring, they suffer the loss of great amounts of blood.

In neonates, the total blood volume varies from 80 to 115 mL/kg, thus, a 10 mL blood sample can correspond to more than 10% of the total blood volume of a premature infant [2]. The first weeks of intensive care usually are the period when the greater amounts of blood are withdrawn [16]. In the NICUs, approximately 15 to 30% of the total blood volume per week are required for laboratory tests. For extremely preterm infants, the iatrogenic blood loss is even greater, representing up to one-third of the total blood volume in the first month of life [13]. Also, there is a so-called "hidden" blood loss due to the sampling for laboratory tests, present in cotton pads, gauze and the dead space of syringes, or tubing of butterfly needles [17].

There is a significant correlation between the amounts of blood collected for laboratory testing and the volume of blood transfused in NICU patients, where the amount of blood transfused can be equal to or greater than the blood drawn, reinforcing the evidence that the iatrogenic blood loss is a major contributor to anemia in premature infants [15].

Besides the iatrogenic blood loss, other non-physiologic conditions can aggravate the development of anemia in the neonatal period, such as blood loss, hemolysis, infections, nutrient deficiencies and infection.

<table>
<tr><th colspan="3">Non-physiological causes of anemia in the neonatal period</th></tr>
<tr><th>Blood loss</th><th>Decreased erythrocyte production</th><th>Increased erythrocyte destruction</th></tr>
<tr><td>• Fetomaternal hemorrhage</td><td rowspan="3">• Infections:<br>Parvovirus B-19<br>Cytomegalovirus<br>Syphilis<br>Toxoplasmosis</td><td>• Immune-mediated hemolysis</td></tr>
<tr><td>• Twin-twin transfusion</td><td rowspan="2">• Red cell membrane deffects:<br>Hereditary Spherocytosis<br>Hereditary elliptocytosis<br>Hereditary pyropoikilocytosis</td></tr>
<tr><td>Fetoplacental hemorrhage</td><td rowspan="2">Nutritional deficiencies:<br>Iron<br>Vitamin B12<br>Folate<br>Copper<br>Vitamins : A, C, E.</td></tr>
<tr><td>• Cord and placental malformations</td><td>• Red cell enzymatic deficiencies:<br>G6PD deficiency<br>Piruvate kinase deficiency</td></tr>
<tr><td>• Internal hemorrhage: head, brains, lungs, liver</td><td rowspan="2">Congenital disorders of erythrocyte production:<br>Diamond-BlackFan Anemia<br>Fanconi Anemia</td><td rowspan="2">Hemoglobinopathies:<br>α and β Thalassemia</td></tr>
<tr><td>• Iatrogenic blood loss</td></tr>
</table>

**Figure 2.**
*Non-physiologic causes of neonatal anemia.*

In the neonatal period, the causes of anemia are classified into 3 groups: blood loss, decreased erythrocyte production and increased erythrocyte destruction. The causes of neonatal anemia are resumed in **Figure 2**.

## 5. Blood loss

Blood loss in the neonates can occur before, during or after the delivery, by several mechanisms, associated with 5–10% of the cases of severe neonatal anemia [18].

During pregnancy, the passage of fetal blood cells to maternal circulation, which is called fetomaternal hemorrhage, occurs in 50% of all pregnancies without promoting any harm to the mother or the fetus. However, in about 1 to 10% of pregnancies, fetomaternal hemorrhage can be substantial, when the blood loss reaches 40 to 100 mL. The fragile separation between the maternal and fetal circulation is described as the main mechanism behind fetomaternal hemorrhage before delivery. Fetomaternal hemorrhage also occurs during delivery, as a consequence of trauma and erosion to placental villi during labour. Other causes of fetomaternal hemorrhage include diagnostic amniocentesis, percutaneous umbilical blood sampling, and traumatic injury to the mother during pregnancy [11, 19].

Another cause of fetal hemorrhage before delivery is the twin-twin transfusion, which occurs in approximately one-third of all monozygotic, monochorionic twin pregnancies, with a mortality rate of approximately 15%. Twin-twin transfusion is responsible for considerable discrepancies of hemoglobin levels between the twins, usually greater than 5 g/dL, leading to simultaneous anemia and polycythemia. The donor twin usually presents low birth weight and may manifest congestive heart failure and shock. The receptor twin is at risk of hyperviscosity syndrome, disseminated intravascular coagulation, hyperbilirubinemia and respiratory distress [11, 20, 21].

In the intrapartum period, fetoplacental hemorrhage can occur, commonly when the infant is held above the placenta, as in cesarean section, before the cord clamping [11].

Cord malformations, such as velamentous insertion are associated with an increased risk of rupture of the blood cord vessels during labour, leading to significant intrapartum hemorrhage. Placental abnormalities such as placenta previa and abruptio placentae can also result in significant maternal and fetal blood loss during delivery [17, 18, 20].

Internal hemorrhage in the newborn can be caused by trauma during birth, bleeding disorders, vitamin K deficiency, and congenital vascular malformations. The most common sites of internal hemorrhage in the neonate are the head, brain, lungs, liver, and less frequently spleen, and adrenals. Traumatic deliveries are frequently associated with subdural/subarachnoid hemorrhage and cephalhematoma [11, 18, 22, 23].

## 6. Hemolysis

An important contributor to neonatal anemia is the increased erythrocyte destruction by hemolysis, which is associated with congenital and acquired conditions. In the neonatal period, hemolytic disorders can be classified as immune-mediated hemolysis, red cell membrane defects, red cell enzymatic deficiencies and hemoglobinopathies.

### 6.1 Immune mediated-hemolysis

Immune-mediate hemolysis occurs when fetal erythrocytes enter the maternal circulation, physiologically, by fetomaternal hemorrhage or amniocentesis. Surface antigens present in fetal red blood cells stimulates the maternal immune system to produce antibodies against them. Those antibodies are usually IgG, which can cross the placental barrier and enter the fetal circulation, marking the fetal erythrocytes for removal by the reticuloendothelial system [18]. The majority of cases of immune-mediated hemolysis in the neonatal period are result of ABO and Rh incompatibilities, where the Rh system is associated with more severe anemia. Other blood group systems like Kell and Duffy can also result in clinically relevant hemolysis [24].

### 6.2 Red cell membrane defects

Quantitative or qualitative disorders in red blood cell membrane proteins can impair erythrocyte deformability and stability, therefore leading to hemolysis.

Hereditary spherocytosis (HS) is the most common hemolytic anemia due to red blood cell membrane defect, which is usually transmitted as an autosomal dominant trait. The central event in HS is the loss of membrane surface area, leading to reduced deformability, due to defects of membrane proteins ankyrin and spectrin, band 3 or protein 4.2. Abnormal spherocytes with impaired deformability are frequently trapped and destroyed in the spleen leading to hemolysis and thus to anemia and hyperbilirubinemia. In the neonatal period, hemolysis can be enhanced because hemoglobin F poorly binds 2,3 diphosphoglycerate, which remains in the free form, contributing to the destabilization of protein interactions in the erythrocyte membrane. Anemia is the most frequent complication of HS in the neonatal period. Other consequences include cholelithiasis, hemolytic episodes, and aplastic crises [10, 18, 25, 26].

Hereditary elliptocytosis (HE) is a group of diseases that are characterized by the presence of elliptical erythrocytes in the peripheral blood, the main defects in this disease concern the alpha and beta spectrin, protein 4.1, band 3 and rarely glycophorin A. Although is more frequent than HS in the general population, HE is less likely to cause significant hemolysis and anemia. HE is usually asymptomatic until 4–6 months of life, although neonatal hemolytic anemia, jaundice and fetal hydrops are also described [10, 27, 28]. Hereditary pyropoikilocytosis is a subtype of HE, where the erythrocyte morphology resembles those seen in the blood smear of patients after severe burns. It is more frequent in individuals of African descendance and causes severe anemia and hemolysis in the neonatal period [9].

### 6.3 Red cell enzymatic deficiencies

Defects in red blood cells enzymes production can induce damage and decrease the lifespan of erythrocytes. The most common is Glucose-6-pyruvate-kinase (G6PD) deficiency, a hereditary X linked recessive disorder that affects over 400 million people in the world. G6PD catalyze the first reaction in the pentose phosphate pathway, responsible for NADPH production, which is essential to maintain adequate levels of reduced glutathione for protecting the erythrocyte from oxidative stress. G6PD deficient erythrocytes are vulnerable to oxidative damage and hemolysis In the neonatal period, G6pD deficiency can result in significant hemolytic anemia and hyperbilirubinemia that may require phototherapy and exchange transfusion [9, 10, 29, 30].

The second enzymatic deficiency associated with neonatal hemolytic anemia is for pyruvate kinase (PK), an enzyme from the glycolytic pathway of the erythrocyte, which is responsible for the generation of adenosine triphosphate (ATP). Therefore, PK deficiency reduces the amount of energy available for the erythrocytes, which cannot maintain their content of potassium and water, resulting in subsequent premature cell death. Most newborns with PK deficiency develop severe jaundice, hemolytic anemia and less frequently, liver dysfunction [10, 18, 31].

### 6.4 Hemoglobinopathies

Erythrocyte hemoglobin defects are also responsible for clinically relevant cases of neonatal anemia. Thalassemia is a group of genetic disorders of globin-chain production, that results in an imbalance between the α-globin and β-globin chain production. The α-thalassemia syndromes result from the deletion of one or more genes of the four α-globin genes, and its clinical manifestation will depend on the number of genes deleted. A single α-globin gene deletion results in a carrier state which is asymptomatic. Deletion of two genes results in α-thalassemia trait, which usually presents as microcytosis and mild anemia. Hemoglobin H disease results from the deletion of 3 α-globin genes. This leads to a significant imbalance between α and β chain production and the formation of hemoglobin H (β4) and hemoglobin Barts (γ4). Patients with hemoglobin H disease are often born with hypochromic, hemolytic anemia, and are at risk for significant neonatal hyperbilirubinemia. The deletion of all 4 α-globin genes results in homozygous α-thalassemia, with a high risk of intrauterine death, severe hemolytic anemia and hydrops fetalis [9, 10, 32].

Hemoglobin H is unstable and has an extremely high oxygen affinity and is thus unable to effectively deliver oxygen. It is also relatively unstable and causes ineffective erythropoiesis and hemolytic anemia, due to membrane injury from oxidative damage resulting in shortened red cell survival [33].

The β-globin chain defects, including sickle cell disease and β-thalassemia, are not usually associated with anemia or hemolysis during the neonatal period, because the erythrocytes of newborn infants contain large amounts of fetal hemoglobin (α2γ2) [9].

## 7. Decreased erythrocyte production

### 7.1 Infections

Congenital infections are an important cause of neonatal anemia by decreased erythrocyte production. Parvovirus B19, a DNA virus with an affinity for erythroid progenitor cells can be vertically transmitted to the fetus during pregnancy in susceptible women, who represent approximately 35% of all pregnancies, leading to severe anemia, miscarriage, non-immune hydrops and stillbirth, although the majority of the cases is asymptomatic [20, 22].

Other congenital infections associated with anemia in the neonatal period are cytomegalovirus, syphilis and toxoplasmosis [34–36].

### 7.2 Nutritional deficiencies

Although iron deficiency is not a common issue in the neonatal period for full-term infants, as the neonatal iron stores are obtained from maternal blood at the last weeks of the third trimester, the preterm neonates have lower iron stores. In consequence, these individuals are more prone to iron deficiency anemia in the

postnatal period, concomitantly to physiologic anemia [37]. Also, low birth-weight infants and those with perinatal blood loss are at risk of developing iron deficiency anemia. Other nutritional deficiencies that can imply the development of anemia in the newborn include folate, copper, vitamin B12, vitamin B6, vitamin A, vitamin C and vitamin E [18, 38].

### 7.3 Congenital disorders of erythrocyte production

Diamond-Blackfan anemia (DFA) is a rare congenital syndrome that affects mostly the erythroid precursor in the bone marrow. DFA is characterized by severe anemia in infants, that is usually macrocytic or normocytic, with reticulocytopenia. Approximately 25% of patients are anemic at birth, and hydrops fetalis occurs rarely. Associated congenital abnormalities include triphalangeal or duplicated digits, abnormal facies, genitourinary, musculoskeletal, cardiac and ophthalmological abnormalities. Various mutations in genes encoding ribosomal proteins are responsible for the pathogenesis of DFA [3, 39].

Fanconi's anemia (FA) is a rare autosomal recessive disorder associated with bone marrow failure and increased susceptibility to leukemia and other types of cancer. Patients with FA usually present congenital malformations and have high sensitivity to alkylating agents and radiation. Various mutations are associated with FA, and all FA genes code for proteins that play roles in various cellular pathways, especially in DNA cross linking and repair. In the neonatal period, FA patients may present with cytopenias, congenital malformations, or both [9, 40].

## 8. Diagnosis of anemia

The diagnosis of anemia in the newborn can be challenging, especially for those in intensive care. However, the accurate diagnosis and the determination of the underlining cause of the anemia is essential for adequate clinical management.

### 8.1 Clinical diagnosis

The diagnostic approach usually includes the patient history (familial, maternal, patient), physical examination and laboratory tests.

The patient history is based on familial and maternal medical history, pregnancy, delivery and postpartum period.

Familial history must be checked for the presence of any relatives with history of chronic anemia because genetic causes for neonatal anemia could be present such as Diamond-Blackfan and Fanconi anemia, enzymatic deficiencies and erythrocyte membrane defects.

Maternal medical history concerns the before pregnancy, pregnancy and postpartum. Bleeding disorders, history of chronic anemia, infections, medication use, trauma or vaginal bleeding, must be included on maternal medical history. Furthermore, maternal laboratory tests such as blood type, screening for antibodies and hemogram can provide useful information for the diagnostic approach of the anemia in the newborn.

As the twin-twin hemorrhage is a substantial cause of neonatal anemia due to blood loss, the type of pregnancy (multiple or singleton) is also relevant for diagnosis [11].

The method of delivery and the presence of intrapartum intercurrences like maternal hemorrhage or newborn distress should also be considered.

The patient history contains information concerning age, gestational age at the delivery, sex, birthweight, history of infections, ventilatory support, medication and transfusions.

Physical examination of the anemic newborn must verify diminished activity, feeding difficulties and dyspnea at rest. Skin examination is necessary for check the presence of pallor, which is present generally in various types of anemia, and jaundice, which is associated with hemolytic anemias. Additionally, hepatosplenomegaly presence is an important finding in physical examination, which is also an indicative of hemolytic anemia [22].

## 9. Laboratory diagnosis

For the laboratory diagnosis of anemia, a stepwise diagnostic approach is recommended to avoid unnecessary tests. This approach is benefic for preventing iatrogenic blood loss of the newborn and other consequences of repeated blood withdrawals, and also reduces the costs for diagnosis. The laboratory tests for the diagnosis of neonatal anemia are presented in **Figure 3**.

The first step on the laboratory diagnosis is the establishment of the anemia, which concerns an adequate interpretation of a complete blood count considering proper reference values for the newborn infant age. Once there is a diagnosis of anemia, the second step is to determine the cause, which can be challenging, especially for preterm infants under intensive care. There are numerous laboratory tests that can be helpful to establish the cause of anemia. However, its choice will depend on the clinical examination, and also on the availability of tests where the NICU is located.

Reticulocyte count will provide useful information concerning the bone marrow capacity to produce new erythrocytes. Automated reticulocyte counts are more accurate than manual techniques because of the standardization of cell size and DNA content, which enables the identification of the number of reticulocytes per microliter of blood [41].

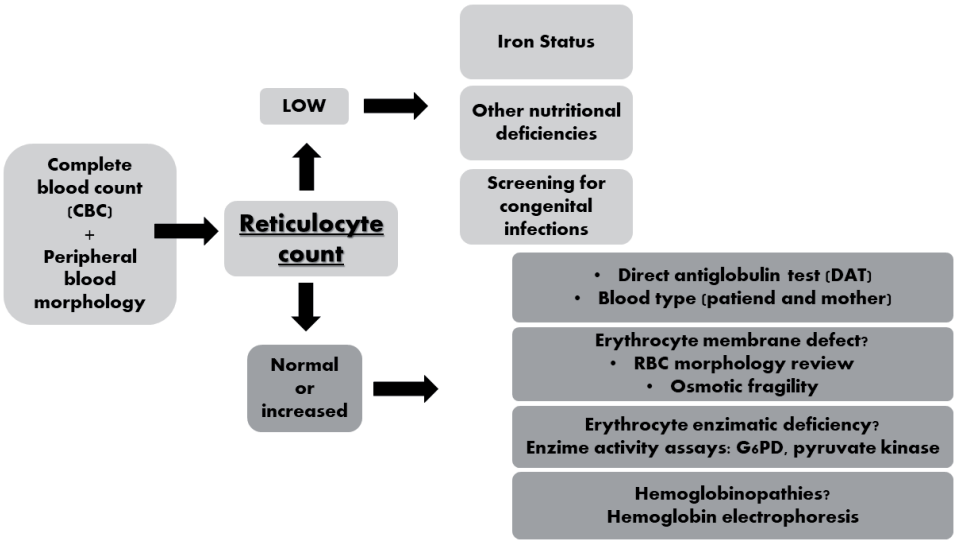


**Figure 3.**
*Laboratory tests for diagnosis of neonatal anemia.*

During the first two weeks of life, the reticulocyte count decreases gradually. In infants with anemia, however, the bone marrow will attempt to compensate through increased erythropoietic activity, characterized by an elevated reticulocyte count. If the erythropoiesis in the bone marrow production is impaired, the reticulocyte count will remain low. In the presence of anemia with low reticulocyte counts, a diagnosis of bone marrow suppression or dysfunction should be considered. The next steps may comprehend the nutritional deficiencies with a complete iron status and vitamin and other elements if necessary [18].

Besides the reticulocyte count, the automated hematologic analyzers have useful parameters for the assessment of iron status, like the reticulocyte hemoglobin content (CHr) and immature reticulocyte fraction (IRF), which reflects the actual availability of iron for erythropoiesis. These parameters are helpful in the assessment and early detection of anemia with no additional costs or need for more blood sampling [42, 43].

To identify iron deficiency anemia, the hemoglobin concentration must be confirmed by other measurements of iron status. Iron status markers include: hemoglobin concentration, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), Chr, total iron-binding capacity, serum ferritin (SF) and transferrin saturation.

As SF is an acute-phase protein, its concentrations may be elevated in the presence of chronic inflammation, infection, malignancy, or liver disease. Because Chr is not affected by these factors or anemia of chronic disease, it is a preferable marker of iron status. It provides a measure of iron available to cells recently released from the bone marrow [41, 44].

Serology for infectious congenital diseases associated with decreased erythrocyte production such as parvovirus B19, cytomegalovirus, syphilis and toxoplasmosis should also be performed. Bone marrow aspirate/biopsy should be performed in the cases where de peripheral blood tests were not enough to establish a proper diagnosis of the cause of anemia. The congenital disorders of erythrocyte production are also characterized by anemia with low reticulocyte counts. If there is a clinical suspicion of genetic disorders such Fanconi anemia and Diamond-Blackfan, specific molecular tests must be required [39, 40].

In the anemic patient with a normal or elevated reticulocyte count, the next diagnostic steps should be focused on the hemolytic anemias.

Evaluation of the hemolytic anemia in newborn infants should include blood typing of the mother and the infant and direct antiglobulin testing (DAT), to detect immune-mediated hemolysis [29].

Examination of the peripheral blood smear is also essential for a proper diagnosis of anemia. Neonatal erythrocytes show considerable heterogeneity, with a greater number of irregularly shaped RBCs, particularly in premature infants, which difficult the morphological analysis. Nucleated RBCs are present in the blood of healthy newborns on the first day of life. Finding 0 to 10 nucleated RBCs per 100 WBCs is typical. In term infants, nucleated RBCs are rapidly cleared from the circulation after birth. However, they persist in peripheral blood in small numbers for up to 1 week of life in preterm infants [2]. Increased counts are often the result of prematurity, anemia of different etiologies, increased erythropoiesis from chronic conditions, acute stress-mediated release from the marrow stores, and postnatal hypoxia [45].

With significant hemolysis, fragmented red blood cells (schistocytes) are usually observed. Additionally, close attention should be paid to the presence of spherocytes or elliptocytes. Hereditary spherocytosis and elliptocytosis, the most commonly inherited membrane defects, can present as hemolytic anemia [25–27]. If the presence of hereditary spherocytosis is suspected, special attention should

be paid to the mean corpuscular hemoglobin content (CHCM), which is usually increased (CHCM >36 g/dL) [28].

If there is clinical suspicion of an erythrocyte membrane defect, the osmotic fragility test will be helpful to confirm the diagnosis. Glycerol lysis and Pink tests are also used as first-line laboratory tests. Other more specialized tests for erythrocyte membrane defects include: flow cytometry, ektacytometry and SDS-PAGE of erythrocyte membrane proteins [28, 29, 46].

Observation of erythrocyte morphology can be useful in cases of erythrocyte enzymatic deficiencies. In the peripheral blood of individuals with G6PD deficiency, bite cells and blister cells are usually seen, as a consequence of oxidative damage to the erythrocyte membrane [30]. The definitive diagnosis of G6PD deficiency will be determined with quantitative tests of enzyme activity. However, falsely negative tests in severe cases of enzymatic deficiency, where great amounts of erythrocytes are removed from the circulation by hemolysis. In those cases, if the clinical suspicion remains, the test for enzymatic activity must be repeated after 3 months [31].

As the hematologic features of pyruvate kinase deficiency are not different from other hemolytic anemias, the definitive diagnosis is obtained with direct quantitative tests for enzyme activity. If there is a clinical suspicion of an erythrocyte enzyme defect, both G6PD and PK tests should be performed [31].

Other laboratory tests that are usually helpful for determining the presence of hemolytic anemia are: increased indirect bilirubinemia, increased LDH and decreased haptoglobin [46].

Thalassemias are usually characterized by low mean corpuscular volume (MCV) and low mean corpuscular hemoglobin (MCH). It is also necessary to exclude iron deficiency anemia by performing a complete iron profile. Morphological features in the peripheral blood are usually helpful to sustain the suspect of thalassemia. Anisopoikilocytosis (variation in shape and size of the erythrocytes) with the presence of target cells are the most typical changes. Tear-drop cells and erythroblasts are usually seen in the most severe forms. Basophilic stippling and polychromasia are also frequent. For the investigation of thalassemia, hemoglobin electrophoresis and hemoglobin HPLC are used as the main screening tests. Confirmatory tests, if necessary, are made by DNA analysis for α and β-globin mutations [47].

## 10. Issues related to preanalytical condition in laboratory diagnosis

The quality of laboratory test results is affected by preanalytical variables such as sample collection, specimen handling, sample size, and analytic interference. The most important precatalytic factors that influence the laboratory results of neonates and infants are the limited blood availability, the variation of results depending on blood sampling sites, and the effect of vigorous crying or exertion on hematologic test results [2].

The total blood volume depends on the height and weight of the individual and, therefore, is markedly low in infants, especially in premature newborns. Repeatedly blood withdrawal is associated with iatrogenic anemia, thus, this practice must be avoided as much as possible [16].

As the blood collection tubes contain a fixed amount of anticoagulant, collecting an amount of blood which is smaller than the recommended by the manufacturer may result in hemodilution or clotting and cause erroneous results. In order to avoid such problems, pediatric collection tubes for reduced volumes can be used. However, as most of pediatric tubes does not follow standard dimensions, its use brings challenges to clinical laboratories, including the need for manual handling,

because the hematology analyzers are not usually adapted to process those tubes automatically. In addition, blood clotting, hemolysis, and insufficient sample volume are common issues when dealing with neonatal specimens [2, 48].

Hemolysis is the most frequent preanalytical issue, especially in neonatal patients [49] The main cause of hemolysis in those patients is the site of collection, where de arterial catheter collections are associated with greater numbers of hemolyzed samples [48].

The blood sampling site is also a relevant factor that influences the complete blood count results. Significant differences between capillary, venous and arterial sites have been reported. Thus, it is useful to concern the sample of collection for proper interpretation of the results [50, 51].

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Chapter 4

# Prolonged Jaundice in Newborn

*Erhan Aygün and Seda Yilmaz Semerci*

## Abstract

Prolonged jaundice is defined as a serum bilirubin level higher than 85 μmol/L (5 mg/dl), which persists at postnatal 14 days in term infants and 21 days following the birth in preterm infants. It affects 2–15% of all newborns and 40% of breastfed infants. Although underlying cause can not be found in the majority of prolonged jaundice cases, this may also be the first sign of a serious causative pathology. Tests performed to determine the underlying cause and failure to determine the etiology cause anxiety for both families and physicians. The most important point is to determine whether prolonged jaundice is of a benign cause or is due to a substantial disease. For this reason, health care providers should not take unnecessary tests in normal infants, but should also recognize infants with a causative pathology. Neonatal jaundice still maintains its importance in neonatal clinical practice, since early diagnosis and treatment is feasible.

**Keywords:** Hyperbilirubinemia, prolonged jaundice, newborn

## 1. Introduction

Prolonged jaundice is defined as a serum bilirubin level higher than 85 μmol/L (5 mg/dl), which persists at postnatal 14 days in term infants and 21 days following the birth in preterm infants. It affects 2–15% of all newborns and 40% of breastfed infants [1].

Although underlying cause can not be found in the majority of prolonged jaundice cases, this may also be the first sign of a serious causative pathology [2]. The most important point is to determine whether prolonged jaundice is of a benign cause or is due to a substantial disease. For this reason, health care providers should not take unnecessary tests in normal infants, but should also recognize infants with a causative pathology. The timing of initial investigations should be between two and four weeks in order to reduce the associated mortality and morbidity. Neonatal jaundice still maintains its importance in neonatal clinical practice, since early diagnosis and treatment is feasible [3–6].

Although jaundice in early infancy is predominantly caused by indirect hyperbilirubinemia, it can also be seen as direct hyperbilirubinemia. Distinguishing between these types of jaundice is crucial in determining the etiology of prolonged jaundice.

To date, the most common cause of prolonged jaundice of indirect hyperbilirubinemia has been identified as breastmilk jaundice. It is known that breastmilk jaundice is seen at a rate of 1.3% in newborn infants and 2.4–25% in infants fed with breastmilk [7–9]. Besides, breastmilk jaundice may extend up to the twelfth week of life [10]. However, the diagnosis of breast milk jaundice is made by excluding other causes. Other possible causes of indirect hyperbilirubinemia include dehydration,

hemolysis, infection, congenital hypothyroidism, inborn diseases of metabolism, and pyloric stenosis [2, 11]. Delayed diagnosis of these causes may increase morbidity.

Despite the jaundiced appearance, newborns with breast milk jaundice appear healthy, grow up in a normal way, and have no signs of an underlying disease. However, the presence of abnormalities in laboratory tests consistent with hepatobiliary dysfunction, such as elevation of aminotransferases and/or γ-glutamyl transpeptidase (GGT), warrants investigating pathological causes [12].

Direct hyperbilirubinemia is defined as serum direct bilirubin >20 μmol/L (>1.2 mg/dl) or direct bilirubin >20% of total bilirubin. Although rare, it usually indicates an underlying pathological cause and requires immediate investigation and prompt intervention. Direct hyperbilirubinemia (cholestatic jaundice) is never physiological. It affects 1/2500 live births and should be suspected in all jaundiced infants with light coloured stools and/or dark urine [4]. Delayed presentation of cholestatic newborns is still an important issue. For the early diagnosis of cholestasis, it is recommended to measure the serum total bilirubin (STB) and direct bilirubin levels of each newborn with prolonged jaundice [13].

Intrahepatic or extrahepatic biliary obstructions, viral infections, inborn diseases of meatabolism and endocrine disorders can lead to hyperbilirubinemia [13]. Extrahepatic biliary atresia is rare. It has been reported to occur at a rate of 1 per 21,000 live births in the UK and 9 per 14,000 live births in New Zealand [14]. Biliary atresia is the most common cause of neonatal cholestasis, and affected infants may appear healthy for a considerable time [15]. Kasai hepatoportoenterostomy should be performed in the first 45 days of life to restore bile flow and reduce further damage to the liver [16]. Early diagnosis of biliary atresia is the most important predictive factor in operated newborns [17].

Various conditions such as intrauterine infections, bacterial sepsis, galactosemia, aminoacidemias, and congenital hypopituitarism can occur with a mixture of increased direct and indirect bilirubin [18].

When severe jaundice goes untreated for too long, it can cause a condition called kernicterus. Kernicterus, or bilirubin encephalopathy, is bilirubin-induced neurologic damage, typically in infants. It can cause athetoid cerebral palsy and hearing loss. Kernicterus also causes problems with vision and teeth and sometimes can cause intellectual disabilities. Early detection and management of jaundice can prevent kernicterus.

## 2. Diagnosis

A global protocol for investigating prolonged jaundice is not defined yet. The incidence of conditions that play a role in prolonged jaundice in terms of etiology varies between countries. This difference is the main reason for the various protocols. The cost of the examinations in cases with prolonged jaundice and the differences in healthcare systems between countries also contribute to this situation. The fact that prolonged jaundice can persist up to 3 months in most of the breastfed newborns and that prolonged jaundice in some newborns can be caused by serious underlying pathologies, even which may lead to liver transplantation, makes the current situation more difficult [19, 20].

The cause of prolonged jaundice seen in 15–40% of newborns is breastfeeding [21]. Breast milk jaundice is highly prevalent among the etiologies of prolonged jaundice. However, the diagnosis of breast milk jaundice is a diagnosis of exclusion. In neonatal prolonged jaundice, at the first step, direct hyperbilirubinemia and indirect hyperbilirubinemia should be differentiated, promptly. This requires

getting a detailed history of the patient and a meticulous physical examination. Especially in the history, stool color and urine color should be questioned. Whether the urine and stool color is normal, the following initial tests should be performed.

### 2.1 Initial tests in patients with prolonged jaundice

| | |
|---|---|
| 1. Direct and indirect bilirubin level and liver function tests, in case of direct bilirubin level is increased | 4. Glucose 6 phosphate dehydrogenase (G6PD) enzyme level |
| 2. Complete blood count, peripheral blood smear | 5. TSH, fT4 |
| 3. Maternal blood group, infant blood group, Direct Coombs test | 6. Urinalysis, urine culture |

If there is no direct bilirubin elevation in the initial tests, these tests are sufficient for the follow-up and treatment of neonatal prolonged jaundice [22]. However, if increased direct bilirubin level is observed, the infant should be examined for cholestasis and specific tests to some cholestatic liver diseases should be done [23].

**These examinations are;**

| **Tests for cholestatic liver disease** | |
|---|---|
| 1. ALT, AST, ALP, GGT, PT, APTT | 6. Serology for TORCH-S infections |
| 2.Abdominal and hepatobiliary ultrasonography | 7. Reducing substance in the urine |
| 3. Urine organic acids and blood amino acids | 8. Alpha feto protein |
| 4. Serum bile acids | 9. Sweat test |
| 5. Alpha-1 antitrypsin level | |

## 3. Etiology

Albeit most of the causes of prolonged jaundice other than breast milk are rare, congenital hypothyroidism and direct hyperbilirubinemia, which require urgent diagnosis (recognition) and treatment, should also be excluded [6]. Determining whether jaundice is hemolytic is important in order to identify the initial approach [2]. The causes of indirect hyperbilirubinemia and direct hyperbilirubinemia regarding etiological investigation in neonatal prolonged jaundice are classified as follows:

**Causes of direct hyperbilirubinemia in neonatal prolonged jaundice** [24]:

| |
|---|
| • Biliary atresia |
| • Sepsis, |
| • TORCH-S infections |
| • Neonatal hepatitis syndromes, |
| • Choledochal cyst, |
| • Galactosemia |
| • Alpha-1 antitrypsin deficiency |
| • Hereditary bile acid synthesis disorders. |

**Causes of indirect hyperbilirubinemia in neonatal prolonged jaundice** [24]:

| |
|---|
| • Breast milk jaundice |
| • Sepsis |
| • Hemolytic diseases |
| • Congenital hypothyroidism |
| • Urinary tract infection |
| • Extravascular blood collection |
| • Pyloric stenosis |
| • Gilbert syndrome |
| • Crigler najjar syndrome |

## 3.1 Hemolytic diseases

The reason for isoimmunization in the mother and the formation of IgG antibodies is the passage of fetal erythrocytes into the maternal circulation. The basis of hemolytic disease in the newborn is the breakdown of fetal erythrocytes by antibodies passing through the placenta [25, 26].

Hemolytic diseases are classified in two groups as immune and nonimmune. Rh, ABO and minor blood group incompatibilities are in the immune group. In the nonimmune hemolytic group, erythrocyte enzyme defects, erythrocyte structural defects, infection, polycythemia and sequestration exist. Rh and ABO incompatibilities are the most common causes of immune hemolytic diseases [27, 28].

### *3.1.1 Rhesus incompatibility*

Rh incompatibility occurs in 30–35% of cases of prolonged jaundice which goes with hemolysis [29, 30]. Rh is a large protein with many antigenic sites. Common antigens are D, C, c, and E antigens. All antigens cause their specific antibody responses. In fact, 90% of this response is against the D antigen [31]. Therefore, a response to the D antigen is required for the diagnosis of Rh positivity [32]. Since the Rh antigen is only present on the erythrocyte membrane, severe hemolysis can be met in the event of Rh compability [33]. Rh system genes are located in the short arm of chromosome 1. The presence of the Rh gene can be learned in the first trimester by polymerase chain reaction (PCR) method via amniocentesis. Alloimmunization occurs when as little as 0.1 ml of blood from the Rh (D) positive fetus passes through the placenta into the circulation of the Rh negative mother [28, 30, 34]. The level of antibodies developed in the mother determines the degree of hemolytic disease. Hemolysis is caused by IgG type antibodies. The level of these antibodies indicates that the mother is sensitized.

In order to prevent the risk of Rh hemolytic disease and maternal sensitization, Rh (−) women whose spouses are Rh (+) should be routinely administered 300 μg anti-D immunoglobulin (Rhogam), whether the indirect Coombs test result is (−) at 28 weeks of pregnancy. This dose provides a prophylaxis for Rhesus disease in the vast majority of deliveries. The risk increases gradually in pregnancies after maternal sensitization [35]. The increase in serum bilirubin level during follow-up determines the severity of the hemolytic event [36].

Prolonged jaundice can be seen in Rh incompatibility. Rh incompatibility should not be forgotten in patients with prolonged jaundice which goes with hemolysis.

### *3.1.2 ABO incompatibility*

The most common blood group incompatibility is the ABO group incompatibility. It does not cause serious problems generally, although it is the most common reason. Unlike Rh incompatibility, the mother does not need to be sensitized beforehand in this entity [37]. Hemolytic disease due to ABO incompatibility is seen in cases where the mother's blood type is O and the infant has A or B blood type. Although ABO incompatibility is seen in 20–25% of pregnancies, fetal sensitization findings (direct Coombs positivity) vary between 3 and 4% [38, 39].

Unlike Rh incompatibility, most of the maternal antibodies for ABO incompatibility are in the IgM form. Therefore, those antibodies do not have the ability of crossing the placenta. In addition, A and B antigens are not only found on erythrocytes, they are also found on different tissue cells. Consequently, not all antibodies passed to the fetus are retained by erythrocytes, but by other tissue cell antigens as well. These situations described, are the main reasons why ABO incompatibility does not cause serious problems compared to Rh incompatibility [40].

The clinical course resulting from hemolysis due to ABO incompatibility is generally mild in many cases and jaundice is observed as the only clinical finding. Jaundice usually develops on the first day and is often kept under control with phototherapy [38]. However, severe hemolysis due to ABO incompatibility is considerably rare [41].

Since it is the most common condition in patients with prolonged jaundice with hemolysis, it should definitely be investigated.

### *3.1.3 Subgroup incompatibility*

Minor blood group incompatibility accounts for as low as 3% of neonatal hemolytic disease cases. Duffy, Kidd and MNS antigens are responsible for this hemolysis. The pathophysiology of hemolysis is similar to Rh and ABO incompatibilities. Minor blood group incompatibilities can usually cause subclinical hemolysis as well as possible severe hemolysis and worsen pictures [30, 34].

Since it usually goes with subclinical hemolysis, it may cause milder manifestations such as prolonged jaundice [42].

### *3.1.4 Erythrocyte enzyme deficiencies*

The most common enzyme defects are; Glucose 6 phosphate dehydrogenase deficiency is a deficiency of 5′ nucleotidase and pyruvate kinase.

### *3.1.5 Glucose 6 phosphate dehydrogenase deficiency (G6PD)*

The most common enzyme deficiency is glucose 6 phosphate dehydrogenase deficiency. The G6PD enzyme acts as a catalyst and helps to reduce oxidative products in erythrocytes.

Due to the X-linked recessive inheritance, it is more common in male. Numerous mutations of the G6PD gene have been detected on the X chromosome. Because of having so many variants of the enzyme, hemolysis caused by G6PD can be present in different scenarios [43, 44]. In infants with G6PD in the neonatal period, hemolysis develops in case of exposure to oxidant stress or infection, and jaundice occurs as a result. Jaundice usually develops within 24–72 hours of life. In newborns with G6PD enzyme deficiency, bilirubin conjugation is much lower than in infants with normal G6PD enzyme. In fact, there are newborns with kernicterus caused by G6PD deficiency in the literatüre [45, 46]. The rate of G6PD deficiency in the

etiology of prolonged jaundice varies according to populations. Various studies reported the rate of G6PD deficiency between 3.8% and 24% [47, 48].

Since prolonged jaundice may be the first sign of G6PD deficiency, the enzyme level should be checked in newborns diagnosed with prolonged jaundice.

*3.1.6 Pyruvate kinase enzyme deficiency*

It is inherited autosomal recessively. It is less common than G6PD deficiency. Unlike G6PD deficiency, signs of hyperbilirubinemia, anemia and reticulocytosis are present from the very beginning. Pyruvate kinase enzyme deficiency should be considered in infants with negative Coombs test and hemolytic anemia, in case of prolonged jaundice without erythrocyte membrane defect [49].

*3.1.7 Erythrocyte membrane defects*

Hereditary spherocytosis, eliptocytosis, stomatocytosis and infantile pycnocytosis, which are erythrocyte membrane defects, can elicit hemolysis in the neonatal period. Hereditary spherocytosis is common in this group, while hereditary elliptocytosis and stomatocytosis are the other rare causes of hemolysis in newborn infants.

*3.1.7.1 Hereditary spherocytosis*

It has an autosomal dominant inheritance. Transforming spherocytic erythrocytes, which are more fragile than normal ones, under osmotic stress, is the characteristic of hereditary spherocytosis. The diagnosis of hereditary spherocytosis is made by detecting spherocytes in the peripheral smear and by osmotic fragility test. ABO incompability can be confused with hemolytic disease. Because microspherocytes can also be seen in the peripheral blood smear in ABO hemolytic disease. The distinction between these two diseases is made with the direct Coombs test [49].

Kocabay et al. found the rate of hereditary spherocytosis to be 0.1% in neonatal jaundice cases. Hyperbilirubinemia occurs in approximately half of newborn infants with hereditary spherocytosis, but this jaundice is usually considered as physiological, and may be overlooked. It can also be a reason for prolonged jaundice in a minority of the newborn infants [50].

**3.2 Bilirubin uptake and conjugation disorders of the liver**

*3.2.1 Gilbert's syndrome*

In Gilbert's syndrome, both hepatocytes' bilirubin uptake is decreased and UDPGT activity is decreased. It is inherited in autosomal dominant or autosomal recessive. It has a prevalence of 2–6%. Although it can cause neonatal hyperbilirubinemia, the diagnosis is usually made at a later stage [25, 50]. It is thought that hyperbilirubinemia, which is observed in newborns with weight loss after insufficient caloric intake, also has a similar etiologic mechanism to Gilbert's syndrome. Phenobarbital can be used as treatment in selected cases of Gilbert's syndrome [51].

Studies have shown that Gilbert syndrome either elicit neonatal prolonged jaundice.

*3.2.2 Crigler Najjar syndrome type 1*

Crigler-Najjar Syndrome type 1 is caused by the complete absence of the hepatic glucuronyl transferase enzyme and is inherited autosomal recessively. It is a chronic non-hemolytic indirect hyperbilirubinemia syndrome and has a severe clinical

course. In the homozygous form, severe indirect hyperbilirubinemia, which may progress to kernicterus, develops within the first three days of life, and bilirubin levels increase gradually, whether treatment is delayed. Diagnosis is made by percutaneous liver biopsy. UGT activity is measured in the biopsy sample/specimen. Phenobarbital is not an effective treatment of choice in Crigler-Najjar Syndrome type 1 syndrome [43, 52].

*3.2.3 Crigler Najjar Syndrome type 2*

Crigler Najjar Syndrome Type 2 is more common than Type 1. Besides, the clinical course is better. The reason for this is that the activity of the UDPGT enzyme is partially present in Type 2. It has an autosomal dominant inheritance. Although indirect bilirubin levels start to increase in the first days of life, they usually do not go above 20 mg/dl levels. Unlike type 1, Crigler-Najjar Syndrome responds to phenobarbital. Therefore, response to phenobarbital can be used as a distinguishing strategy for type 1 and type 2 disease [25, 53]. Crigler-Najjar syndrome is also an important reason for prolonged jaundice.

In Crigler-Najjar syndrome type II, UDPGT activity is reduced in the same way as is found in infants with prolonged jaundice due to Gilbert's syndrome [54]. Therefore, it is an etiology that should be kept in mind in newborns with prolonged jaundice.

*3.2.4 Lucey Driscoll Syndrome*

It is a rare disease of newborn, which goes with high bilirubin levels in the postnatal first two days of life. Bilirubin levels are above 20 mg/dl and may rise to levels that can require exchange transfusion. These high bilirubin levels can persist for longer than 14 days [40]. Most of these infants develop kernicterus, whether exchange transfusion is not performed.

### 3.3 Hypothyroidism

It is one of the substantial causes of neonatal prolonged jaundice. Prolonged jaundice is seen in approximately 10% of infants with congenital hypothyroidism. Decreased UGT activity is blamed for the pathophysiology of hyperbilirubinemia seen in congenital hypothyroidism. In this case, hyperbilirubinemia may persist for several months. Treatment with thyroid hormone leads rapid resolution of jaundice [55, 56].

### 3.4 Galactosemia

Galactosemia may present with hyperbilirubinemia in the neonatal period. In the clinical picture of the disease; there are findings such as vomiting, dehydration, hepatomegaly, splenomegaly. Diagnosis is made by detecting the reducing substance in urine, sugar chromatography and enzyme levels [30].

Galactosemia is one of the etiologies of prolonged conjugated hyperbilirubinemia as a hereditary and metabolic disease. In newborns with galactosemia, hyperbilirubinemia becomes evident in the first week of life and can proceed with prolonged jaundice in many patients [57].

### 3.5 Infections

In the neonatal period, infections can be accompanied by jaundice. Particularly, urinary tract infections, and sepsis are common causes of jaundice. Indirect hyperbilirubinemia can develop in sepsis due to hemolysis caused by endotoxins [44].

Direct and indirect hyperbilirubinemia is seen in congenital infections such as TORCH-S group infections [30].

The incidence of urinary tract infection in asymptomatic infants, under two months of age with jaundice, but without fever, has been shown to be 7.5%. Therefore, prolonged jaundice may be the unique finding in urinary tract infection [58]. In a study, the most common infection associated with jaundice in the neonatal period was found to be urinary tract infection [59].

### 3.6 Extravascular blood collection

Extravasation of blood in the body leads to increased bilirubin production by enhanced heme protein metabolism via destruction of erythrocytes. Accumulation of red blood cells in tissue layers surrounding the brain and skull (cephalo hematoma, subdural hematoma, subgaleal hematoma) or in any part of the body in traumatic deliveries can lead severe hyperbilirubinemia [60].

#### *3.6.1 Cephal hematoma*

Cephal hematoma defines a bleeding into the subperiosteal region and occurs in approximately 1–2% of all live births. Rupture of vessels results in a blood collection extending from the bone to the periosteum during labor [61]. Cephal hematoma can be causitive for jaundice due to the increase in bilirubin synthesis in the first 48–72 hours of life because of the destruction of erythrocytes in the extravasous blood collection [41].

## 4. Treatment of neonatal jaundice

The aim of the treatment is to reduce the increased bilirubin levels in order to prevent the damage to the central nervous system by the formation of kernicterus. Timely and prompt treatment is crucial to prevent the permanent effects of bilirubin toxicity such as kernicterus [62].

Whether the underlying cause of hyperbilirubinemia is known, treatment should be arranged according to that etiology. The most commonly used methods in the treatment of jaundice are: Phototherapy, intra venous immunoglobulin (IVIG) administration, exchange transfusion, and phenobarbital. Treatment indications vary according to the gestational week of the infant, postnatal age, bilirubin level, and the presence of hemolysis [63].

| **Treatment of Neonatal Jaundice** | |
|---|---|
| 1. Phototherapy | 2. Phenobarbital |
| 3. Intravenous Immunoglobulin | 4. Metalloporphyrins |
| 5. Exchange Transfusion | |

It is appropriate to use the curves designed by American Academy of Pediatrics, which evaluate either the gestational week, and risk factors for infants whose gestational age is greater than 35 weeks. Instead, the tables include the cut-off bilirubin levels according to birth weight, for the infants whose gestational age is less than 35 weeks [64].

In case of a rapid rise in bilirubin levels within the first 24 hours, newborn infants should be evaluated for hemolysis. The ETCOc measurement directly indicates heme

catabolism and bilirubin production [65]. The hourly rate of increase in bilirubin levels is also an important indicator for hemolysis. An increase of 0.2–0.5 mg/dl per hour in the bilirubin level is thought as an indicator of hemolysis. With a positive direct Coombs test (+), higher reticulocyte count, a decrease in hemoglobin and hematocrit levels are laboratory findings supporting hemolysis [66].

### 4.1 Phototherapy

Phototherapy is used to reduce increased serum bilirubin level and reach a normal bilirubin level, to reduce the need for exchange transfusion and to prevent the development of kernicterus. Phototherapy lowers bilirubin levels by using certain wavelengths of light energy [67]. Blue light at a wavelength of 440–460 nm is the value at which bilirubin is best absorbed. In order for phototherapy to be effective, the energy must be a maximum of 5u/cm/nm. In order to obtain this energy, ideally, 440–460 nm, which is the best absorbed wavelength, and blue light should be used. The distance of the light should be adjusted to be 30–40 cm away from the infant. It should be given at the ideal wavelength and distance of 40 uw/cm/nm [68, 69]. In the same way, multidirectional administration of one-way phototherapy, from different angles enhances the success of the treatment [70].

In phototherapy, treatment decision was determined by bilirubin level, rate of increase in bilirubin level over time, birth weight, gestational age and postnatal age, and the presence of risk factors such as Rh, ABO and minor blood group incompatibility, G6PD enzyme deficiency, presence of asphyxia, hypothermia, acidosis, sepsis, and lower levels of albumin. Phototherapy is started when the serum bilirubin rises to a level that pose a risk for neurotoxicity. Phototherapy should be started in bilirubin values that exceed the determined limits. Risk factors that should be considered when determining the phototherapy limit are: Rh, ABO and minor blood group incompatibility, G6PD, albumin levels below 3 g/dl, presence of asphyxia, pronounced tendency to sleep, sepsis, hypothermia, presence of acidosis in blood gas analysis. In order for a newborn to be considered risk-free, all risk factors must be excluded, otherwise the newborn is considered as under risk. Decision of phototherapy, depends on the TSB.

Phototherapy is considered as safe. But it can have some undesirable effects. These side effects can be listed as follows: Retinal degeneration without an eye protective cover, fluid loss, bronze baby syndrome, rash, lactose intolerance, hypocalcemia, an increased risk of PDA particularly in preterm infants [40, 68]. There are studies showing that phototherapy increases the risk of conjunctivitis, and it predisposes to asthma and allergic rhinitis in long term [71].

### 4.2 Exchange transfusion

Exchange transfusion is a successful but risky treatment method used in severe neonatal hyperbilirubinemia [72]. When TSB exceeds the cut-off level that is determined for exchange transfusion, the procedure should be performed without a delay.

Whether the expected decrease is not reached in TSB level despite intensive phototherapy or in case of a gradual increase, or when the risk of kernicterus is greater than the risk of exchange transfusion, or whether kernicterus has developed and signs are present, transfusion should be performed quickly [73].

When albumin levels are low, more care should be taken in terms of exchange transfusion. Because the amount of free bilirubin that cannot be bound to albumin will increase and there will be a high amount of free bilirubin which can increase the risk of kernicterus. The bilirubin/albumin ratio does not make a decision for exchange transfusion, but is used to support the treatment decision in conjunction

with TSB levels in newborn infants. Albumin infusion is not recommended in patients with either hyperbilirubinemia and hypoalbuminemia [66].

A central catheter is placed before the patient for blood exchange. Then, blood collection from the newborn and replacement of the patient's own blood with whole blood or erythrocyte suspension mixed with plasma are performed through this catheter. Approximately twice of the blood volume of the infant, which corresponds to 160–170 ml/kg, is exchanged by this way. The volume of blood taken or given once during the exchange transfusion process should not exceed 5 ml/kg. Besides, the volume rate of blood given or taken in the exchange process should not be more than 2 mL/kg/min. The reason for this is that if this rate is exceeded, sudden changes in intracranial pressure can occur due to blood pressure fluctuation [74]. The exchange transfusion process ensures that the majority of the infant's sensitized erythrocytes are replaced by unsensitized erythrocytes. This change provides a significant decrease in serum bilirubin level.

Serum electrolytes, bilirubin and blood glucose level should be checked at regular intervals during exchange transfusion. Because hypocalcemia and hypoglycemia may occur depending on the content of the blood product used during the procedure.

Exchange transfusion is not an innocent procedure and complications may befall. During the procedure, the newborn can have apnea, blood pressure imbalances can be met, the heart rate can slow down, electrolyte disturbances may be observed, disturbances in blood glucose level can be observed, thrombocytopenia, coagulation disorders, disseminated intravascular coagulation, metabolic acidosis, thromboembolism, malnutrition, necrotizing enterocolitis, sepsis can also be observed. While the risk of death is 1% in healthy infants, it is 12% in infants with risk factors [75].

The use of exchange transfusion has greatly decreased due to the frequent and ideal use of phototherapy. This has also reduced the incidence of possible mortality and morbidity risk arising from the exchange transfusion procedure.

### 4.3 Pharmacological treatment

Agents used to treat neonatal jaundice can be classified according to their action of mechanism as follows; Inhibition of bilirubin (Tin protoporphyrin and mesoporphyrin, Zinc protoporphyrin and mesoporphyrin), accelerating bilirubin excretion process (Phenobarbital, Ethanol, Chloroquine, Antihistamines, Clofibrate, Antipyrine), inhibiting the enterohepatic circulation (Agar, Activated charcoal, Cholestyrylpyrron, bilirubin oxidase) and IVIG [75]. IVIG, phenobarbital, and metalloporphyrins are the most preferred ones in the treatment of hyperbilirubinemia.

#### *4.3.1 Phenobarbital*

Phenobarbital is a potent inducer of microsomal enzymes. It makes this strong induction by inducing the enzyme glucuronyl transferase. By this mechanism, it increases bilirubin conjugation, excretion and bile flow, which means that it affects all steps of bilirubin metabolism. In addition, phenobarbital is used in the diagnosis and treatment of Crigler Najjar disease [76]. It is recommended for use only in high-risk conditions.

#### *4.3.2 Intravenous immunoglobulin*

Intravenous immunoglobulin therapy can be used in Coombs test positive Rh or ABO incompatibility, minor blood group incompatibility, and newborn infants

who received intrauterine transfusion/s. IVIG should be given timely at a dose of 0.5–1 g/kg and within 2 hours, without delay, in newborn infants with hyperbilirubinemia that do not decrease despite intense phototherapy, and whose bilirubin level is close to the limits of exchange transfusion [77].

High-dose IVIG therapy in newborn infants, such as a dose of 0.5 g/kg, reduces the need for exchange transfusion. It does this by slowing the rate of bilirubin rise and lowering maximum bilirubin levels. IVIG is thought to prevent hemolysis by its mechanism of blocking reticuloendothelial Fc receptors [78, 79].

As a special case, the management of hemolytic disease due to Rh incompatibility is as follows; Preparation should be made before birth. First of all, preparation of the blood product to be given should be done. Since exchange transfusion can be required as soon as in the delivery room, equipment should be ready for use and IVIG should be kept, if needed. A staff experienced in neonatal resuscitation should be present. As soon as the infant is born; intensive phototherapy treatment is started and IVIG is given to the patient as a pharmacological treatment promptly. Hemoglobin (Hb) and STB levels are checked from the blood sample taken from the umbilical cord. Intensive phototherapy should be started in newborn infants born above 38 weeks, if the bilirubin level is above 6 mg/dL and/or the Hb value is less than 10 g/dL in the blood sample taken from the umbilical cord, as well as the preparation begins for exchange transfusion. In case of the STB increase is more than 0.5 mg/dL per hour despite intensive phototherapy and IVIG therapy, rapid exchange transfusion should be performed [80].

#### *4.3.3 Metalloporphyrins*

Metalloporphyrins, which competitively inhibit heme oxygenase enzyme, slow down bilirubin synthesis. The most effective metalloporphyrin is Tin (Sn) because it lowers bilirubin [81, 82]. A single dose of 6 μmol/kg is used in the treatment of neonatal jaundice. It has been observed that Sn-mesoporphyrin applied together with phototherapy can elicit transient erythema in some of the newborn infants. Currently, metalloporphyrins are not a part of routine practice of treatment for neonatal jaundice [76].

## 5. Conclusion

Prolonged jaundice can become an intensive care problem, if not noticed early. Extreme hyperbilirubinemia (TB of 25 to 30 mg/dL) can cause bilirubin encephalopathy, Kernicterus, which is usually characterized by the deposition of unconjugated bilirubin in brain cells. Neuronal necrosis/damage occurs in the basal ganglia, hippocampus, hypothalamic nuclei as a bilirubin-phosphatidylcholine precipitate, diencephalon, midbrain, neurohumoral and electrolyte control, brainstem nuclei for oculomotor and auditory function, and in the cerebellum. Clinical manifestations include cerebral palsy, deafness, seizures, etc.

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Chapter 5

# Retinopathy of Prematurity: A NICU Based Approach

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## Abstract

Retinopathy of prematurity is a fibrovascular proliferative disorder affecting the peripheral retinal vasculature in premature infants. It is one of the leading causes of preventable childhood blindness across the globe. The world is currently experiencing ROP as third epidemic, where majority of the cases are from middle-income countries. With intensive use of in-vitro fertilisation (IVF) and multiple births, ROP emerging as a significant problem globally. High quality neonatal services, better equipment, improved training, evidence-based screening protocols and access to ROP specialists preventing blindness due to ROP in most of the countries. For more than three decades, improvement in treatment strategy for severe ROP markedly decrease the incidence of ROP related blindness. Current international screening guidelines recommend ROP screening for all premature infants based on birth weight of less than 1501 g or a gestational age of 30 weeks or less, while latest Indian screening guidelines includes all premature infants with birth weight of <2000 grams or gestational age of <34 weeks. Current strategies include adoption of newer screening guidelines, telemedicine and vision rehabilitation.

**Keywords:** NICU- neonatal intensive care unit, ROP- retinopathy of prematurity, GA- gestational age, LBW- low birth weight, RBSK- Rastriya bal swasthya karyakram

## 1. Introduction

Retinopathy of prematurity (ROP) is a vasoproliferative disorder of the retina occurring in premature babies, originally designated as retrolental fibroplasia by Terry in 1952 [1]. The term ROP was coined by Heath in 1951 [2]. It is a disorder of development of retinal blood vessels in premature babies and is a major cause of preventable childhood blindness. Normal retinal vascularization happens centrifugally from the optic disc to the ora serrata, the outer edge of the retina. Vascularization up to the nasal ora is completed by 8 months (36 weeks) and temporal ora by 10 months (39–41 weeks) [3]. ROP begins to develop between 32 and 34 weeks after conception, regardless of gestational age at delivery and has two distinct phases. During the acute first phase, the normal vasculogenesis of the retina is disturbed by the relative hyperoxia of the extrauterine environment. This causes vaso-obliteration and non-vascularization of some areas of the anterior retina. Subsequent hypoxia causes a second chronic phase, characterised by the

proliferation of vascular and glial cells arteriovenous shunt formation, occasionally leading to involution or permanent cicatricial changes and visual impairment [4].

It is estimated that of about 15 million children born preterm worldwide, about 53,000 develop light-threatening ROP requiring treatment, and 20,000 suffer blindness or severe visual impairment. In the Early Treatment for Retinopathy of Prematurity (ET-ROP) study in the United States, the incidence of any stage ROP was 68% among infants weighing <1251 g. Among infants with ROP, clinically-significant (prethreshold) ROP developed in 36.9% [5]. More than 60% of preterm births occur in Africa and South Asia. India accounts for the most preterm births in the world (3.5 million). The occurrence of severe blinding ROP is related to poor neonatal and ophthalmic care, more common in middle and low socio-economic countries with regional variations and technology and capacity. India has the third highest in terms of LBW, with about 1.7 million weighing <2500 g and about 0.4 million <1500 g. Crucially, premature birth and LBW predispose a newborn to develop ROP, for which India is evidently the hot bed. It is also known that ROP can develop in bigger and more mature babies in India, which may be attributed to the suboptimal quality of neonatal care [6]. As a general rule first screening should be done at 1 month of postnatal age.

## 2. Why should we screen for ROP?

There are several necessary reasons to have a screening programme for ROP. Firstly, the premature child is not born with ROP and retinal disease is not present at birth. Each child has a potential for normal vision, even if the retina is immature at birth. Screening aims to identify those infants who have reached or have the potential to reach threshold ROP, which if untreated may cause visual impairment or blindness, which has serious medico-legal implications. There are indefensible legal repercussions should an infant develop ROP and retinal detachment, but had not received eye examination. Secondly, besides the economic burden of such childhood blindness, the grief and the personal tragedy for the family is tremendous. Early recognition of ROP by planned screening provides an opportunity for timely and effective treatment [7].

## 3. How can we start a screening programme in an NICU?

It is the duty of the treating neonatologist to ensure that the neonates at risk are screened by a trained ophthalmologist at regular intervals. One of the key factors in establishing and maintaining a successful programme is to convince the administrative body, and nursing staff of the neonatal intensive care unit (NICU) about the necessity and effectiveness of the programme.

A responsible person in the nursery should be designated to coordinate the selection of the 'at risk' preterm infants according to the guidelines of the ophthalmologist and ensure their eye evaluation at the appropriate time. An airtight system should be decided for initial evaluation as well as for follow-up visits so as to avoid any eligible infant falling out of the strict screening criteria. The moral and legal responsibility of getting the baby to the ophthalmologist for screening at the appropriate time rests solely with the paediatrician. Written guidelines and criteria for ROP screening provided to the NICU staff (**Table 1**) help streamline the programme. Once the programme is accepted, a trained ophthalmologist is needed to conduct the eye examination. This could be a paediatric ophthalmologist or a retina specialist.

| Country | Cut-off criteria |
|---|---|
| **Where to screen** | |
| **India** | If GA is known: ≤34 wk GA<br>If GA is not known/unsure – All infants with </=2000 g birth weight.<br>Other preterm infants at the discretion of the neonatologist |
| **China** | GA <34 wk,<br>BW <2000 g<br>Any infant, irrespective of BW or GA, who may have been ventilated for at least 1 wk. or received<br>Supplemental oxygen for >30 days |
| **Taiwan** | GA <31 wk<br>BW <1500 g<br>Larger babies if unstable clinical course, based on paediatrician discretion |
| **Thailand** | GA <30 wk<br>BW 1500 g |
| **Malaysia** | GA <32 wk<br>BW <1500 g<br>Infants with an unstable clinical course who are at high risk (as determined by the paediatrician) |
| **Indonesia** | GA <32 wk<br>BW <1500 g |
| **Vietnam** | GA <33 wk<br>BW <1500 g |
| **USA** | GA <30 wk<br>BW <1500 g<br>Larger babies to be screened if they had unstable clinical course |
| **UK** | GA <32 wk<br>BW <1501 g |
| **When to perform the first screening** | |
| **India** | Before discharge from the NICU or 30 days of life, whichever is earlier infants with period of gestation less than 28 weeks (gestation age) or less than 1200 grams birth weight should be first screened at 2 to 3 weeks after delivery.<br>No examination needed in first-32 weeks |
| **China** | 4-6 wks after birth or 31-32 wk of PMA<br>Babies with BW >2000 g may screen at 3 wk |
| **Taiwan** | 4-6 wk after birth or 31-32 wk of PMA |
| **Thailand** | 4-6 wk after birth or 31-32 wk of PMA |
| **Malaysia** | 4-6 wk after birth |
| **Indonesia** | 4-6 wk after birth |
| **USA** | 4 wk after birth of at 31 weeks of PMA whichever is late |
| **UK** | Babies with GA <27-screening at 31-32 wk. of PMA<br>Babies with GA 27-32 wk-screening to be undertaken at 4-5 wk. post natal age<br>All eligible babies should undergo screening before discharge |
| **Screening methods** | |
| **India** | Indirectophthalmoscopy by a trained ophthalmologist Retinal Imaging using a wide-field retinal camera (eg, 3 Nethra Neo or RetCam) by a trained ophthalmologist/ trained technician/DEIC optometrist<br>Smartphone based widefield retinal imaging by trained ophthalmologist or optometrist |

| Country | Cut-off criteria |
|---|---|
| **China** | Indirect ophthalmoscopy by a trained ophthalmologist Retinal Imaging using a wide-field retinal camera |
| **Taiwan** | Indirect ophthalmoscopy by a trained ophthalmologist |
| **Thailand** | Indirect ophthalmoscopy by a trained ophthalmologist |
| **Malaysia** | Indirect ophthalmoscopy by a trained ophthalmologist (RetCam examination is no sufficiently sensitive to be a substitute indirect ophthalmoscope |
| **Indonesia** | Indirect ophthalmoscopy by a trained ophthalmologist |
| **USA** | Indirect ophthalmoscopy by a trained ophthalmologist (digital retinal imaging is good for documentation and counselling but not used as primary method for screeing) |
| **UK** | Indirect ophthalmoscopy by a trained ophthalmologist (using infant speculum and depressor |

*BW indicates birth weight; GA, gestational age; NICU, neonatal intensive care unit; PMA, postmenstrual age; SNCU, special/sick newborn care unit.*

**Table 1.**
*Comparison of Asian Nations Screening Criteria with the United States of America and United Kingdom [8].*

## 4. Whom should we screen?

The aim of screening premature babies for ROP is to timely detect all treatable neonates, with minimal expense of time and resources. This also aims at not screening those babies who are unlikely to get a severe form of ROP. The criteria for screening babies are based on two critical factors – the birth weight and the gestational age. Other additional contributing factors should be also taken into consideration (**Table 1**) [7].

Although much has been written about the association of oxygen use and ROP, it has been found that oxygen is not the cause of ROP. On the contrary, low levels of oxygen and slow weaning from oxygen may help regression of early stages of ROP [7]. The targets for oxygen therapy will be discussed in the section on prevention.

## 5. When should screening begin?

A premature infant is not born with ROP. The retina is immature, but this is perfectly natural for their age. It is the post-natal mal-developments in the retinal vessels that could lead to ROP. The sequence of events leading to ROP usually takes about 4-5 weeks except in a small subset of premature infants who develop Rush disease early by 2-3 weeks. Routine screening criteria listed in **Table 1**. We strongly recommend that one session of retinal screening be carried out before day 30 of the life of any premature baby.

## 6. Follow-up protocol after initial examination

The ophthalmologist plans for further follow-up examinations based on the initial fundus findings (**Table 2**). Further evaluation for ROP is not needed if the retina is fully mature (defined as retinal vessels seen up to temporal ora serrata, which usually occurs by 40 weeks post-conceptional age [7]. Since there preterm babies are at higher risk for developing refractive errors, delayed visual maturation and squint, these babies, need to see a paediatric ophthalmologist for refraction,

| **How frequently to examine** | |
|---|---|
| 1. Mature retina | Follow-up 3-12 months |
| 2. Immature retina | Follow-up bi-weekly |
| 3. Immature Zone I retina | Follow-up weekly |
| 4. Pre-threshold ROP | Follow-up 3-7 days |
| 5. Threshold ROP | Early treatment within 72 hours |
| 6. Retinal Detachment in ROP | Early surgical treatment ROP |

**Table 2.**
*Follow-up schedule for ROP screening/treatment.*

- Media clear
- Pupil dilates fully
- No new vessels in the iris
- No new vessels in the retina
- All retinal/preretinal and vitreous haemorrhages cleared
- Regression of dilatation and tortuosity of retinal vessels
- No increase in retinal traction manifested by disc/macula drag
- No elevation of retina/ridge at or posterior to area of laser
- Feeder vessels to area of active new vessels achieve normal calibre
- Demarcation between laser-treated and normal retina is quiet and flat in terms of vasculature, with adequate scar effect of the laser

**Table 3.**
*Signs of regression of retinopathy of prematurity.*

vision assessment, and ocular alignment (squint) at 3-12 months of age. If there is an obvious squint, nystagmus, tearing, discharge, photophobia, leucocoria or vision loss, then early evaluation is needed. But, if there is no apparent squint or vision problem, the child can be seen at one year of age. If the retina is immature (retinal vessels are not seen up to nasal ora serrata) then baby must be screened every two weeks till the retina is mature [9]. In eyes with retinal vessels seen only up to the Zone I area at initial visit, weekly evaluation is needed. These eyes are at high risk of developing aggressive posterior ROP or Rush disease very quickly, (and not necessarily the classical stages 1-3 ROP). If there are early signs of ROP then the child must be examined every week for any progression or regression of the disease. If child develops pre-threshold ROP, then the child should be seen every 3-7 days for progression. In case of threshold ROP, urgent peripheral retinal laser ablation should be done within 48-72 hours [10]. In eyes with ROP stage 4 or 5, early surgical treatment such as belt buckling or vitreous surgery can help save some vision, though the majority have a guarded prognosis [11]. Signs that indicate disease that have reached a quiescent stage of ROP are shown in **Table 3**.

## 7. How do we dilate?

The recommended eye drops are tropicamide 0.5% - 1% with phenylepherine 2.5%. Two to three instillations of each of these drops, five minutes apart are usually sufficient to dilate the pupils in 15-20 minutes; and the effect remains for 30-45 minutes. Cyclopentolate 0.5% to 1.0% can also be used safely. Care should be taken to wipe (with sterile cotton/tissue) any eye drops that spill onto the cheeks, as they can be absorbed from the skin of the babies and can cause tachycardia. It is not

advisable to use 10% phenylepherine or atropine (drops or ointment) in premature babies for screening, as severe tachycardia, and fatal hyperthermia and dehydration can occur due to systemic absorption. [Note: diluting commercially available adult formulation of tropicamide 0.8% and phenylephrine 5% drop with methylcellulose eye drops or distilled water in 1:1 dilution can also match the recommended dose.

## 8. Where should the examination be done?

The place of screening must be warm and clean enough for the baby. This is often the nursery/office of the neonatologist but can also be the office of the ophthalmologist. The baby should be well wrapped; and the baby should be preferably fed and burped an hour before evaluation. Check functionality of all the screening material before starting screening the baby (**Table 4**). Critically ill babies should be evaluated preferably in NICU /incubator under the guidance of the neonatologist, monitored by a pulse oximeter. A quick flashlight evaluation of adnexa and anterior segment (to rule out any congenital ocular anomaly) is done before starting screening. At the end of the evaluation, rest of the forms/diagrams are completed and discussion with the parents/paediatrician/staff about the retinal status carried out. *Errors in dilution of dilating drops can prove fatal for the baby*.

## 9. The Examination

Examination is performed with an indirect ophthalmoscope with a condensing lens of +20D or +28 D/30D may be used for this purpose (**Table 4**) [7, 8, 10]. The advantage of using +28D/30D is its wider area of view, though the magnification is less. An infantile speculum (after instilling topical anaesthesia drops) may be used to keep the eye open or the examiners may open it with their fingers. Oculocephalic reflex, wherein the head of baby is turned towards the side to be evaluated, can be used to examine the peripheral retina. Turning the eye to desired direction or scleral depression to see ora serrata with either a simple non-serrated wire vectis may be needed in only few cases. Examination for ROP does not require any sedation or general or even topical anaesthesia.

The anterior segment is first examined with the condensing lens focussed on the cornea, iris, pupil and lens to look for any media opacity, tunica vasculosa lentis or dilated tortuous iris new vessels. Next, retinal evaluation is done starting with

1. Indirect ophthalmoscope
2. Spare bulb
3. +20 D/+28 D lens
4. Paediatric wire speculum
5. Paediatric depressor/wire vectis
6. Dilating ROP drops
7. Sterile cotton
8. Literature (pamphlets/flyers) for parents education
9. ROP documentation forms
10. Receipt book (optional)

**Table 4.**
*Checklist of materials needed for ROP screening.*

evaluation of media clarity. The posterior pole over the area of Zone I is first examined for disc, macula and retinal vessels to rule out any evidence of Plus disease, vascular loops or retinal avascularity. Any evidence of immaturity or ROP in the nasal periphery would qualify the disease for Zone II. Complete vascularisation of the nasal periphery with the avascular area in the temporal periphery would qualify the disease for Zone III. Vascularisation in temporal zone III periphery confirms complete vascularisation, thus, the time to stop screening. Thus, screening can be stopped when a baby is no longer at risk of sight-threatening ROP.

ROP screening examination can have short term effects on blood pressure, heart rate and respiratory function in the premature babies. The examination should be kept as brief as possible and precaution must be taken to ensure that emergency situations can be dealt with promptly and effectively. The screening examination can be stressful for both babies and parents. In addition to oral communication, parents should be given written information about the screening process prior to each examination of their baby. Ophthalmological notes should be made after each ROP examination, detailing zone, stage, and extent in terms of clock hours of any ROP and the presence or absence of any pre-plus or plus disease. These notes should include a recommendation for the timing of the next examination and be kept with the baby's medical record.

## 10. Classification of ROP

All the findings of the examination must be well documented according to the international classification for retinopathy of prematurity (ICROP) [12] recommendations specifying the location (Zone I-III) and severity of the disease (Stage I-V), with or without Plus component and the extent of clock hours.

Location of the disease (Zones): The normal blood vessels of the retina progress from the optic nerve posteriorly to the edge of the retina (ora serrata) anteriorly [12]. The location of ROP is a measure of how far this normal progression of blood vessel development has reached before the disease takes over. Three circular zones are defined with the optic disc at the centre (**Figure 1**). Zone I is a small area around the optic nerve and macula. The radius of Zone I is equal to twice the distance between the disc and the fovea. Disease in Zone I is the most dangerous. Zone II is up to the equator on the temporal side and up to the ora serrata on the nasal side. Zone III is the remaining crescent of retina from the equator to the ora on the temporal side. (**Figure 1**).

Extent of the disease (clock hours) - The eye is divided into twelve sectors similar to a clock. The extent of ROP is defined by how many clock hours of the eye's circumference are diseased. The extent can vary from 1 to 12 clock hours (**Figure 1**).

Plus disease - any stage or zone of ROP may be associated with additional component of Plus Disease. Plus disease is characterised by abnormal dilated vessels on the iris and/or engorgement and tortuosity of the blood vessels in the retina (**Figure 2**). Additional findings include retinal haemorrhages, poorly dilating pupil and hazy media.

## 11. Stages of the disease (severity)

ROP is a progressive disease [12–14]. It starts slowly, usually anywhere from the third to the tenth week of life and may progress very fast or very slowly through successive stages, from stage 1 through 5 (**Figure 2**). It may cease at stage 1, stage 2, or mild stage 3 and finally disappear completely, without affecting vision.

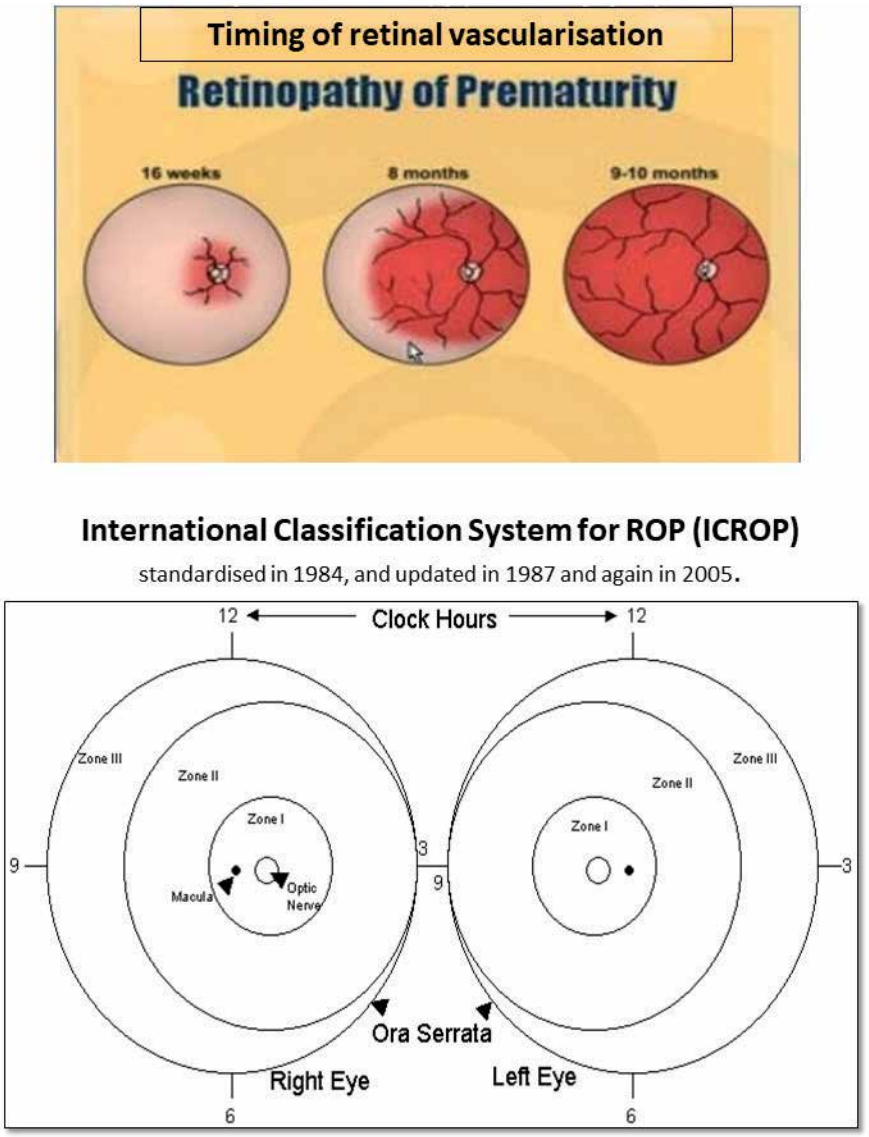


**Figure 1.**
*Timing of retinal vascularisation.*

Stage 1 ROP is characterised by a white line separating the clearly normal red retina from the sharply contrasting underdeveloped white/grey retina.

Stage 2 ROP displays a rolled ridge of scar tissue instead of only a line. It may be limited to a small area or encircle the entire inside of the eye like a belt.

Stage 3 ROP is characterised by the development of abnormal new blood vessels on the edge of the ridge seen in stage 2. These vessels are lifted off from the surface and project into the vitreous cavity. Since more than 50% eyes with stage 3 will progress to stage 4 or 5, treatment with anti-VEGF injection or laser is considered in this stage.

Stage 4 ROP occurs due to pulling of the retina by the scar tissue resulting in partial retinal detachment (RD). Depending on the extent of RD stage 4 is further divided into stage 4A (sparing macula) and 4B (involving macula). In stage 4 A, the eyes have reasonably good chance of achieving usable vision if the retina can be re-attached. The involvement of the macula in stage 4B severely limits the prospect of usable vision. In stages 4A and 4B, surgery at the earliest may help to salvage some useful vision.

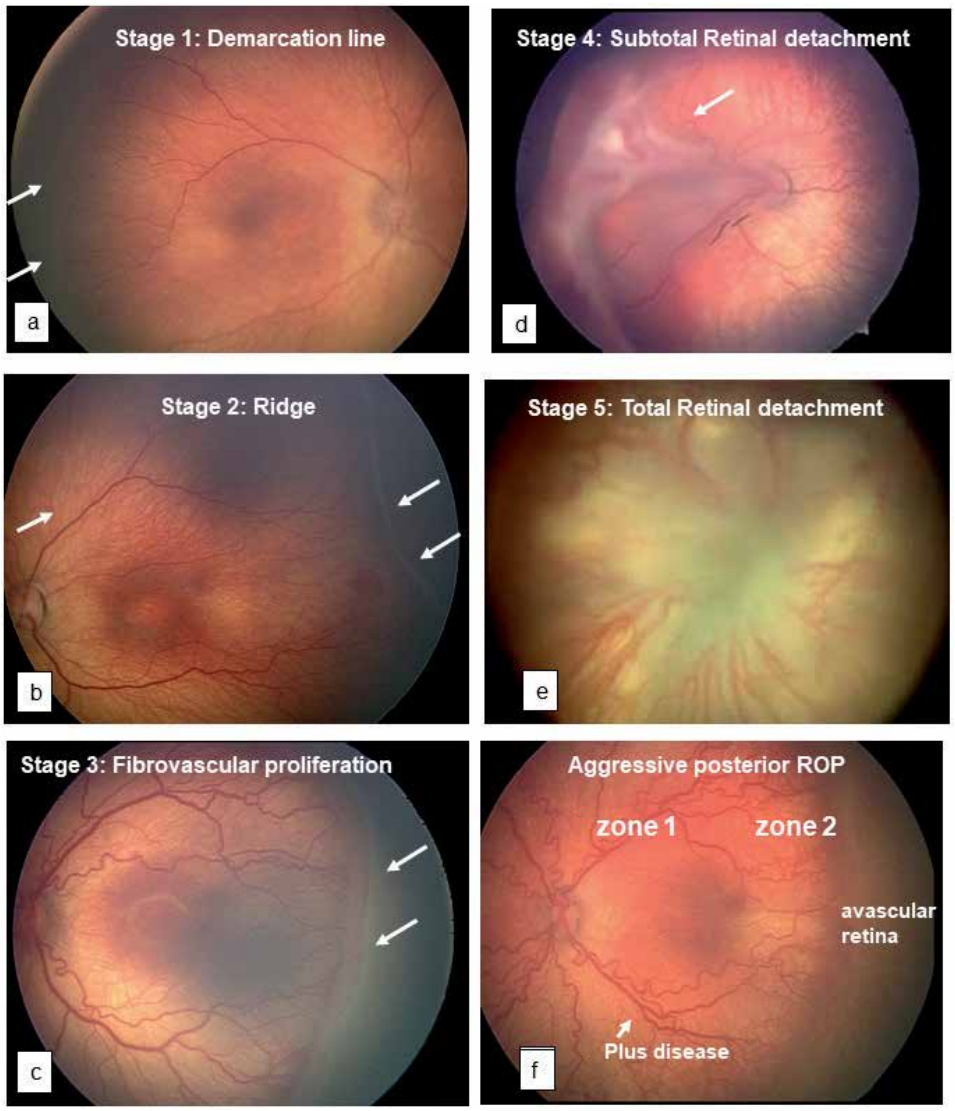


**Figure 2.**
*Various stage of ROP. (a) stage 1 ROP, (b) stage 2 ROP showing ridge (white arrow), (c) Stage 3 ROP showing fibrovascular proliferation at junction of vascular and avascular retina (white arrow), (d) stage 4 ROP showing subtotal retinal detachment, (e) stage 5 ROP showing total retinal detachment, and, (f) Aggressive posterior ROP showing detailed and tortuous vessels in all quadrants without standard stage signs.*

Stage 5 ROP involves complete retinal detachment, with the retina assuming a partial or closed funnel configuration, clinically seen as white reflex in the eye (leucocoria). Treatment at this stage involves surgery to reattach the retina. Some vision may be recovered after this surgery but usually the eye becomes legally blind.

Aggressive posterior ROP (APROP), also known as Rush disease, is a severe form of ROP, if untreated, usually progresses rapidly to stage 5 ROP. The characteristic features of this type of ROP include its posterior location, prominence of plus disease, and the ill-defined nature of the retinopathy. These eyes do not have the classical ridge or extra retinal fibro vascular proliferation, but rather have innocuous looking retina and dilated tortuous vessels forming arcades. This type of ROP is likely to get missed by in-experienced examiners. Observed most commonly in Zone I, it may also occur in posterior Zone II. (**Figure 2**).

| Imaging modality | Field of view | Setting for use | Staffing requirement | Advantages | Disadvantages |
|---|---|---|---|---|---|
| SROP camera [14]<br>Condensing lens<br>• +20D<br>• +28D<br>• +40D<br>(attached with MIIRETCAM device) | 46 degree<br>53 degree<br>90 degree | • Special care baby unit<br>• Outpatient department<br>• Theatre | • Ophthalmologist<br>• Nursing staff to hold baby and monitor vital signs. | • Non-contact based<br>• Portable<br>• Wide field of view<br>• Cost effective<br>• Able to image till ora serrata through sclera depression<br>• High-resolution images | • Only colour imaging available |
| NIDEK Camera [3] | 30 degree | • Special care baby unit<br>• Outpatient department<br>• Theatre | • Ophthalmologist | • Non-contact based<br>• Portable | • Low resolution images<br>• Narrow field of view<br>• Only colour imaging available<br>• Unable to image till ora serrata |
| Video indirect ophthalmoscopy [3] | 53 degrees (28D lens)<br>46 degrees (20D lens) | • Special care baby unit<br>• Outpatient department<br>• Theatre | • Ophthalmologist<br>• Nursings staff to monitor vital signs | • Non-contact based<br>• Portable<br>• Cost effective<br>• Able to image till ora serrata through scleral indentation | • Low resolution images<br>• Only colour imaging available |
| 3Netra Neo widefield camera [15] | 120 degrees | • Special care baby unit<br>• Outpatient department<br>• Theatre | • Ophthalmologist<br>• Nursings staff to monitor vital signs | • Portable<br>• Wide fundal field of view<br>• Fast image acquisition | • Contact based<br>• Heavy weight camera<br>• Unable to image out ora serrata<br>• Costly |

| Imaging modality | Field of view | Setting for use | Staffing requirement | Advantages | Disadvantages |
|---|---|---|---|---|---|
| RetCam Widefield camera [16, 17] | 130 degrees | • Special care baby unit<br>• Outpatient department<br>• Theatre | • Ophthalmologist<br>• Nursings staff to monitor vital signs | • Portable<br>• Wide fundal field of view<br>• Fast image acquisition<br>• Colour and fluorescein angiographic imaging available | • Contact based<br>• Heavy weight camera<br>• Sedation or general anaesthesia essential only for high quality angiograms<br>• Unable to image out to ora serrata<br>• Costly |
| Optos ultrawidefield camera [18] | 200 degrees | • Outpatient department | • Ophthalmologist<br>• Nursings staff to monitor vital signs<br>• Ophthalmic photographer | • Non-contact based<br>• Fast image acquisition<br>• High-resolution images<br>• Widel field of view<br>• Colour and angiographic imaging available<br>• No sedation required | • Non-portable<br>• Unable to image out to ora serrate<br>• Costly<br>• Ophthalmic photographer needed for image capture |

**Table 5.**
*Comparison of different imaging modalities for retinopathy of prematurity imaging.*

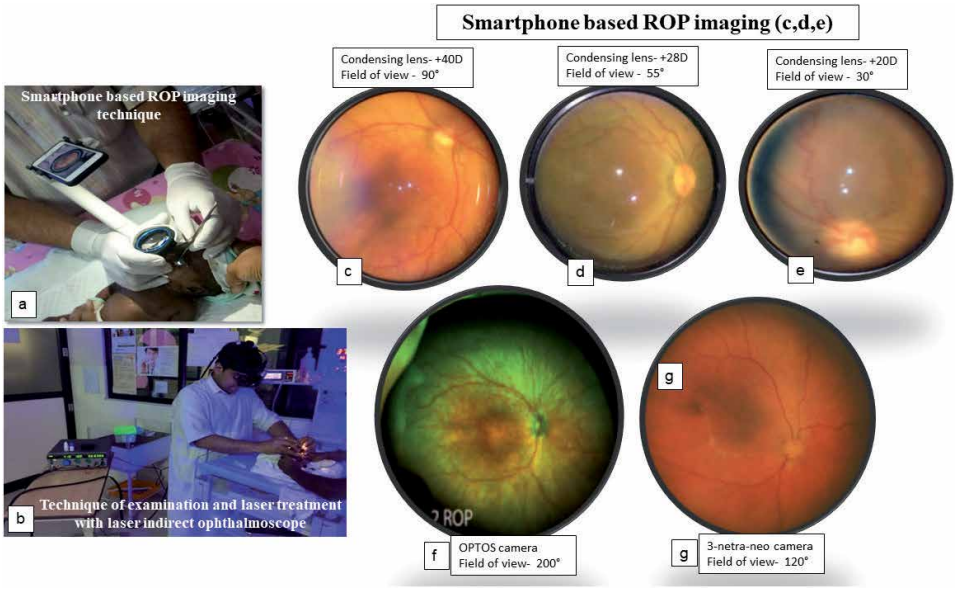


**Figure 3.**
*Comparison of various ROP imaging, (a) Smartphone based ROP imaging technique, (b) Technique of examination and laser treatment with laser indirect ophthalmoscope, Smartphone based ROP imaging(c,d,e) (c) 90⁰ field with +40D condensing lens, (d) 55⁰ field with +28D condensing lens, (e) 30⁰ field with +20D condensing lens, (f) 200⁰ field with OPTOS fundus camera, (g) 120⁰ field with 3-netra neo fundus camera.*

## 12. Imaging for ROP screening

The current gold standard method for ROP screening requires indirect ophthalmoscope with the +20D/28D condensing lens. But this method is subjective and correct diagnosis can be missed by inexperienced hands or when the infant is too sick to allow adequate fundus examination. Nowadays, digital fundus photography is used in ROP screening to facilitate consultation in difficult cases and also contribute to medicolegal affairs. Fundus photography is possible by various professional equipment as the RetCam (Natus USA, formerly Clarity MSI, USA) "shuttle", which is costly and unaffordable in most of the local medical healthcare systems. "3 netra neo" is another recently developed wide-field ROP screening camera developed in India with 120-degree field of view, is also a contact camera available for tele-screening, in the rural program of Karnataka Internet Assisted Diagnosis of Retinopathy of Prematurity (KIDROP) and some other states, with better resolution and lighter probe as compared to RetCam [15]. Optos is another non-contact based imaging with 200-degree field of view. Since, smartphone has become basic necessity in today's world, high-quality optical system and coaxial light source of modern smartphone cameras can also be used for ROP imaging and diagnosis as shown in **Table 5** (**Figure 3**) below [14]. **Table 5** and **Figure 3** shows comparison of various ROP imaging cameras with their field of view.

## 13. A note on prevention

We can alleviate the burden of visual morbidity from retinopathy of prematurity (ROP) to a great extent by primary prevention. Strategies include rigorous adoption of inexpensive evidence-based protocols on temperature control, prevention of sepsis and support for breast-milk feeding, and oxygen monitoring. Several trials

have looked at the optimal oxygen concentration which maximises the survival of the infant and minimises the risk of ROP.

The Neonatal Oxygenation Prospective Meta-analysis (NeOProM) collaboration [16] has reported analysis of five trials of oxygen saturation (SpO2) targeting in very preterm infants and shown that a SpO2 target of 85-89% compared to 91-95% was associated with less incidence of ROP, but increased mortality [16, 17]. Currently, the scientific consensus suggests that a Target Oxygen Saturation in between 90 and 94%, with a lower alarm limit at 89% and higher alarm limit at 95% allows optimal outcome for the neonates with minimum risk of ROP [19]. Other methods of pharmacological prevention will be shortly dealt with in the recent advances section.

## 14. Treatment

The major modalities used in the treatment of ROP currently are LASER, intravitreal Anti-VEGF injections and finally surgery.

### 14.1 Role of LASER

The stimulus for neovascularisation comes from the avascular retina which releases angiogenic factors including Vascular Endothelial Growth Factor (VEGF). Therefore, ablation by cryotherapy or laser photocoagulation destroys the avascular retina and in turn decreases the levels of VEGF. This leads to the regression of new vessels. The level 1 evidence for cryotherapy in ROP comes from the CRYO-ROP study [18]. CRYO-ROP study advocated treatment of threshold ROP which was defined as at least five contiguous or eight cumulative clock hours of stage 3 ROP in zone I or II in the presence of Plus disease.

LASER photocoagulation achieves retinal ablation with much more precision and less side effects with more than 90% successful outcomes. It is essential to treat the entire avascular retina from the ridge/vascular part of the retina up to the ora serrata for 360 degrees in both eyes, without leaving any untreated 'skip area' [7]. Visual outcomes reported after laser are better than those after cryotherapy. Therefore, after the advent of Early Treatment for Retinopathy of Prematurity (ETROP) study [13], there has been a paradigm shift from cryotherapy to LASER in the treatment of ROP.

Early Treatment for Retinopathy of Prematurity (ETROP) study [13] found that a subset of ROP prior to reaching threshold level carries high risk of progression to threshold disease and early treatment of the same can prevent vision loss. This was termed Type I ROP or High-risk pre-threshold ROP, which includes

- Zone I, any stage ROP with plus disease or
- Zone I, stage 3, with or without plus disease or
- Zone II, stage 2 or 3 ROP, with plus disease

The clinical algorithm also indicates that continued serial examinations, as opposed to peripheral retinal ablation, should be considered for any eye with:

Type II ROP or low risk pre-threshold ROP was defined as

- Zone I, stage 1 or 2 with no plus disease or
- Zone II, stage 3 with no plus disease

Eyes with type II ROP can be safely observed and treated only when progression to type I status or threshold ROP occurs.

Once threshold ROP or high-risk pre-threshold (type1) ROP is identified, treatment has to be initiated in 24-72 hours of diagnosis. LASER can be performed under topical anaesthesia in Neonatal Intensive Care Unit settings under neonatologist monitoring. Laser indirect ophthalmoscope is used to give laser treatment. Near-confluent LASER burns have to be applied to the whole of avascular retina [7]. 24% dextrose solution can be given orally to minimise the pain during the procedure. General anaesthesia or sedation may be required in selected cases. Precautions have to be taken to prevent apnoea, hypoxia, bradycardia, and hypothermia during and after the procedure. Oral feed can be avoided 30 minutes pre and post laser procedure.

## 15. Role of anti-VEGF intravitreal injections

Intravitreal injection of Anti-VEGF agents is being increasingly used as a treatment for ROP. The purported advantages include relatively shorter time of administration and therefore less stress on the baby, faster regression of PLUS disease, no destruction of peripheral retina and a lower risk of myopia. But the adoption of Anti-VEGFs has not been universal due to certain limitations which include higher rates of late recurrence, persistent avascular retina in the periphery, delayed onset retinal detachment, and a concern about systemic absorption and related side effects.

The Bevacizumab Eliminates the Angiogenic Threat of Retinopathy (BEAT-ROP) study [20] in 2011 was the first study to provide evidence for the use of the Anti-VEGF agent, Bevacizumab (0.625 mg intravitreal injection) in the treatment of ROP. Subsequent studies have demonstrated the efficacy of intravitreal Ranibizumab injections also in the treatment of ROP [20]. However, the role of Anti-VEGF as monotherapy is limited in view of higher rates of late recurrences.

The dose de-escalation evaluation done in CARE-ROP study of bevacizumab for ROP, which found that dosing between 2.5% and 20% of the adult dose of bevacizumab may be effective in controlling acute ROP though these dose levels may lead to higher rates of recurrence [21]. The rate of ROP recurrences after anti-VEFF injection were significantly higher in patients with APROP or zone I ROP as compared to type 1 ROP or zone II ROP [22].

Ocular profile and short-term efficacy of anti-VEGF is comparable to the standard of care, i.e. LASER. But systemic and long-term risks are still being evaluated. Anti-VEGFs are particularly useful in situations where fast regression is clinically beneficial like in zone 1 disease and aggressive posterior ROP. Many clinicians follow a combination approach where initial control of PLUS disease in APROP is achieved with Anti-VEGF and subsequent rescue treatment with LASER after a few weeks to prevent the possibility of late recurrences [23, 24].

In comparison with intravitreal bevacizumab and conventional laser ablative therapy, recurrence after intravitreal ranibizumab has been observed more frequently than either intravitreal bevacizumab or laser monotherapy. Because ranibizumab is an antibody fragment with a shorter half-life, it is possible that the rate of recurrence after initial injection may be higher in eyes treated with ranibizumab because it is more rapidly cleared from the eye compared to bevacizumab [21].

In patients with recurrence, additional treatments included a second intravitreal ranibizumab injection, supplemental diode photocoagulation, and extreme cases required surgical intervention in form of external scleral buckle and vitrectomy [21]. The combination of indirect laser photocoagulation and intravitreal

anti-VEGF injection (bevacizumab or ranibuzumab) was well tolerated and can induce effective and prompt regression of aggressive zone I ROP [25].

## 16. Role of surgery

Surgical management is considered for advanced stages of ROP i.e. cicatricial ROP (stage 4 and 5). Best surgical outcomes can be attained if surgery is performed at stage 4A when the macula is still uninvolved. The surgical options for stage 4 ROP include lens sparing vitrectomy and scleral buckling procedure. Stage 5 ROP needs a more aggressive approach of vitrectomy with lensectomy. Visual outcomes of stages 4B and 5 remain poor [26].

In summary, currently, the first line of treatment for ROP is LASER photocoagulation. Anti-VEGFs are increasingly being used as monotherapy, but the general consensus is that Anti-VEGFs should be reserved for cases requiring quick response like zone 1 disease and APROP and can be used in combination with LASER for optimal results. Surgery is reserved for advanced stages of ROP with retinal detachment. Treatment and follow-up protocol is summarised in **Table 2**.

## 17. Role of telemedicine and artificial intelligence in ROP screening

Indirect ophthalmoscopy for ROP screening has limitation of examination by experts. Wide-field imaging can be performed ny optometrists and non-physicians, enabling ROP screening in resource-poor settings, which lack trained personal and accessibility to care. Image -based medicine not only offers advantage of longitudinal records, opportunity of second/expert opinion, training and education, but also can be used to assess outcome of treatment and provide medicolegal protection. Artificial intelligence (AI) in ROP has recently received attention. It is computer-based automated image analysis and deep learning system having potential to improve the efficacy and accuracy for diagnosis and risk-assessment in ROP [8].

## 18. Other recent advances

### 18.1 Newer predictors of ROP

Currently, the guiding parameters for ROP screening are gestational age and birth weight. However, only 10% of the screened babies require any form of treatment. This apparent wastage of resources has driven the search for more accurate predictors of severe ROP, to identify babies who may need more frequent screening or early treatment.

### 18.2 Low weight gain proportion

Proportion of weight gain is defined as weight at 6 weeks of age minus birth weight, divided by the birth weight. A value of <50% is considered a strong predictor of severe ROP [27].

### 18.3 WINROP algorithm

Lofquist et al. developed an algorithm based on weekly measurement of body weight and serum IGF-1 levels from birth to a post conceptional age of 36 weeks. They have demonstrated a sensitivity of 85-100% in detecting severe ROP [28].

### 18.4 ROP score

ROP score is another predictive tool formulated by Eckert et al. based on birth weight, gestational age, weight gain and blood transfusions in the first 6 weeks of life, and use of oxygen [29]. They have concluded that ROP score is a more useful tool in predicting ROP than birth weight and gestational age alone. Moreover, it is easy enough to be administered by nursing staff and does not require additional blood investigations.

### 18.5 Other biochemical tools

In addition to the above serum IGF-1 has been independently studied as a predictor for ROP and found useful [30]. Another marker found useful as a predictor of ROP is plasma soluble E selectin (sE-Selectin) [31].

These predictive tools may help reduce the burden of screening on the ophthalmologist and also reduce the need of routine stressful examination on the neonates in the future. Further studies may be required for their validation before adding them in to the routine neonatal care protocol.

## 19. Pharmacological prevention of ROP

Several agents have been studied for the prevention of ROP in high risk newborns. Propranolol has a known anti-angiogenic property and PROP-ROP study looked at its role in prevention of ROP [32]. However, serious adverse effects like bradycardia was noted in the treatment arm and the study halted.

Recombinant IGF-1 [33]. Granulocyte Colony Stimulating Factor (G-CSF) [34], Omega 3 Polyunsaturated Fatty Acids [35] have shown antiangiogenic property in animal models. Further studies are required to evaluate safety and efficacy in humans.

### 19.1 Gene therapy

Mutations and polymorphisms in several genes have been found to be associated with severe ROP and failure of treatment (eg. Norrin, Frizzled 4, Lrp5). These could be future therapeutic targets for gene therapy [36]. Rat models of ROP have demonstrated successful local gene transfer using adenoviral vectors to retinal blood vessels.

Therefore, though the incidence of ROP is rising every year, newer tools are being added into the armamentarium of the team involving the neonatologist, nursing staff and the ophthalmologist to help prevent blindness related to ROP.

## 20. Summary

- ROP is on the rise recently due to improvement in neonatal care and related higher survival of preterm infants all over the world.
- Timely detection and treatment can prevent blindness.
- ROP screening can be done by a well-trained ophthalmologist in any NICU setting with minimal cost and equipment.

- Whom to screen? - American Association for Paediatric Ophthalmology and Strabismus stipulate screening all infants ≤30 weeks GA or ≤1500 g BW, while Indian screening guidelines as per RBSK stipulates screening all neonates <34 weeks GA and <2000 g BW, OR other risk factors like Respiratory Distress, Sepsis etc.

- When to screen? – 1 screening session before 'Day 30' of infant birth **"Gold rule"**. At 2-3 weeks afterbirth for high risk babies with gestational age<28 weeks and birth weight <1200 g.

- When to treat? - Threshold ROP, High risk pre-threshold ROP within 72 hours of detection, APROP within 24-48 hours of detection.

- Treatment modalities- LASER, Anti-VEGF injections and surgery depending on stage and severity of disease.

- LASER is the first line of treatment.

- Anti-VEGFs useful in conditions requiring quick response like zone 1 disease and APROP.

- Surgery. Useful for cicatricial ROP though visual outcomes are poor in advanced disease.

- Telemedicine and artificial intelligence aids in distant screening even without trained personal and utilise computer based deep learning for risk prediction.

- Optimal neonatal care with strict adherence to oxygen protocols can prevent severe sight threatening ROP while ensuring survival.

- WHO recommends a SpO2 target of 88-94%.

- Predictors of severe ROP like WINROP algorithm and ROP score can ensure optimise utilisation of limited resources and avoid unnecessary stressful screening of infants at low risk.

- Pharmacological methods of prevention using agents like IGF-1, G-CSF etc., and advanced treatment options like gene therapy may materialise in the future.

## 21. Conclusion

ROP is recognised world-wide as it is one of the preventable cause of blindness in children. The current approach includes timely screening and documentation using paediatric fundus camera for early diagnosis and timely appropriate treatment. Recently, use of anti-VEGF agents to combat severe forms of ROP–APROP and zone 1 ROP with a caveat of recurrence and therefore need for longer follow-up. Combination of anti-VEGF and laser could work better to reduce the recurrence and need for long-term follow-up. Teleophthalmology is now becoming popular in areas with limited trained manpower and expertise for managing ROP. Artificial intelligence is coming up as excellent distant learning tool in ROP for diagnosis,

follow-up, management, and academic purpose. Though not discussed much, visual rehabilitation is an important aspect of ROP management.

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Chapter 6

# Reducing Toxic Phthalate Exposures in Premature Infants

*Randall Jenkins*

## Abstract

Phthalates are a ubiquitous group of industrial compounds used as industrial solvents and as additives to plastics to make products softer avnd more flexible. Phthalates are found in a variety of products including medical devices, personal care products, flooring, and food packaging. Infants in the neonatal intensive care unit are exposed to phthalates both in the building materials, but more importantly in the medical supplies and devices. Toxicity from phthalates has been of concern to researchers for many decades. Toxicity concerns to neonates includes male reproductive toxicity, hepatotoxicity, cardiotoxicity (including hypertension), neurotoxicity, and neurodevelopmental abnormalities. Limited recommendations have been given for reducing phthalate exposures to premature infants. These include avoiding infusing lipids or blood products through intravenous tubing containing phthalates. Storage of blood in containers made with phthalates has been a strong recommendation and has largely been accomplished. A comprehensive plan for phthalate reduction has heretofore been missing. This chapter has the goal of identifying the problem of phthalate exposure in premature infants, with some practical solutions that can be done today, as well as suggestions for manufacturers to complete the work.

**Keywords:** phthalates, neonate, hypertension, toxicity, di-2-ethylhexyl phthalate (DEHP)

## 1. Introduction

After the year 2000, we began observing a pattern of hypertension in premature infants [1]. In 2019, we reported on an evaluation of 97 premature infants revealing common features including near-universal low plasma renin activity, transient time course, and a good clinical response to treatment with spironolactone. Meanwhile, Trasande had recently shown an association with phthalate compounds and elevated blood pressure in children [2]. A few years earlier, Zhao proposed a possible mechanism for elevated blood pressure related to phthalates. Zhao documented in human microsomes how monoester phthalate metabolites cause sodium retention via inhibition of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) [3]. This is the same enzyme responsible for licorice-related hypertension and the syndrome of apparent mineralocorticoid excess [4].

When considered together, these concepts led to a prospective study to evaluate the hypothesis that phthalates increase blood pressure in premature infants, and may cause the heretofore unexplained hypertension in this population [5]. The study showed phthalate exposure was associated with increased blood pressure and

hypertension in premature infants. Evidence was presented for activation of the mineralocorticoid receptor among hypertensive premature infants [5]. This experience raised our awareness of other potential toxic effects of phthalates in premature infants, and has spurred a more comprehensive examination of phthalates' effect on premature infants.

Phthalates are a ubiquitous group of industrial compounds related to phthalic acid [6]. These compounds are used as industrial solvents and additives to make plastic softer and more flexible. Phthalates are found in a variety of products including medical devices, personal care products, flooring, and food packaging [7]. Infants in the neonatal intensive care unit are exposed to phthalates in the room's construction materials, but more importantly in the department's medical supplies and devices [7, 8].

Toxicity from phthalates has been of concern to researchers since the 1970s. Research showing male reproductive toxicity in animals raised alarms for human exposures, especially as phthalates are known to be endocrine disruptors [9, 10]. Although steroidogenesis in rodents is quite different from that of humans, concerns have been validated for various endocrine effects of phthalates on developing humans [10]. Toxicity concerns to neonates include hepatotoxicity, hypertension, neurotoxicity, and neurodevelopmental abnormalities [7].

Limited actions have been recommended for reducing phthalate exposures to premature infants [11, 12]. These include avoiding infusions of lipids or blood products through phthalate-containing intravenous tubing, as well as avoiding blood storage containers which are comprised with phthalates [11–13]. Of these recommendations, only the proper storage of blood has been broadly adopted [13]. A comprehensive plan for phthalate reduction has heretofore been missing. This chapter's goal is to identify the problem of neonatal phthalate exposures and provide a blueprint for phthalate exposure reductions that can be achieved immediately, as well as suggest how manufacturers can further this work for the benefit of premature infants.

## 2. Phthalates: what are they?

Phthalates are a group of low-cost compounds which are used in the industrial production of commercial products [6, 7]. In 2015, almost 8 million metric tons were produced [14]. Phthalates are used as solvents, and as a softening agent (plasticizer) in polyvinyl chloride (PVC). Phthalates are not covalently bound to the plastic and act by preventing the full polymerization of the PVC, thus imparting elasticity and flexibility to the plastic [15]. Phthalates are used in the production of vinyl flooring, wall coverings, food packaging, personal care products, toys, upholstery, fragrances, pharmaceuticals, fragrances, baby-care products, and medical supplies and devices [6, 7, 16–20]. PVC products may be comprised of up to 50% phthalates [16].

Di-2-ethylyhexyl phthalate (DEHP) is the most common phthalate used in the medical setting. DEHP is the only phthalate approved by the US Food and Drug Administration (FDA) for use in medical products. In the EU, where DEHP has been banned, alternative phthalate plasticizers are used, including di (isonyl) cyclohexane-1,2-dicarboxylate (DINCH), tris (2-ethylhexyl) trimellitate (TOTM), di (2-ethylhexyl) terephthalate (DEHT), di-(2-ethylhexyl) adipate (DEHA), acetyl tri-n-butyl citrate (ATBC), di-isodecyl phthalate (DiDP), di-isonlyl phthalate (DINP), and di (2-prophylheptyl) phthalate (DPHP) [21, 22]. DINCH and ATBC are both used for blood product storage as these prevent excessive hemolysis during storage [23]. Toxicity data is limited on many of these alternatives, making safety

determination difficult to ascertain [21, 24]. This chapter will focus primarily on DEHP, as it is the most-studied and most prevalent plasticizer in the world [7].

Humans are exposed to phthalates via food (from packaging), dermal absorption, and air inhalation [7, 15–20]. In the hospital setting, infants receiving respiratory therapy are exposed to phthalates through three primary means; from indwelling catheters and tubes, from intravenous fluid; and from the room's air [5, 7, 8, 11, 12, 16].

## 3. Metabolism and excretion of phthalates

Phthalates are quickly metabolized and excreted in urine [19, 24], making urine an ideal medium for monitoring phthalate exposures [7, 19, 24]. DEHP is metabolized to MEHP via pancreatic and other lipases. MEHP is the primary metabolite in urine along with two secondary metabolites, MEOHP and MEHHP. Further metabolic pathways are detailed by Koch et al. [19, 24], but include glucuronidation and excretion, leaving little tissue accumulation [20]. In adults, 44.2% of a DEHP exposure is excreted in 24 hours via these three metabolites [25]. This knowledge has allowed quantitative predictions of DEHP exposure based on urine metabolite measurements using an equation by David [26] as modified by Koch [24, 25]. It is not known how these predictions might vary when considering premature infants. Understanding the metabolic pathways is important, as there is ample evidence that the metabolites of DEHP are more toxic than the original compound [3, 8, 16].

Metabolism of DEHP is likely to be different in premature infants as compared to adults or older children [9]. Pancreatic enzymes are not full mature until 6–12 months of age [27, 28]. Glucuronidation activity is also not mature until three months of age, which may increase the half-life of MEHP in these infants [29].

Monitoring studies have found detectable levels of phthalates in almost all populations on the planet, with levels in children typically several times higher than in adults [7]. Zhang et al. showed that the median concentration of DEHP metabolites in children is 38.5 mcg/Liter, and 43.3 in adults [30]. This concentration can be double in pregnant women [31]. Premature infants, by virtue of their small size, and exposure to invasive medical products may have exposures that are more than 4,000 times the levels considered safe for reproductive toxicity [12]. Also, of great concern to premature infants, phthalates have been shown to cross the placenta, and have been measured in human amniotic fluid [32, 33].

## 4. Adverse effects from phthalates

### 4.1 Early animal studies on cancer and reproductive risks of DEHP exposures

Postnatal phthalate exposures have raised concerns of potential carcinogenic, mutagenic, reproductive, hepatic, and cardiotoxic effects [34–37]. Some of the first concerns raised from animal studies included carcinogenic risks. Rodents treated with DEHP had increased risk of hepatocellular carcinoma [38]. This risk prompted the EPA to classify DEHP as a possible human carcinogen [7]. As these studies were performed only in animals, it remains unclear as to the actual human carcinogenic risk from DEHP.

Other early animal (rodent) studies have shown that phthalate exposure can result in abnormal anogenital distance, pathologic changes in rodent testes, hypospadias and cryptorchidism, and reduced circulating testosterone [39]. Reviews of phthalate anti-androgenic reproductive toxicity are available [40–42]. Prenatal

exposures to phthalates have been associated with endocrine disruption, and have been reviewed by Martinez-Arguelles [10]. As steroidogenesis is different between rodents and humans, it remains unclear the magnitude of reproductive toxicity phthalates cause in humans.

### 4.2 Cardiovascular toxicity

Intrauterine DEHP exposure has been associated with cardiac malformations and alteration of key cardiac transcription factors in animals [43]. Both Wang and Snijder separately reported an increased risk of cardiac defects with increased parental exposure of phthalates [44, 45]. DEHP has been shown to inhibit gap junction intercellular communication in lung fibroblasts [46], sertoli cells [47], and cardiomyocytes [48].

DEHP exposure can decreased cardiac contractility in animals. Chick myositis exposed to 4 mg/ml of DEHP stopped beating after 30 minutes, and 97–98% of cardiomyocytes died after 24 hours of exposure [49]. DEHP exposures 6 times this level can be seen in children on ECMO [50]. Similar findings occurred when rat hearts were perfused with blood from blood bags containing DEHP [51]. Lastly, embryonic human cardiomyosites showed a decline in spontaneous beating after exposure to DEHP at 50 mg/ml [52]. A more complete summary of the effects of DEHP on electrophysiology and contractility was published in 2020 by Ramadan et al. [53].

In 2013, Trasande reported that DEHP exposures were associated with increased blood pressure in children [2]. Other reports showed the same in adults [54, 55]. Just a few years earlier, Zhao et al. provided a possible explanation how DEHP might raise blood pressure [3]. He demonstrated in human microsomes that MEHP (a DEHP metabolite) inhibits 11β-HSD2, the enzyme that converts cortisol into cortisone, the less potent mineralocorticoid. Excess cortisol activates the mineralocorticoid receptor, resulting in sodium retention. This is the mechanism of action for licorice-related hypertension, or the syndrome of apparent mineralocorticoid excess [4].

Our group reported that DEHP exposure in premature infants is associated with increased blood pressure and hypertension [5]. In a cohort of premature infants, we showed evidence of inhibition of 11B-HSD2 via changes in measurement of urinary cortisol/cortisone ratio which is a marker for 11β-HSD2. We also showed that markers of sodium channel activity were increased in infants exposed to DEHP. Hypertension in these neonates responded to treatment with spironolactone, and resolved over 10–20 weeks [5].

A subsequent study showed hypertension virtually disappeared when DEHP-containing intravenous fluid (for both mothers and infants) was removed for a two-year period [56]. Subsequently the DEHP-containing IV fluid returned and the hypertension returned to the same level prior to removal. There remain two large unknowns: first, it is unknown as to what is the crucial time period during which DEHP exposure might result in hypertension; second, it is unknown if prenatal DEHP exposure may have an epigenetic effect necessary for, or potentiating the development of subsequent hypertension in premature infants.

### 4.3 Metabolic and genetic toxicity

Phthalates can upregulate gene expression [57, 58]. One such gene which has been reported to upregulate after DEHP exposure is peroxisome proliferator-activated receptor alpha (as well as its cofactor) to increase utilization of fatty acid substrates in cardiomyocytes [57]. Aronson reported increased lactate levels and

lower ATP levels following DEHP exposure [51]. Martinelli suggested that DEHP exposure to animal skeletal muscle could disrupt glucose metabolism [58]. Amara showed that DEHP exposed mice have altered lipid profiles with higher triglycerides, cholesterol and high and low lipoproteins [59]. Human children aged 6–18 exposed to the metabolite MEHP had increased obesity, triglycerides, and increased blood pressure [60].

### 4.4 Immunity and oxidative stress effects

MEHP (the principal metabolite of DEHP), has been found in neonates to inhibit neutrophil apoptosis and chemotaxis [61]. Synthesis of integrin CD11b is doubled by exposure to DEHP at concentrations of 0.1–0.3 mg/L [62]. This integrin is involved in leukocyte adhesion and can thereby increase inflammation. Exposure of human neutrophils to MEHP can increase H2O2 content and inhibit apoptosis and chemotaxis [61]. Exposure of mice to inhaled MEHP results in a significant increase in lymphocytes, neutrophils and eosinophils in broncho-alveolar lavage fluid [63]. DEHP exposure has been linked to increased oxidative stress, both as indicated directly above, and also by increased malondialdehyde levels in infants and children receiving lipids and hyperalimentation fluid containing DEHP [64].

### 4.5 Pulmonary toxicity

Rat pups exposed to maternal DEHP during the final week of gestation showed marked enlargement in terminal airspaces, and a reduction in the number of airspaces, along with decreased surface area for gas exchange [65]. These findings closely resembled those seen with bronchopulmonary dysplasia. A similar study in rat pups also showed pathologic changes similar to BPD when analyzed during the postnatal period [66].

### 4.6 Hepatic toxicity

Rabbits were exposed to similar lipid infusions through IV tubing with and without DEHP. The non-DEHP tubing was made of polyethylene. After a three-week infusion of these lipids, only the DEHP exposed rabbits showed liver fibrosis, cell necrosis, and other features of oxidative stress. The conclusion was that DEHP was responsible for hyperalimentation-related cholestasis [67]. Von Rettberg et al. performed a similar study in human premature infants [68]. One group of 30 neonates receiving parenteral nutrition via DEHP-plasticized PVC tubing for three years. A second group of 46 neonates receiving parenteral nutrition via PVC-free tubing for three years. When comparing incidence of TPN-associated cholestasis they found that the incidence of cholestasis decreased from 50–13% after the change to PVC-free tubing. This translated to an increased risk of cholestasis with use of DEHP-plasticized PVC tubing by a factor of 5.6 [68].

### 4.7 Neurologic toxicity

Gestational and postnatal DEHP exposure has adverse effects on rat brain development and function [69]. Rat pups were exposed to intraperitoneal infusion of DEHP (10 mg/kg/day x 7 days) during the crucial time of hippocampal development. These pups at day 26 showed decreased innervation and neuronal density in specific hippocampal regions in males but not females exposed to the DEHP [70] In a similar study, the same abnormal hippocampal development was also seen only in males exposed to DEHP [71]. DEHP exposure in utero caused metabolic

disturbances of the lipid metabolome of the fetal rat brain, causing anomalous brain growth [69]. Other animal studies on DEHP and brain development are detailed in a review by Rowdhwal et al. [72].

Prenatal exposure to phthalates has been associated with poorer infant executive function, attention, and motor reflexes [73, 74]. In-utero DEHP exposure has also been associated with childhood impairments of cognitive dysfunction, motor function, executive function, as well as hyperactivity [75, 76], and autism spectrum [77] in term infants [75, 78]. Another study looked at IQ in 7-year-old children as a function of phthalate levels of pregnant mothers in their third trimester. IQ was 6–7 points lower in the mothers exposed to the highest levels of two phthalates, DnBP, and DiBP. Stroupstrup has begun a large study to study DEHP exposure in premature infants [79], given the similarity of the above abnormalities, and similar "preterm behavioral phenotypes" seen in premature infants as described by Montagna and Nosarti [80].

## 5. Exposures of DEHP in premature infants

Children and adults are exposed to phthalates primarily through ingestion of food, inhalation of dust, chewing or sucking on objects, inhalation of vapor, skin absorption from personal care products [7, 9]. In contrast, premature infants are exposed both trans-placentally before birth, and through exposure to medical devices after birth [5]. There are little data on the magnitude of trans-placenta transfer of phthalates. Data on postnatal phthalate exposure is much more robust, and dates back almost 50 years to 1973 and 1974 when DEHP was shown to be present in blood plasma and cryoprecipitate products [81, 82]. Of importance, DEHP is the only FDA-approved phthalate allowed in medical products for use in the USA.

### 5.1 Early determinations of DEHP exposures in premature infants (1980–2003)

In the late 1980s, Schneider et al. was one of the first to report high levels of DEHP exposure in ECMO circuits [83]. Also during this time, Barry et al. showed presence of DEHP in cardiac bypass circuits [84]. Roth et al. and Latini et al. also presented data showing presence of DEHP in respiratory tubing and endotracheal tubes in mechanically ventilated preterm infants [85, 86]. A few years later, Latini and Chellini showed that PVC endotracheal tubes were about 23% DEHP by weight, and that almost half of the DEHP can leach into the infant over several days time [87, 88]. Chellini et al. estimated the exposure to a 2 kg infant could be a mean of 49 mg/kg over several days-time [87].

In 2000, Loff et al. were the first to demonstrate that PVC IV infusion lines expose infants to large amounts of DEHP [89]. Several years later, Loff showed that when lipids were administered through PVC IV lines (containing DEHP) could leach about 6.5 mg/kg/day for a 24 hour lipid infusion [11]. Loff advised that PVC IV lines containing DEHP should be abandoned – this has not happened. Similarly, Kambia et al. demonstrated large DEHP exposure in patients receiving parenteral (IV) nutrition [90].

### 5.2 Quantitative DEHP exposures in the NICU

In 2004, Antonio Calafat et al. were the first to report quantitative evidence that premature infants receiving intensive medical treatment are actually exposed to higher concentrations of DEHP than the general population [8]. Calafat's group examined 33 urine samples from 6 premature infants between 4 and 92 days of age.

Most of the samples were obtained after the first month. Samples were analyzed for MEHP as well as the two principal oxidative metabolites, MEOHP and MEHHP. During the hospital stay, these infants were exposed to a variety of devices, but the data was not robust enough to show the effect of particular interventions or devices. DEHP metabolites were measureable in all 33 samples, demonstrating the ubiquity of DEHP exposure in this population. Metabolite levels varied widely from sample-to-sample—up to 100-fold. The median concentration of MEHP in these 6 premature infants was 129 ng/mL, which is about 26-fold higher that the US median of 3.43 ng/mL seen in children aged 6–11 years of age [91, 92]. Similar findings were seen for MEOHP and MEHHP levels in premature infants as compared with the US median for children, although these two oxidative metabolites are typically about 10-fold higher than that for MEHP across all samples [8].

A follow-up study was done soon thereafter by the same group, this time dividing 54 neonates (from two institutions) into low, medium, and high DEHP exposure [93]. The "low" DEHP exposure group was comprised of infants receiving bottle or gavage feeds with no other interventions. The "medium" group was comprised of infants who either had indwelling feeding tubes, parenteral nutrition, or were on continuous positive airway pressure devices. The "high" DEHP exposure group was comprised of infants intubated and on a mechanical ventilator while also receiving parenteral nutrition. As expected, infants in the "low" category showed urine DEHP metabolite levels only slightly more than the US averages noted above. Exposures as measured in the median group were about 7-fold higher, with exposures in the "high" group another 3-fold higher than seen in the "medium" group. Interestingly, urine metabolite levels varied greatly, with DEHP metabolite levels in one NICU being about 4-fold that of the other. This was speculated to be due to a difference in the frequency two DEHP-containing devices used: PVC endotracheal tubes and PVC umbilical catheters [93].

A year later, this group examined two other phthalates, dibutyl phthalate and monobenzyl phthalate, both used in personal care and construction products [94]. Metabolites of these two phthalates were detected in all 54 of the samples from the prior study, but these levels did not vary by gender, institution, or level of intensiveness of care. This information suggests that the premature infants are not only exposed to phthalates through medical products, but also from other parts of their environment, whether it be in the air, in their feedings, or in materials they contact.

Using a different approach, Mallow used data from the above-reported DEHP exposures in the NICU to estimate a typical daily DEHP exposure for a prototypical infant receiving blood, intravenous nutrition (including lipid infusions), mechanical ventilation, and a feeding tube [12]. Mallow estimated that a 2-Kg infant would receive 16.3 mg/day of DEHP while on all of this intervention.

### 5.3 A recent comprehensive quantitation of DEHP exposures in an NICU

Over more than twenty years since many of the potentially toxic exposures of DEHP have been reported, manufacturers have come out with some alternatives to DEHP, which include use of other polymers aside from PVC, as well as alternative phthalate plasticizers [95]. Braun, USA, markets DEHP-free intravenous fluid in the USA. We have observed that feeding tubes, IV administration tubing, and endotracheal tubes are readily available in DEHP-free material, although DEHP-containing products are still marketed. The only medical devices for which DEHP-free materials have not been observed are bags of sterile water for respiratory humidification, suction devices, urine collection bags, and most tubing for respiratory devices [5].

This last year, our group sought to examine the current state of DEHP exposure in a single large Northwestern US NICU [96]. This was accomplished both by

direct and indirect measurement of DEHP exposure in premature infants. For all intravenous products, including IV fluid, hyperalimentation fluid, lipid emulsions, and tubing sets, we directly measured the amount of DEHP leaching into IV fluid collected in a glass container at the "patient" end of sham IV circuits. Indirect measurement of DEHP exposure was based on urine metabolites collected in 12 premature infants receiving one specific respiratory therapy for two days or more (mean of 13 days). Koch's method was used to predict 24-hour DEHP intake based on a formula estimating 44.2% of DEHP intake is excreted in the three main metabolites (MEHP, MEHHP, and MEOHP) [24].

These results (**Table 1**) showed that IV DEHP exposures were zero when DEHP-free IV fluid was tested using DEHP-free tubing. Similarly, lipid emulsions (other than a fish-oil product) when administered through DEHP-free tubing DEHP exposure was measured at zero (below level of detection). Use of intravenous fluid gave varying DEHP exposures, magnified many-fold when administered through DEHP-positive tubing. Most striking was the enormous exposure related to hyperalimentation and lipid infusions when administered through DEHP tubing. In this case, DEHP daily exposures were in the range of 12 mg per day (range of 3.4–45 mg/day).

DEHP exposures from respiratory devices, including patients receiving no known DEHP exposures, are shown in **Table 2**. A wide range of exposures was observed. Most exposures were low, and not statistically different from "baseline" patients in room air who were not receiving any intravenous fluid (or any other known or suspected DEHP exposure). The "baseline" patients had a median DEHP exposure of 25.5 mcg per day. The baseline exposure was presumed to be related to environmental (likely air vapor) in the NICU. Among these "low-level" exposures, the DEHP exposures from mechanical ventilators appeared the highest at 61.4 mcg/day. Given the small numbers of tests, this was not statistically different from the baseline patients.

DEHP exposure related to continuous positive airway pressure (CPAP), specifically the bubble CPAP system, was remarkably higher than any other device tested. The median daily DEHP exposure was 7843 mcg per day, which is about 300-fold that seen in baseline patients. There was strong evidence that the bubble CPAP exposures were not spurious, given no significant variation was seen among six samples obtained in 4 patients, and tested at two unrelated laboratories. This report did not specifically test CPAP systems separate from bubble CPAP, but noted a 2019 study where two mask (non-bubble) CPAP patients had urine metabolite levels below the level of detection [5]. Lastly, no data is available for DEHP exposures from oscillator, jet ventilator, or non-invasive ventilation systems.

This study estimated DEHP exposure in 14 premature infants based on a chart review of all recorded IV and respiratory DEHP exposures. The value from the tables above were used to tabulate the daily DEHP exposure for each patient. **Table 3** shows the mean exposures for these 14 infants for each fluid or device. The mean DEHP exposure from IV fluid including hyperalimentation and lipid infusions was 4,039 mcg over the course of the NICU stay. The mean respiratory DEHP exposure was much higher than the intravenous exposures at 221,369, with 97% of that exposure attributable to bubble CPAP therapy. The total DEHP exposure for the NICU stay was a mean of 182,369 mcg/kg.

### 5.4 Determination of safe levels of DEHP exposure

Determination of safe levels of DEHP exposure has proved to be a complex and daunting task. One of the difficulties is that extrapolation of animal data to humans can be misleading. Animal studies may be done with different levels of exposure, and may differ in timing from human exposure (acute vs. chronic). For example,

| Intravenous Product | Median | IQ Range | Range | Median | IQ Range | Range | Median | IQ Range | Range |
|---|---|---|---|---|---|---|---|---|---|
| | From Container | | | DEHP-Negative IV Set | | | DEHP-Positive IV Set | | |
| DEHP-negative IVF | 0.0 | 0.0 | 0–0.2 | 0.0 | 0.0 | 0–0 | 5.2[b] | 12.1 | 2.4–15.0 |
| DEPH-unlabeled IVF | 27.0 | —[a] | 26–40 | 2.4 | 1.4 | 1.4–3.2 | 11.0[b] | 16.2 | 2.3–26.0 |
| DEHP-positive IVF | 560.0 | —[a] | 560–620 | 32.0 | 24.1 | 7.6–40 | 15.0 | 24.8 | 4.4–38.0 |
| HA fluid | 5.1 | —[a] | 3.4–13.0 | 6.7 | 13.1 | 0–16 | 500.0[b] | 1430 | 420–2300 |
| Mixed lipid emulsion | 1.9 | 4.4 | 0–5.9 | 0.0 | 0.0 | 0–0 | 9300[b] | 4300 | 6100–13,000 |
| Fish oil lipid emulsion | 8.3 | 10.8 | 0–15 | 92.0 | 62.6 | 74–170 | 4000[b] | 3350 | 2100–6900 |
| Soybean lipid emulsion | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 12,000[b] | 23,050 | 3400–45,000 |

[a]*Unable to calculate interquartile range due to n = 3.*
[b]*Denoting signifant difference (p, 0.05) in median DEHP concentration between fluid without and with DEHP in the IV set using Wilcoxsen ranked sum test.*
*IV, intravenous; IVF, Intravenous fluid; DEHP, di-2-ethylhexyl phthalate; ND, not dectected; HA, hyperalimentation fluid, IQ, interquartile range. Reproduced from [96] 2021, Toxics.*

**Table 1.**
*DEHP content (mcg/L) in three types of IV fluid, one type of HA fluid, and three types of lipid emulsion.*

| Respiratory Device | *n* | Median (mcg/day) | IQ Range (mcg/day) |
|---|---|---|---|
| Bubble CPAP | 5 | 7843.5[b] | 6500.5 |
| Room Air (baseline) | 5 | 25.5 | 42.5 |
| HFNC | 5 | 21.6 | 20.3 |
| LFNC | 1[a] | 7.3 | NA |
| Ventilator with DEHP-negative ETT | 5 | 61.4 | 174.1 |

[a]*Four samples excluded due to additional IVF received by the patient.*
[b]*Signifant difference ($p < 0.05$) in median DEHP exposure between the baseline and other respiratory device using Wilcoxsen ranked sum test.*
*Reproduced from [96] 2021, toxics.*
*CPAP, continuous positive airway pressure; HFNC, high-flow nasal cannula; IQ, interquartile range; LFNC, low-flow nasal cannula; DEHP, di-(2-ethylhexyl phthalate); ETT, endotracheal tube; NA, not able to calculate an interquartile range when N = 1.*

**Table 2.**
*Daily DEHP estimated exposures of respiratory therapy device based on urine metabolites of DEHP.*

| Mean cumulative DEHP exposure by IV product or respiratory device | Quantity (mL-days) | Mass (mcg) | Totals (mcg/Kg) |
|---|---|---|---|
| Conventional intravenous fluid | 454 mL | 5 | |
| Starter (initial) hyperalimentation | 133 mL | 67 | |
| Hyperalimentation fluid | 2283 mL | 1141 | |
| Lipid emulsions | 274 mL | 2847 | |
| Total intravenous DEHP | | 4039 | |
| Mechanical ventilator[a] | 23 days | 6616 | |
| Bubble CPAP | 28 days | 219,338 | |
| NIPPV | 2 days | 127 | |
| Low flow nasal cannula | 1 day | 4 | |
| High flow cannula | 3 days | 69 | |
| Mean respiratory DEHHP | | 221,369 | |
| Mean IV + respiratory DEHHP | | 230,207 | |
| Mean IV + respiratory DEHHP per Kg | | | 182,369 |

[a]*Using non-DEHP endotracheal tube.*
*All IV tubing was DEHP-positive in these patients. Reproduced from [96] 2021, toxics.*
*DEHP, Di-2-ethylhexyl phthalate; VLBW, Very low birth weight; IV, Intravenous; CPAP, Continuous positive airway pressure; NIPPV, Noninvasive positive pressure ventilation.*

**Table 3.**
*Mean cumulative DEHP exposures for 14 VLBW infants based on actual IV and respiratory exposures using above derived values for DEHP exposure for each device.*

early work in animals suggesting that DEHP was a carcinogen, and that it might result in injury to male testicular tissue with resultant fertility reduction [7]. These problems have not occurred thus far in children.

Cardiac contractility and viability may only be affected by the extreme exposures, such as with cardiac bypass or extracorporeal membrane oxygenation circuits [50]. Still, increased blood pressure can be seen with much lower levels [2]. Hypertension has been seen with actual and currently occurring DEHP exposures [5]. It is usually seen in patients with substantial postnatal DEHP exposures, although the contribution of prenatal phthalate exposures remains unknown, and safe levels of postnatal DEHP exposure is unknown.

Liver problems, however, related to actual DEHP exposure were substantiated as cholestasis in premature infants was markedly reduced when DEHP IV tubing was avoided [68]. In this case, animal studies may also be relevant. Mallow showed daily intake of DEHP exposure was 162,459 times that deemed as acceptable by the U.S. Consumer Product Safety Commission [12].

The US Environmental Protection Agency (EPA) established an estimate called the DEHP reference dose (RfD), as the estimate of safe daily intake of DEHP. This was set in 2003 at 20 mcg/kg/day [91]. A Canadian study in 1994 estimated DEHP exposure in infants at 9 mcg/kg/day [92]. A more recent study in a subset of the U.S. estimated that typical exposure is 0.71 mcg/kg/day [97]. The mean U.S. DEHP level associated with this exposure was 2.7 ng/mL [97].

The Consumer Product Safety Improvement Act was passed by the US Congress in 2008. This act limited the concentration of six phthalates including DEHP to a limit of 0.1% for toys and childcare products [98]. In 2007, the European Union effectively banned DEHP from use in medical products [99]. Despite this directive, when evaluating European infusion fluid labeled DEHP-free, only two of nine devices were indeed DEHP-free [22]. Concern was also raised that the alternative phthalate plasticizers have not been fully studied as to risk of adverse effects in humans [22, 99].

France, in 2015, banned used of DEHP-containing tubes in neonatal, pediatric, and maternal units [100]. The FDA in the US limits phthalate use in medical products to just DEHP. The FDA has not yet set a limit for DEHP, or designated safe levels of DEHP use in the US, leaving decisions about use of products containing DEHP to providers and hospital administrators. Our experience is this effectively leaves the decision up to manufacturers and distributors, as toxicity concerns are rarely raised by either hospital staff, providers, or administrators.

## 6. Actions to reduce DEHP exposures in premature infants

Since premature infants are so small and are still in the midst of development it would seem prudent to avoid potential toxicants whenever possible. At a minimum, one should strive to reduce neonatal exposure to less than 20 mcg/day, the estimated safe limit set by the EPA. Lower levels of exposure should be achieved if possible. In the past, arguments were made that the benefits of life-saving medical devices outweigh the unknown and ill-defined risks [9]. Now, much of the exposure can be eliminated by choice of products currently available by manufacturers.

The easiest step to take would be to not use IV administration sets containing DEHP, especially for administration of blood products, hyperalimentation fluid, or lipid emulsions. DEHP-free infusion sets are readily available, with little cost differential.

The second step would be to use only DEHP-free IV fluid, at least for premature infant and maternal use. DEHP-free commercial IV fluid is available in the US and abroad.

The third, and potentially the most crucial step would be to limit exposure to respiratory devices found to have very large DEHP exposures. We tested only one bubble CPAP system, and currently have no data on that from other manufactures of bubble CPAP. Advice on CPAP alternatives is beyond the scope of this publication, but modification of current bubble CPAP, or providing CPAP using either a ventilator device or with a linear pressure generator might be preferred from a toxicity viewpoint. Specific testing of suspected high-exposure devices may be helpful in assessing the risk/benefit ratio for clinical use.

These changes in medical products should result in marked reductions in DEHP exposure, but one has to be vigilant about: 1.) New products that contain DEHP and may not be labeled concerning its phthalate content. 2.) Supplies that change on a daily basis without providers' and staff's knowledge. This author has seen this occur on many occasions. 3) Current products in clinical use which do not have available testing data (such as oscillator or jet ventilators).

We suggest periodic checking of IV supplies used in maternal units, and all supplies used in the NICU for products labeled to contain DEHP. It may also be prudent to have some system for checking a random patient's urine for DEHP metabolites, as a screen for unexpected DEHP exposure. Lastly, building materials (primarily flooring and wall coverings) should be selected to minimize phthalate environmental exposure in the NICU.

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## Acronyms and abbreviations

| | |
|---|---|
| DEHP | di-(2-ethylhexyl) phthalate |
| PVC | polyvinyl chloride |
| IV | intravenous |
| NICU | newborn intensive care unit |
| MR | mineralocorticoid receptor |
| 11β-HSD2 | 11β-hydroxysteroid dehydrogenase type 2 |
| AME | apparent mineralocorticoid excess |
| MEHP | mono-(2-ethylhexyl) phthalate |
| PRA | Plasma renin activity |
| MEHHP | mono-(2-ethyl-5-hydroxyhexyl) phthalate |
| MEOHP | mono-(2-ethyl-5-oxohexyl) phthalate |

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Neonatology is one of the areas of greatest development and evolution within pediatrics. Every year there are advances in the management of the different diseases that newborns develop, which makes it necessary to refresh knowledge on traditional and other emerging issues. This book includes six chapters that address critical and relevant issues in neonatal care and seeks to contribute to the clinical work of health teams in neonatal units.

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