VILNIUS UNIVERSITY

FACULTY OF MEDICINE

Medicine

Institute of Clinical Medicine, Clinic of Children's Diseases

Hannah Celina Schoellhorn, Year 6, group 9

INTEGRATED STUDY MASTER'S THESIS

Anemia and Red Cell Transfusions in Very Low Birth Weight (VLBW) Babies

Supervisor Prof. Dr. Arūnas Liubšys

Head of the department Prof. Dr. Augustina Jankauskienė

Vilnius, 2025

hannah.schoellhorn@mf.stud.vu.lt

1. Abbreviations

AOP	Anemia of Prematurity
BPD	Bronchopulmonary Dysplasia
CBS	Cord Blood Sampling
C-UCM	Cut-Umbilical Cord Milking
CMV	Cytomegalovirus
CPAP	Continuous Positive Airway Pressure
DCC	Delayed Cord Clamping
ELBW	Extremely low birth weight (<1000g)
EPO	Erythropoietin
GA	Gestational Age
Hb	Hemoglobin
Hct	Hematocrit
IVH	Intraventricular Hemorrhage
I-UCM	Intact-Umbilical Cord Milking
LBW	Low birth weight (<2500 g)
NEC	Necrotizing Enterocolitis
NICU	Neonatal Intensive Care Unit
PDA	Patent Ductus Arteriosus
POC	Point-of-care
RBCT	Red Blood Cell Transfusion
RBC	Red Blood Cell
ROP	Retinopathy of Prematurity
TRALI	Transfusion-related Acute Lung Injury
UCM	Umbilical Cord Milking
VLBW	Very low birth weight (<1500g)

2. ABSTRACT

This thesis evaluates the issue of anemia in very low birth weight newborns, with an emphasis on the effects of regular blood sampling and red blood cell transfusions. After establishing the significance of anemia in very low birth weight infants, the causes and risk factors will be explained. The thesis then portrays the consequences of anemia for this group of infants, and leads to its main chapter, dedicated to prevention and treatment methods for anemia for these patients. Treatment and prevention methods are categorized as either pharmacological or non-pharmacological, with the main method being red blood cell transfusion. The last chapter covers complications associated with red blood cell transfusion and offers a view towards future steps in science as well as treatment and prevention especially by improved blood sampling protocols.

3. KEYWORDS

Anemia, Prematurity, Blood Sampling, Red Blood Cell Transfusion, very low birth weight (VLWB)

4. INTRODUCTION

Anemia in very low birth weight (VLBW) newborns is a major health concern, especially given the high frequency with which blood is drawn in neonatal settings. VLBW newborns, defined as those weighing between 1000 to 1499 grams at birth, are more prone to anemia due to their immature hematopoietic systems and the large number of blood tests required for their medical care. Anemia not only causes acute health issues, but it also has long-term implications for growth and neurodevelopment. The frequent requirement for Red Blood Cell Transfusions (RBCT) to treat anemia in these newborns complicates their care, thereby increasing concerns about transfusion-associated hazards and the best management techniques. Regular blood sample and RBCT are both necessary and possibly hazardous, requiring a delicate balance of monitoring and minimizing interventions. The significance of this thesis lies in its comprehensive summary of this medical problem and the assessment of treatment and prevention effectiveness for the survival and quality of life of VLBW babies. Despite advances in neonatal care, anemia in VLBW newborns remains a widespread issue, emphasizing the need for better techniques to reduce iatrogenic blood loss and to optimize RBCT protocols. The aim of this thesis is to assess the outcomes of regular blood sampling on anemia in VLBW babies and to identify effective ways for lowering the need for RBCT.

5. LITERATURE REVIEW

5.1 RELEVANCE OF THE PROBLEM

Prematurity has been the top cause of newborn mortality for more than a decade, and it is currently the greatest cause of pediatric mortality up to the age of five, making it both a societal and an emotional burden for affected families worldwide. Every year, an estimated 15 million infants are delivered prematurely across the world, accounting for around 11% of all births. Preterm birth rates appear to be rising in numerous nations, mostly high-income countries (1). It can therefore be assumed that these rates could further increase with positive global development of Gross Domestic Product (GDP) and income.

Although various socio-demographic, dietary, medical, obstetric, and environmental variables have been proven to raise the risk of spontaneous preterm delivery, its etiology is still poorly understood (2). Given the significant worldwide burden of preterm births, which are a main cause of neonatal and pediatric mortality, it is crucial to explore the incidence of anemia in severely preterm newborns. It is important to understand, while prematurity does not equal low birth weight, there is a high correlation between the two. Preterm newborns, particularly those with very and extremely low birth weight, have a variety of distinct risk factors that make them particularly vulnerable to anemia. This sensitivity is increased by their immature hematopoietic system and the many interventions they require. Since blood sampling for diagnostic purposes is the most common procedure performed on newborns, this exacerbates the already existing challenge of anemia in the Neonatal Intensive Care Unit (NICU). The overall effect of regularly drawing blood from the organism of a newborn is obviously related to the ratio of the amount of withdrawn blood relative to the weight of the infant. Therefore, the amount of venous and arterial blood samples is especially important, while capillary blood sampling is a factor, too. Understanding and managing anemia and the influence of blood sampling is therefore crucial for improving neonatal care and lowering morbidity and death rates in this vulnerable population.

This literature review focuses on VLBW newborns. These are infants weighing 1000 to 1499 grams at the time of birth. Extremely low birth weight (ELBW) newborns are defined as weighing under 1000 grams. They are considerably more vulnerable to anemia, nonetheless, they account for fewer than 1% of all births, whereas VLBW newborns account for around 1.4% of all births, depending on the country (3). A study from Thailand, which analyzed ELBW newborns between 2015 and 2020 even reports a figure as low as 0.175% of all live births (4). Given their higher incidence and increased susceptibility to anemia and related challenges, VLBW babies are a particularly vulnerable population that requires targeted study and clinical care.

5.2 CAUSES AND RISK FACTORS OF ANEMIA IN VLBW INFANTS

5.2.1 MATERNAL FACTORS

Research indicates that neonatal anemia is influenced by various maternal factors, including the mother's anemia status, age, preterm birth, type of delivery, as well as health and lifestyle factors, such as inadequate intake of iron, folate, and vegetables (5).

Anemia is the most common nutritional deficiency in pregnant women, affecting approximately 40% of pregnant women worldwide. This prevalence has reached levels of moderate to severe public health importance as defined by the World Health Organization (WHO), indicating that any adverse effects of maternal anemia on neonatal anemia could have substantial public health consequences. Therefore, understanding the relationship between maternal and neonatal anemia is critical (5).

The increased metabolic demands of pregnancy make women particularly vulnerable to iron deficiency, which, if untreated, can lead to maternal anemia. Studies have shown that pregnant woman reach their lowest point of serum ferritin levels in week 35-38 of pregnancy (5). Since the fetus depends on maternal iron stores, insufficient iron levels in the mother increase the risk of neonatal anemia. These findings emphasize the importance of adequate maternal nutrition, including iron and folate supplementation and sufficient vegetable and fruit intake during pregnancy, to reduce the risk of anemia in both mothers and their newborns. For instance, studies from Ethiopia have found that low vegetable consumption and insufficient iron and folate supplementation during pregnancy are significantly associated with neonatal anemia (6). Additionally, mode of delivery plays a role: research from Afghanistan has shown that Cesarean delivery is linked to lower levels of hemoglobin (Hb) in the umbilical cord compared to vaginal birth, potentially raising the risk of neonatal anemia (7).

5.2.2 PHYSIOLOGICAL PECULARITIES

Hb levels in postpartum physiologically decrease significantly and reach a lowest point (9.5-11.0 g/dl) between the age of six to twelve weeks. This postnatal decline of Hb in term newborns is generally tolerated and does not require intervention, hence it is usually referred to as 'physiological anemia of infancy'. In contrary, preterm and low-birth-weight newborns exhibit higher levels of anemia than term neonates. The Hb decrease in premature infants is happening earlier (nadir at 4-6 weeks) and the Hb level falls to lower levels. (to ~ 8 g/dL in 1000 to 1500 g VLBW infants, to ~ 7 g/dL in ELBW infants <1000 g). The frequently developed anemia in preterm infants is highly related to their gestational age (GA) and develops from a combination of physiological and pathological

factors. This so-called ‘anemia of prematurity’ requires intervention and it is not seen as a physiological or benign phenomenon (8-11).

The physiological decline in Hb can be explained with the short red blood cell (RBC) lifespan, reduction of erythropoiesis, and quick growth after birth in newborns.

1. As opposed to 120 days in adults, neonatal RBC life span is about 90 days. With around 35-50 days, the neonatal RBC life span in preterm infants is even shorter (12).

2. Erythropoietin (EPO) synthesis and erythropoiesis are negatively impacted by the sudden spike in tissue oxygen levels brought on by the increase in oxygenation that comes with regular breathing in an oxygen-rich extra-uterine environment. Although anemia stimulates EPO synthesis in preterm newborns, plasma levels in anemic infants are lower than those in comparable anemic older patients. The processes responsible for decreased plasma EPO levels in preterm newborns are still insufficiently understood, and they are likely to be numerous. The liver, which is less sensitive to tissue hypoxia and anemia, being the primary production site of EPO in preterm infants, is just one element of explanation. Reduced EPO production cannot fully explain the low plasma EPO levels in anemic newborns, as some infants have been found to have very high plasma EPO levels. Other processes indicating increased metabolism, such as fast plasma clearance and fractional elimination, as well as short mean plasma residence times, likely contribute to reduced EPO plasma levels (10,12).

3. During the early months of life, extrauterine body development is extraordinarily fast, and RBC production by neonatal marrow must increase at the same pace (10,12). Hemodilution brought on by an increase in blood volume following weight gain could be another contributing factor in physiologic anemia (13)

5.2.3 BLOOD SAMPLING

As interesting as physiologic mechanisms after delivery are for anemia in infants, they are not the primary cause of anemia in the NICU. Non-physiologic variables including clinical diseases with sepsis, obstetrical complications, poor nutrition, cardio-respiratory illnesses, as well as iatrogenic blood loss from repeated laboratory tests, exceed the impact of physiologic decline by far. Especially the frequent laboratory testing required in preterm newborns leads to additional iatrogenic blood loss resulting in extremely low levels of Hb. The infants who were born most prematurely, require most blood sampling, resulting in the greatest loss of red blood cells in absolute terms, but even more when looked at in relation to their small circulating blood volume. Laboratory testing in the NICU during the first 6 weeks of life can cause blood loss, ranging from 15% to 30% of an infant's total blood

volume (11). To put it in absolute numbers: Out of around 80 ml/kg total circulating volumes in neonates, 11-22 ml/kg of blood is lost weekly in the first six weeks of age, due to blood sampling in VLBW infants (14).

It is crucial to understand the particular blood tests that are most commonly administered to VLBW and other critically ill newborns when thinking about neonatal laboratory blood loss. Understanding the importance of each laboratory test can help establish strategies to reduce anemia. In a study the analytes drawn most in 50 ill and ventilated VLBW neonates included pH, PCO2, PO2, electrolytes, glucose, Hb, calcium, lactate, bilirubin, creatinine, blood urea nitrogen, and Hb, arranged in decreasing order of frequency. Other tests that were conducted least often included blood culture, as well as type and cross-matching. Neonatal caregivers may be able to reduce needless blood sampling by carefully evaluating each laboratory blood sample for its need and urgency in terms of care measures (14).

5.2.3 PATHOLOGIC CONDITIONS CAUSING ANEMIA

In addition to iatrogenic blood loss, anemia in neonates can be exacerbated by various non-physiological conditions. The Etiology of anemia in newborns can be classified in three categories:

1. Anemia due to Hemorrhage
2. Anemia due to Hemolysis
3. Anemia due to Failure of red cell production.

Blood loss/ Hemorrhage can occur before delivery (prenatal), during birth (intranatal), or after birth (postnatal), and can come from transplacental, intraplacental, or extraplacental sources. It is the most common cause and includes obstetrical etiology (placental abruption, placenta previa, and trauma to the placenta or umbilical cord during delivery), fetomaternal hemorrhage, fetoplacental hemorrhage, twin-twin transfusion, internal hemorrhage, as well as iatrogenic blood loss by phlebotomy. Fetomaternal hemorrhage can occur spontaneously or result from maternal trauma, traumatic amniocentesis, external cephalic version or a placental tumor. In twin-twin transfusion the Hb levels between the twins differ by 5 g/dl and may cause anemia in the donor twin (15).

Hemolysis, defined as RBC destruction, can be caused by immune hemolysis (Rh incompatibility, ABO incompatibility and minor antigen incompatibility) and nonimmune hemolysis (Infections, Toxic exposure, Vitamin E deficiency). Other rare causes include hereditary disorders such as RBC enzyme defects (G6PD deficiency, Pyruvate kinase deficiency), RBC membrane defects (Hereditary

spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis, hereditary stomatocytosis), and hemoglobinopathies (α-thalassemia) (15).

Impaired red blood cell production can be caused by either congenital etiologies, such as Diamond-Blackfan anemia, Dyskeratosis congenita, Fanconi anemia and sideroblastic anemia, or by acquired etiologies such as infections (CMV, Rubella, Parvovirus B, Malaria, Toxoplasmosis, Syphilis) and Nutritional Deficiencies (Iron, Folate, Vitamin B12) (13,15,16).

Table 1: Etiologies of anemia in newborns

Hemorrhage	**Hemolysis**	**Impaired RBC Production**
Fetomaternal Hemorrhage Fetoplacental Hemorrhage Twin-twin Transfusion Obstetrical Causes (Placenta Previa, Placental Abruption, Trauma to Placenta or Cord) Internal Hemorrhage Iatrogenic Blood Loss	Immune: - Rh Incompatibility - ABO Incompatibility - Minor Antigen Incompatibility Non-Immune: - Infections, Toxins, Vitamin E Deficiency - RBC enzyme defects (G6PD Deficiency, Pyruvate Kinase Deficiency) - RBC Membrane Defects (Hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis, hereditary stomatocytosis) - Hemoglobinopathies (α-thalassemia)	Congenital: - Diamond-Blackfan anemia - Dyskeratosis congenita - Fanconi anemia Acquired: - Infections - Nutritional Deficiency (Iron, Folate, Vitamin B12)

5.3 CONSEQUENCES OF ANEMIA

Anemia of prematurity can lead to various clinical symptoms including tachycardia, tachypnea, poor weight gain, need for supplementary oxygen, as well as increased apneic episodes. These symptoms are primarily caused by inadequate oxygen delivery to critical tissues. Due to the resulting inadequate oxygen supply, anemia in newborns can cause acute organ dysfunction, such as heart failure, respiratory distress, or multi-organ failure in severe cases. E.g.: Intestinal tissue hypoxia caused by anemia can cause intestinal cell injury, and thus may result in Necrotizing Enterocolitis (NEC), a severe gastrointestinal disease (17). What's more, the hypoxia caused by anemia could result in reduced brain oxygenation which may negatively impact neurodevelopment in short and long

term (18). Besides, decreased neurocognitive development, growth restriction and increased susceptibility to infection in life could be long-term effects of chronic hypoxia (19).

Therefore, Some VLBW newborns may require therapies such as RBCT, iron supplementation, erythropoiesis-stimulating medications, and regular laboratory monitoring to manage the anemia. The exact management of anemia in newborns is usually determined by etiology, severity, and gestational age. Possible methods to prevent and treat anemia will be further explored in the next chapter.

5.4 METHODS TO PREVENT AND TREAT ANEMIA

As discussed in the preceding chapters about causes of anemia, there are many factors involved and consequently, many possible prevention strategies are available.
When looking at the maternal factors, vaginal delivery as opposed to a cesarean section may help prevent anemia. Improving maternal nutritional status, especially vegetable consumption, can have a positive impact on reducing anemia among newborns. Additionally, Iron and Folate substitutions can have a positive impact regarding anemia prevention or reduction.

Significant progress has been achieved in reducing preterm anemia by techniques to maximize and preserve neonates' circulatory volume, such as placental transfusion at delivery and the minimum sample procedure. The majority of VLBW preterm infants with AOP recover within 3–6 months of age, but some require measures like close laboratory monitoring together with packed RBCT, iron supplementation or erythropoiesis-stimulating agent administration (11). Methods to reduce and treat anemia in newborns can be divided into non-pharmacological and pharmacological interventions.

5.4.1 NON-PHARMACOLOGICAL INTERVENTIONS

5.4.1.1 DELAYED CORD CLAMPING

Delayed Cord Clamping (DCC) is a widely used method to ensure adequate newborn blood volume and thus preventing anemia. Following the “radical” transition from intra-uterine to extra-uterine life, a sufficient circulatory volume is crucial as a start into the neonatal phase. A placental blood transfusion of whole blood, red blood cells and stem cells is therefore an excellent means to accomplish that for the newborn right after a natural vaginal delivery, just before the umbilical cord is clamped. Term infants undergoing immediate cord clamping can lose up to 30% of their blood volume, whereas preterm infants under 30 weeks of gestation may lose up to 50% (20,21).

The arguments supporting DCC include hematologic benefits, hemodynamic benefits such as improved blood pressure, decreased need of inotropes and improved systemic and cerebral blood flow, additional iron that has been shown to increase the growth of the infant`s white matter of the brain, and improved neurodevelopmental outcomes for term and preterm infants in general. However, the exact mechanism behind these outcomes are still not fully understood. (22). According to a meta-analysis, DCC reduces all-cause mortality among preterm newborns by 30% (20).

The prolonged placental transfusion for at least 30 to 60 seconds is recommended by the World Health organization and results in a blood volume of approximately 93ml/kg after three minutes. The Neonatal Resuscitation Program (NRP) recommends 60 seconds of delay for preterm and term infants not needing resuscitation, while the American Congress of Obstetricians and Gynecologists recommends delayed cord clamping for 30 to 60 seconds for preterm infants without the need to resuscitate. When it comes to extremely preterm children, the best time to clamp the cord is more complex and multi-factorial to determine and should optimize placental transfusion without compromising stabilization and resuscitation efforts (23).

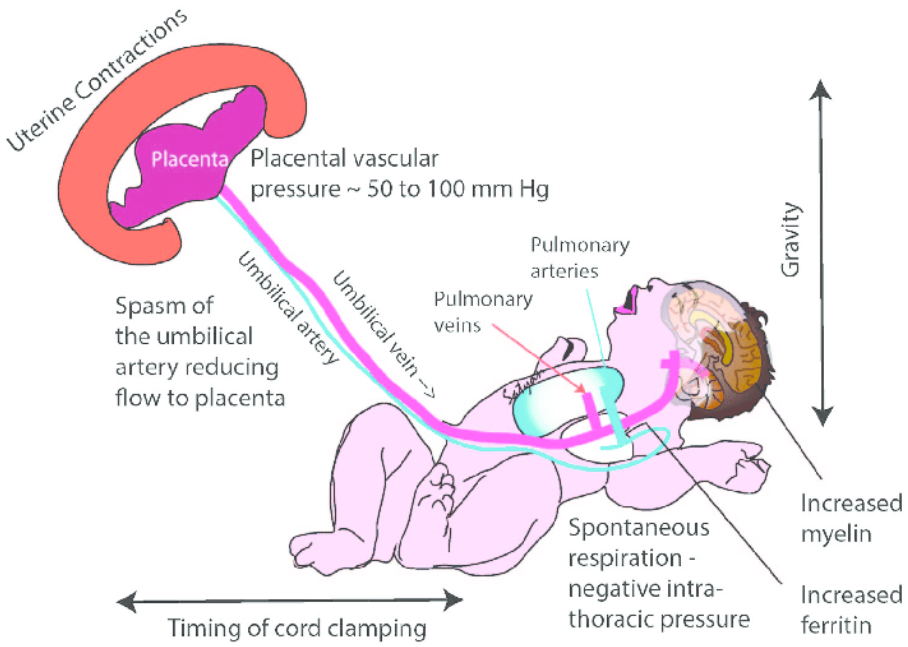
Eighteen randomized trials on delayed and early cord clamping were systematically reviewed by Fogarty and colleagues. In these trials for newborns born before 37 weeks, DCC was defined as 30 or more seconds and early cord clamping as under 20 seconds. In addition to increasing peak hematocrit (Hct) by approximately seven percent and decreasing the percentage of newborns requiring blood transfusions by ten percent, DCC significantly decreased hospital mortality by 30% (24).

Another observational study from California comparing DCC after 60 to 75 seconds with 30 to 45 seconds in preterm infants (<32 weeks) found immediate outcomes in the delivery room, as well as general neonatal outcomes. The study group with prolonged placental transfusion showed higher Hct in the first 2 hours after delivery, reduced the need for intubation in the delivery room and in the first 24 hours, as well as less intubation in general. Other findings included fewer newborns with hypothermia, reduction in surfactant therapy and red cell transfusion during hospitalization (23). DCC has been demonstrated to enhance myelin content in brain regions related to motor, sensory and visual function at 4 months of age when compared to early cord clamping (20).

It is important to take into consideration that a wide variation in the definitions and methods of early and delayed clamping across studies may influence any quantitative difference in observed outcomes. The positive effects are qualitatively demonstrated in each of them.

Hence, DCC is recognized as a standard procedure around the whole world. In full-term newborns, there are only very few minor negative outcomes recorded, including jaundice and polycythemia. The optimal duration of the delay is however not defined yet (23,25).

Figure 1: Placental transfusion with delayed cord clamping (DCC)



5.4.1.2 CORD MILKING

Umbilical Cord Milking (UCM) can be a useful alternative, especially when DCC is not possible and/or not recommended in unstable patient. It can accelerate placental transfusion when the situation does not allow DCC. The benefits mentioned above for DCC also apply for UCM.

Umbilical Cord Milking can be divided into two types: Intact-UCM (I-UCM) and Cut-UCM (C-UCM).

In I-UCM the umbilical cord is clamped after being firmly milked with the thumb and forefinger, forcing blood from the placental end toward the baby multiple times. In C-UCM, a 30 to 40 cm long section of umbilical cord is clamped and cut right away (either at the placenta's cord insertion site or close to the introitus), untwisted, and gradually milked toward the baby before the cord is clamped at the base (20).

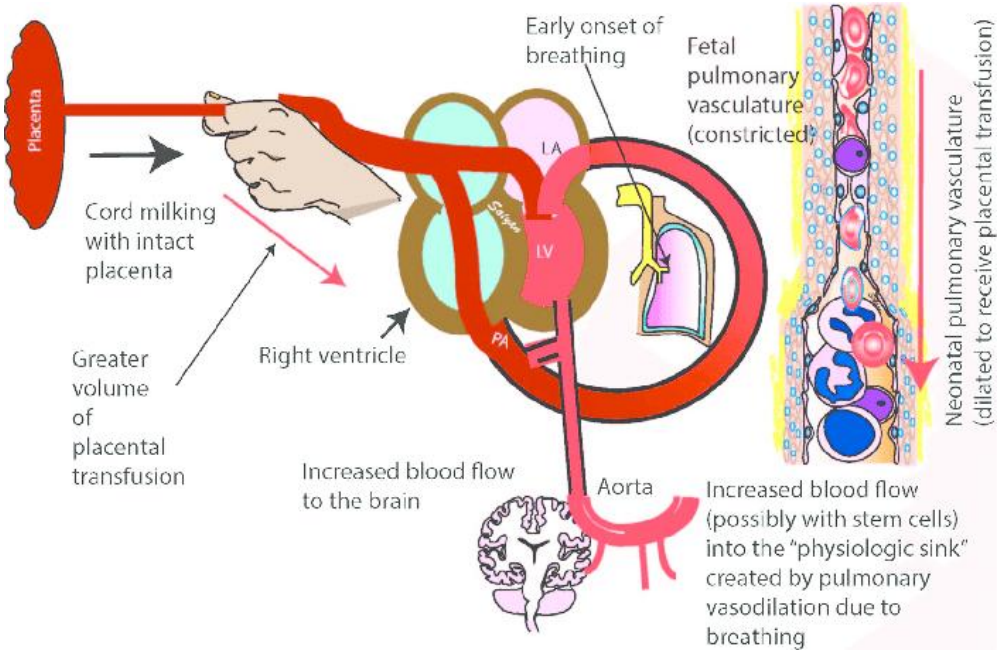
Neonates who receive I-UCM have greater Hb levels, lower risks for chronic lung illness, and lower IVH of all grades than those who receive immediate cord clamping, according to a recent meta-analysis of seven randomized-controlled trials in preterm newborns under 33 weeks (26).

A study comparing outcomes between I-UCM and C-UCM in term infants report no significant differences in terms of hemodynamic and hematological adaptation, as well as cerebral oxygenation (27).

To summarize, UCM (I-UCM and C-UCM) and DCC are effective methods for enhancing newborn outcomes, with UCM providing as a viable alternative when DCC is not possible. Ongoing research

is improving our understanding of the safest and most effective method for a variety of clinical scenarios.

Figure 2: Placental transfusion through Intact- Umbilical Cord Milking



5.4.1.3 MINIMIZING IATROGENIC BLOOD LOSS

Minimizing iatrogenic blood loss is another effective way to avoid anemia in newborns. It has been observed that critically sick preterm newborns lose eleven to twenty-two ml/kg/week for laboratory testing, which is comparable to fifteen to thirty percent of their blood volume (28).

A study conducted at Leiden University Medical center in 2016, analyzed the parameter of gestational age in correlation to anemia caused by phlebotomy in an observational study including 20 extremely preterm infants born before 28 weeks of gestation. This study recorded the volume of blood sampling in the first 4 weeks, the exact number of punctures performed either for phlebotomy or IV access, as well as the amount of blood loss by these procedures was recorded. A blood loss of 30.2 ml/kg at 24 weeks of GA and 15.9 ml/kg at 27 weeks showed that blood loss is highest in the most preterm infants. A median blood loss of 28.5% of the circulating blood volume was recorded, the number of punctures per patient during the initial 4 weeks of life was 47, and volume of RBCT administered was 30 ml/kg. In the first month of life, blood loss for tests and procedures causes extreme preterm infants to lose nearly one-third of their total blood volume. 90% of the infants under 1000 grams received one or

more transfusions, with a median of two, while 40 % of low birth weight infants receive RBCT (29, 30).

A retrospective study from Taiwan compared 20 VLBW infants (under 1500 g/ GA less than 32 weeks) that had undergone normal blood sampling with a study group of 84 infants who underwent small volume blood sampling. Before the introduction of small volume blood sampling in 2016, 1ml for each complete blood count, electrolytes, as well as blood gas analysis was required by the laboratory. After the introduction of new devices, the required amount for the total analysis of Hct, Hb, Na+, K+, Ca2+ and gas analysis decreased to 0,2 ml. The blood sampling loss (22,1 ± 8,4 vs. 52,5 ± 18,4 in ml) as well as the total RBCT volume (12,8 ± 16,0 vs. 26,0 ± 16,0 in ml) after 30 days was significantly lower in the study group, while Hct (29,8 ± 4,0 vs. 21,4 ± 13,2 in %) and iron levels were significantly higher. The protocol of small volume blood sampling lead to a higher bone marrow function after 30 days of age (8, 11).

Thus, reducing blood loss from phlebotomy and assessing the need for testing is an essential step in avoiding AOP. Microsample point-of-care (POC) devices can now measure a wider variety of analytes thanks to recent technological advancements. Electrolytes, hemoglobin, bilirubin and blood gas analysis are routinely tested via capillary POC devices. It is a device that makes it possible to conduct tests outside of a hospital's laboratory, while only needing a tiny amount of blood taken from capillary sources from the heels. Compared to standard blood tests requiring 3.5 ml, the POC device in pediatrics requires only 18 µl of blood. Research comparing a POC analyzer to a standard blood analyzer found POCs to be sufficiently trustworthy to detect Hb concentration. Readings of the platelet count and red blood cell count, however, should be taken with caution due to a small overestimation of these results. Nevertheless, the POC device can reduce anemia in newborns. After the introduction of a POC system at Stanford Medical University, which is able to measure pH, pCO2, Hb, K+, Ca2+ and Na+, a 30% decrease in phlebotomy performed was seen. Consequently, a drop of 43% in transfusions in newborns aged two weeks was observed, due to the decreased amount of phlebotomy (27-29)

Furthermore, it is possible to get first laboratory investigations using the easily accessible umbilical cord blood, which would otherwise be wasted. This eliminates the need to collect sizable amounts of blood from the newborn for admission laboratory testing. There are two methods of Cord Blood Sampling (CBS): Method A (see figure 4) method uses the vein / artery of an isolated segment of the cord that was clamped and cut before or after the delivery of the placenta. Method B (see figure 4) uses a fetal vessel on the placental surface close to the umbilical cord (34).

Typically, complete blood count, arterial blood gas/ venous blood gas, blood type, antibody screening as well as blood cultures are obtained for admission laboratories. If necessary, metabolic screening, genetic testing, serum bilirubin, coagulation, as well as ABO and Rh typing may be added. Although there is some minimal variability in laboratory values from CBS and neonatal blood sampling in Hb levels, they should not influence further steps in the majority of cases (14, 34). Regarding platelet count, white blood cell count and Hct, no differences between direct sampling and CBS could be evaluated in several studies (34).

For obtaining blood culture from CBS, there are concerns, since the placenta is delivered through the vaginal canal/ abdominal incision and thus could be contaminated. Studies show contamination rates ranging up to 12% (34). A 1981 study comparing CBS and direct infant sampling showed promising results: Direct sampling cultures from sick appearing newborns were negative, however, larger blood volumes taken from the placenta for blood culture were positve. This implies that this method may be a more reliable source for blood cultures than the small amout taken from newborns directly (14).

Figure 3: Obtaining blood for admission laboratory from the placental surface

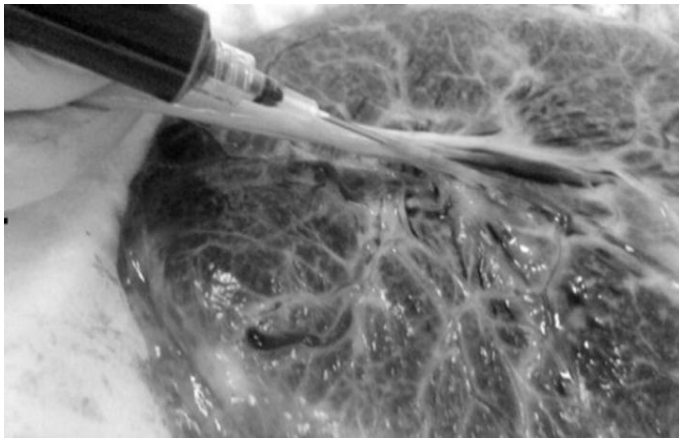
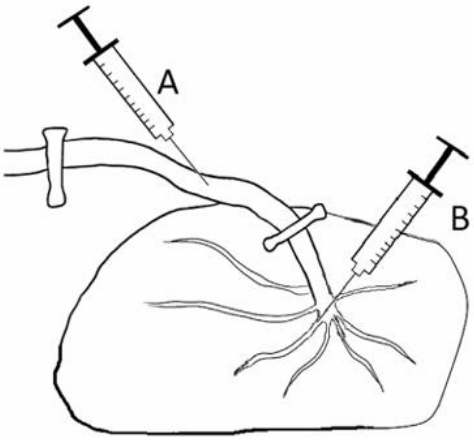


Figure 4: Two Approaches of CBS



Supporting the utilization of cord blood is given by study from US military NICUs. Researchers found that using umbilical cord blood for admission laboratories resulted in higher Hb levels (15,5 g/dL vs 14,0 g/dL before) measured at 12 to 24 hours of life in VLBW newborns (35).

The goal of being able to reduce blood sampling or even eliminate it is tangible in the future by methods like Infrared Spectrophotometric. In this way, clinicians can obtain tissue Hb, arterial and tissue saturation, as well as bilirubin without any use of blood. A 2023 study tested the SAMIRA device, that is capable of measuring Hb, bilirubin and SpO_2 from a newborn's vascular bed underneath the nail. Although it overestimated bilirubin levels above 15 mg/dL by 5mg/dL, it showed a close correlation with values from blood tests. SAMIRA'S accuracy in identifying anemia, hypoxia and

heart diseases makes it a potential tool to reduce the burden of blood sampling, however it is important to take into account the certain limitations such as high bilirubin (36).

Essential to consider is the fact that newborns who have central venous catheters or arterial lines are the most vulnerable since blood collection is easier and more convenient. The median daily blood sample count is 2.3–4 times greater when arterial lines or central venous catheters are present. In comparison to peripheral venous catheters, blood samples taken from central venous catheters often have higher overdraw rates in addition (8, 31).

A study from Jung et al. analyzed neonatal outcomes before and after introduction of a modified blood sampling protocol, focusing on laboratory testing based on needs and point-of-care testing utilizing capillary blood to analyze differences in neonatal outcomes. The study comparing extremely low birth weight neonates born before and after the modification showed successfully a decreased amount of iatrogenic blood loss without having an adverse effect on the health of the newborn. The total sampling volume after one month (16.7 ± 4.1 mL vs. 15.6 ± 4.4 mL), as well as the total sampling volume during hospitalizations (51.4 ± 29.7 mL vs. 44.3 ± 27.5 mL) were significantly lower in the second group after 2016' modifications. However, no significant difference was observed in terms of anemia (Hb 10.8 ± 2.2 vs. 11.0 ± 1.9) , mortality or morbidity (17).

While the effects of clinical blood sampling on AOP is confirmed, the question of the effects of trial related blood sampling needs to be looked at. The positive contribution of such trials for survival and outcomes of preterm infants has clearly been demonstrated over the past few decades and has contributed to clinical and scientific advancements in neonatal care and treatment. However, many trials involve additional blood sampling, which increases sample-related blood loss in participating infants. The FortiColos trial analyzed if trial-related blood sampling plays a role in increased RBC demand for neonates. The number of trial related and clinical related blood samples, as well as volume (ml/kg) were counted. These figures were compared to the necessity of RBCT during the first 28 days of their lives. Sampling volumes consisted of 193 very preterm infants. Mean gestational age of the neonates was 28+/- 1weeks with a mean birth weight of 1168 +/- 301 g. A total of 8.1 ± 5.1 ml/kg or 11% of the total blood volume was collected for sampling (clinical and trial related), of that 1.6 ± 0.6 ml/kg were conducted for trials. When it comes to trial-related blood sampling, no additional RBCT was found to be needed, showing that this type of blood sampling is safe under the trial criteria of Europe and is therefore not listed as a area of prevention or reduction of AOP (37). However, given the significance of each additional blood draw, it should be carefully assessed in each newborn.

In conclusion, reducing iatrogenic blood loss in the clinical environment is essential to prevent neonatal anemia, especially in very sick or very light preterm infants who are already at a higher risk. Numerous tactics, including the use of POC devices and small volume blood sampling methods, have demonstrated promising outcomes in lowering blood loss and, consequently, the requirement for RBCT. Research findings indicate that these methodologies not only result in reduced blood collection volumes and possibly transfusion frequencies, but also enhance Hct and iron levels, thereby enhancing prognoses for neonates, particularly for those born with severe preterm birth. Healthcare practitioners may improve overall neonatal care and optimize long-term health outcomes by implementing these cutting-edge treatments and practices.

This will help to successfully decrease the burden of anemia and its related consequences in vulnerable infant populations. Future developments in lab technology are expected to keep up the current trend of enabling the detection of more analytes on ever smaller amounts of blood. Thus, by lowering the requirement for RBCT, this is anticipated to enhance the care of critically sick newborns(14).

5.4.2 PHARMACOLOGICAL INTERVENTIONS

5.4.2.1 ADMINISTRATION OF ERYTHROPOIESIS-STIMULATING AGENTS

Pharmacological methods to avoid and treat neonatal anemia include administering synthetic erythropoietin to counteract the physiological Hb decline in infants. In the late 1980’s erythropoietin agents firstly became available and opened new treatment modalities for anemia in newborns (13).
The deficiency of erythropoiesis-stimulating hormone (endogenous EPO) occurs along with the normal decline of Hb following delivery. Theoretically, the use of long-acting darbepoetin or synthetic EPO might decrease the incidence of newborn anemia.
Numerous studies have examined EPO's potential as a neuroprotective agent as well as its effect in preventing anemia and reducing the need for RBCT in preterm and/or low-birth-weight newborns. Early EPO administration (up to 7 days after birth) and late EPO administration (from 8 days after birth) were evaluated for efficacy and safety in two different Cochrane studies. RBC transfusions and the number of RBCs transfused decreased as a result of EPO therapy, although individual outcomes may differ, and the overall impact is thought to be of minimal significance. Although a higher risk of ROP (Retinopathy of prematurity) with late administered EPO was seen and raised safety concerns, in summary the incidence of ROP was not significant, after further trials were conducted.

Additionally, Intraventricular Hemorrhage (IVH) and NEC incidences were significantly decreased with early administration, but not after late EPO treatment (38).

According to a Indian study from Bangladesh, short-term recombinant human erythropoietin therapy (rHuEPO) resulted in blood transfusion reduction and improvement of hematological values in VLBW newborns, making it an effective method to prevent AOP. The study compared group I, that received folic acid, iron and rHuEPO with group II, that received only folic acid and iron. In study group II hemoglobin levels decreased more from baseline levels (7 days before the administration of rHuEPO) than in group I. Additionally, 5 out of 23 participants from group II required blood transfusion in either the first (6 weeks) or second follow up (10 weeks), while no participant from group I required blood transfusion at any time. Lastly, the rHuEPO receiving group showed higher linear growth (39).

5.4.2.2 IRON SUPPLEMENTATION

Another pharmacological method used to prevent and treat anemia is iron supplementation. The main source of iron in infants is transported from the mother through the placenta in the third trimester. As a result, the more prematurely an infant is born, the greater deficiency of iron can be found. NICU's worldwide use enteral iron supplementation as a standard therapy as soon as enteral feeds above >100 ml/kg are well tolerated. The regular dose of iron supplementation is 2-3 mg/kg/day, although prematurely born infants receiving EPO treatment or suffering from acute blood loss may need 5mg/kg/day (25,40).

There may be advantages in neurocognitive and psychomotor development to starting iron supplementation earlier (before 28 days of age) as opposed to later (after 28 days of age), according to a single trial's limited data (41).

The effectiveness of the previously listed methods for avoiding anemia in newborns can be seen in a retrospective study from 2018 in India, New Delhi. It included all intramural births before or at 32+0 weeks of gestation from the NICU. The mean gestational age of the 43 infants (16 female, 27 male) was 29.49 ± 2.35 weeks with a mean birth weight of 1234.93 ± 368.737 grams. 17 of 43 required RBCT during their stay. Blood transfusion in preterm neonates of the Indian study has been reduced through to a stringent blood transfusion policy, prudent use of blood analysis, microsampling, POC utilization for e.g. capillary blood gas, iron supplementation, early start of enteral feeding, sepsis prevention, as well as careful use of venous and arterial lines. An important finding regarding this chapter is that early iron supplementation and earlier enteral feeding reduced the incidence of iron deficiency anemia, improved ferritin levels, and avoided the need for RBCT (42).

5.5. RED BLOOD CELL TRANSFUSION

Despite the development of alternative treatment methods, the most effective and most used way to treat anemia in newborns remains the RBCT. RBCs are the most frequently transfused blood product in newborns (9). An estimated 260,000 RBCT's are given to VLBW newborns in the US each year (43). The rapid increase of RBC's through RBCT enhances the newborn's oxygen carrying capacity, leading to improved tissue oxygenation, as well as improved hemostasis (44).

Preterm infants' neurocognitive outcomes may be improved by optimizing vital organ oxygenation by administering RBCT during the period of growth and development, but more research is needed to confirm this as well (25). RBCT advantages that were collected in studies include lowering the heart rate, improving respiratory stability, and consequently increasing weight gain by improved oxygenation status (25). Up to 90% of preterm infants in the NICU require at least one RBC transfusion (45). Depending on the product utilized and the volume the newborn can bear, the typical dose of red blood cells is 10 to 20 ml /kg (46). For newborns with GA under 32 weeks or birth weight ≤1500 g, some recommend a volume of 15 ml/kg (47).

In general, the newborns' Hb concentration should increase by around 20 g/l at a dosage of 15 ml/kg (46). The exact formula to calculate the transfusion volume in milliliter is as follows: newborns' weight (kg) x estimated blood volume (ml/kg) x (desired Hb (g/l) – patient Hb (g/l)/ Hb of donor unit). The estimated blood volume in extremely preterm neonates is 100-120 ml/kg, while in term neonates the estimated blood volume is 80-85 ml/kg (48).

It used to be standard procedure to transfuse newborns with relatively fresh RBC, because stored RBC have higher plasma potassium levels, and because prolonged storage of RBC results in lower levels of 2,3-diphosphoglycerate (2,3-DPG). For newborns receiving large volume transfusions above 20 ml/kg, high potassium concentration from stored blood can be fatal. Newborns that do not get large-volume transfusions, specifically less than 20 ml/kg, at a slow rate of three to four hours are not at risk and do not require fresh RBC (49).

According to a recent study, using fresh RBC stored less than 7 days instead of those obtained from standard blood banks, stored up to 42 days, did not improve outcomes for major neonatal morbidities such as IVH, ROP, bronchopulmonary dysplasia (BPD), or NEC in VLBW premature newborns (50, 51).

The risk of high potassium levels in stored blood can be managed by washing the RBC aliquots or reducing supernatant by centrifugation (46,47). Unfortunately, the process of washing removes up to 20% of RBC, explaining why this approach is not generally suggested for newborn small-volume transfusions.

Furthermore, 25 gray gamma- irradiated blood products contain damaged T-lymphocytes, inhibiting replication in immune-weakened or immature recipients. This procedure reduces transfusion-associated graft-versus-host disease. On the other hand, the risk of hyperkalemia is higher in irradiated blood products and thus fresh RBC should be considered. To conclude, it is of great importance to analyze every patients' needs and risks in detail before deciding which type of transfusions is the best (9). Reducing iatrogenic blood loss in preterm infants may not only reduce the necessity for RBCT but also the risk of complications, therefore strict indications may help counteract the overuse and complications of RBCT (45).

5.5.1 INDICATIONS FOR RED BLOOD CELL TRANSFUSION

An erythrocyte transfusion is a common technique in newborn critical care units. Several recent research and national guidelines in this area have resulted in new recommendations for the preparation, indication, and administration of erythrocyte transfusions in infants. Until recently, there was no scientific evidence about transfusion thresholds for premature newborns. Few randomized controlled trials have evaluated the safety and effectiveness of different transfusion Hb thresholds in newborns with VLBW.

According to the 2011 Cochrane study, there are no significant differences between liberal/high and restrictive/low transfusion groups in terms of short- and long-term outcomes, including death or serious morbidity at first hospital discharge and death or bad neurodevelopmental outcomes up to twenty-one months of age (51).

ETTNO (Effects of liberal vs restrictive transfusion thresholds on survival and neurocognitive outcomes in extremely low-birth-weight infants) and TOP (transfusion of prematures) are two significant clinical trials that compare higher and lower Hb thresholds while transfusing extremely low-birthweight infants. The ETTNO trial used a Hb threshold of <9.5 g/dl as restrictive and <11.9 g/dl as liberal in stable newborns between three and seven days of age. For eight to twenty-one days of age, the threshold was <8.2 g/dl (restrictive) and <10.5 g/dl (liberal). Above twenty-one days, the thresholds decreased to <7.1 g/dl and <9.5 g/dl. In case of unstable newborns, the threshold was 2-2.1 g/dl higher. The TOP trial used more restrictive thresholds in unstable newborns than the ETTNO trial. These studies yielded conclusive and consistent findings. There was no difference between the two Hb threshold groups in the primary outcome, neurodevelopmental impairment at 2 years age or death before evaluation. Evaluated outcomes included cognitive deficits, hearing or vision impairments and cerebral palsy (52).

The studies conclude that the Hb transfusion thresholds, which were 11–13 g/dl for severely ill or ventilated babies and 7–10 g/dl for babies that do not require significant respiratory support, is safe to use without anticipated negative effects on neurodevelopment outcomes or survival (53).

The majority of NICUs worldwide now follow transfusion protocols based on Hb or Hct levels. Criteria such as post-natal age and the type of respiratory support are included and cause different thresholds. A summary of guidelines shown in Table 2 point out the variations in RBCT indications in newborns in different countries (25).

The Swiss Society of Neonatology makes a clear distinction between critically sick neonates without acute blood loss and non-critically sick babies without acute blood loss. Invasive mechanical ventilation, Continuous Positive Airway Pressure (CPAP), Noninvasive Positive Pressure Ventilation (NIPPV) with a $FiO_2 > 0.25$ for more than 12/24 hours, an open ductus arteriosus, acute sepsis, NEC requiring further treatment, and severe apnea requiring caffeine and CPAP treatment are all criteria for critically ill neonates. The Swiss recommendation is divided into three groups based on age. Up to the seventh day of life, the threshold for critically ill newborns is Hct < 34 % and Hb of 11.3 g/dl, while for non-critically ill newborns, the Hct threshold is <28 % and Hb is 9.3 g/dl. From the eighth to the twenty-first day of life, the thresholds for critically ill newborns are <30 % and 10.0 g/dl, and for non-critically ill newborns, <24 % and 8.0 g/dl. The last category concerns newborns beyond the twenty-first day of life: for critically ill newborns, the threshold is <27 % and 9.0 g/dl, and <21 % and 7.0 g/dl for non-critically ill newborns (40).

Recommendations of the German Medical Association are divided into categories according to the ventilation status and age. Premature and mature Newborns' Hb threshold up to 24 hours of age receiving invasive ventilation, or non-invasive ventilation is <12 g/dl, whereas newborns without any respiratory support have a threshold of <10 g/dl. In the next category from one to seven days of age, newborns with invasive ventilation therapy have a transfusion threshold of <12 g/dl, but with non-invasive ventilation or oxygen therapy, or even no respiratory support the threshold is <10 g/dl. Newborns from eight to fourteen days of age receiving invasive ventilation have a threshold of <10 g/dl, while with non-invasive therapy it is <9.5 g/dl and with no therapy <7.5 g/dl. The last age category is for newborns over 14 days of age: In this case, invasive respiratory therapy baby’s threshold stays same with <10 g/dl, while with non-invasive therapy the threshold decreases to <8.5 g/dl and in non-supported newborns again 7.5 g/dl (52).

The British Committee for Standards in Hematology divides into the same categories according to respiratory support and into the same age groups as the German Medical Association, except for a one-day difference in the last age group. Additionally, the British recommendations specifically

divide into premature and full-term newborns. The Hb thresholds are equal in every category and age group. This recommendation was published 2016 and the same thresholds are used in German recommendations from 2024 (48).

The Canadian Pediatric Society has set transfusion thresholds specifically for neonates with anemia of prematurity, but in contrary to the German and British guidelines, the Canadian guidelines do not specifically define thresholds for up to twenty-four hours of age. This recommendation is divided into two groups, according to the need of respiratory support (inspired oxygen requirement in excess of 25% /need for mechanical assistance increase airway pressure. Up to seven days of postnatal age, Hb threshold lies at 11.5 g/dl with respiratory support and at 10.0 g/dl without support. From eight to twenty-one days of age, the threshold is 10.0 g/dl for respiratory supported newborns and 8.5 g/dl for not supported newborns. The last category is for newborns above 14 days of age: If these newborns receive respiratory support, the threshold is 8.5 g/dl and without it is 7.5 g/dl (46).

Table 2: Overview of RBC transfusion guidelines of Switzerland, Germany, UK, Canada

1. Swiss Society of Neonatology, 2022:

Age	Critically ill newborn Hb (g/dl)	Non critically ill newborn Hb (g/dl)
0 – 7 days	11.3	9.3
8 – 21 days	10.0	8.0
>21 days	9.0	7.0

2. German Medical Association Hemoglobin thresholds, 2024:

Age	Invasive ventilation	Oxygen therapy/ non-invasive ventilation	No respiratory support
0 - 24 hours	<12.0 g/dl	<12.0 g/dl	<10.0 g/dl
1 - 7 days	<12.0 g/dl	<10.0 g/dl	<10.0 g/dl
8 - 14 days	<10.0 g/dl	<9.5 g/dl	<7.5 g/dl
>14 days	<10.0 g/dl	<8.5 g/dl	<7.5 g/dl

3. British Committee for Standards in Hematology (BCSH), 2016:

	Age	
Premature	24 hours	Without oxygen <10.0 g/dl With oxygen/ non-invasive ventilation <12.0 g/dl Ventilated <12.0 g/dl
	1-7 days	Without oxygen <10.0 g/dl With oxygen/ non-invasive ventilation <10.0 g/dl Ventilated <12.0 g/dl
	8-14 days	Without oxygen <7.5 g/dl With oxygen/ non-invasive ventilation <9.5 g/dl Ventilated <10.0 g/dl
	>15 days	Without oxygen <7.5 g/dl With oxygen/ non-invasive ventilation <8.5 g/dl Ventilated <10.0 g/dl

4. Canadian Pediatric Society, updated in 2024:

Postnatal Age	With respiratory support Hb (g/dl)	Without respiratory support: Hb (g/dl)
0 – 7 days	11.5	10.0
8 – 14 days	10.0	8.5
>14 days	8.5	7.5

Study results from the Indian study, mentioned before, showed the connection between increased need for RBC transfusion in newborns with respiratory support. Observable is the study was the significantly higher need of RBC transfusion in septic babies, after surfactant therapy and correlation to the number of days on ventilation or CPAP (34). The disparity in transfusion practices among NICUs highlights the lack of consensus on the best RBC transfusion method for preterm and term newborns.

5.5.2. POSSIBLE COMPLICATIONS OF RED BLOOD CELL TRANSFUSION

Red blood cell transfusion is a widely used measure in the NICU. Newborns who are the smallest and least developed typically require the most transfusions (54). Around 90 % of ELBW infants require RBCT, while around 40% of LBW require a transfusion (9). Large, randomized trials are being conducted to determine the optimal transfusion thresholds for pre-term infants, as there is still much controversy surrounding the potential benefits and risks of RBCT.

Transfusion of RBCs to newborns carries a range of risks, both infectious and non-infectious risks. Although the risks of transfusion are well known for adult recipients, these risks are not so often reported for newborns. Receiving blood transfusions increases the risk of contracting infectious diseases like HIV, hepatitis, Cytomegalovirus (CMV), Trypanosoma cruzi, Plasmodium spp., West Nile virus, as well as parvovirus B19 (42).

Since the 90s these risks have been decreasing significantly, resulting in a low risk of infection by viruses, parasites, bacteria, parasites, and prions. Nevertheless, these kinds of infections can be fatal in newborns, due to their immature immune system and low levels of maternal IgG. Specifically, transfusion-transmitted cytomegalovirus (TT-CMV) infections can be reduced by using CMV-seronegative and leuko-reduced blood products (9). Leukoreduction of blood products reduces the concentration of leukocytes in blood components, eliminating a considerable number of cells that may be infected with CMV (55). In a prospective multicenter cohort analysis of 539 VLBW babies, no CMV infections were observed after transfusion with CMV-seronegative or leuko-reduced blood. For these patients, breast milk is the primary source of CMV infection after birth (56).

To conclude, transfusion-transmitted infectious diseases are rarely found in developed countries, but new pathogens and false negative testing of blood products can still occur.

Regarding non-infectious risks, a German study of 132 VLBW infants confirms significant correlation of erythrocyte transfusion with unfavorable neonatal outcome parameters, including lower birth weight, longer hospital stays, sepsis, infection, patent ductus arteriosus (PDA), NEC, ROP and death (21). According to the Serious Hazards of Transfusion Report 2016, there were an estimated 14.6/10,000 components of adverse outcomes from RBC transfusions in the UK, and there were an estimated 1.05/100000 components of mortality from blood transfusions that year.

Packed RBCT comes with several potential risks. These include exposure to blood-borne infections, graft-versus-host disease, metabolic issues, cardiovascular issues, iron overload, hypothermia, and elevated oxidative stress. The increase in oxidative stress is particularly concerning, as it may

contribute to a higher risk of complications in preterm infants, including conditions like BPD, ROP, IVH and NEC (11).

Transfusions in preterm infants specifically have been associated with acute lung injury, NEC, extension of IVH, ROP, longer hospital stays, and fatalities in some studies (42). This correlation can be seen in a Taiwan cohort study, in which clinical outcomes of RBCT in 120 preterm infants were observed. The amount of RBCT the ELBW newborns received in the first seven, thirty, and sixty days of life were recorded. Clinical outcomes were evaluated with a follow-up period of up to two years of age. These included in-hospital mortality, ROP, chronic lung disease (CLD), NEC, BPD and neurodevelopmental outcome (54).

The following paragraph will discuss the possible dangers of RBC transfusions for neonates, specifically ROP, NEC, IVH, TRALI, BPD, PDA and lastly, neurodevelopmental outcomes.

5.5.2.1 RETINOPATHY OF PREMATURITY (ROP):

Preterm newborns are more likely to have changes in visual function, such as myopia, strabismus, nystagmus, and poor stereopsis compared to term-born children (57). Due to compromised retinal vascularization and thus elevated vasoproliferation, preterm newborns may suffer from a potentially fatal neurovascular condition known as Retinopathy of Prematurity (ROP). To avoid blindness and irreversible vision damage, early diagnosis and treatment are essential. Major risk factors for ROP include low birth weight, as well as low gestational age, and exposure to varying exogenous oxygen concentrations, oxygen therapies and respiratory distress syndrome (58).

RBCT is an independent risk factor for the development of ROP in VLBW infants, according to a study by Ghirardello et al. that specifically examined the risks of RBC transfusion in VLBW infants. The risk of complications rises as the number of transfusions increases, with three or more transfusions increasing the risk of ROP by 4.88 times (59).

A study comparing 505 very preterm infants that received RBCT in the first 4 weeks of life with 327 non-transfused very preterm infants found a significant correlation between RBCT and the incidence of ROP and > Stage 2 ROP. Furthermore, the study showed supports the finding of the above-mentioned study, that the risk of ROP increased with the number and volume of transfusion (60).

Not only ROP incidence, but also ROP progression is associated with RBCT. This can be observed in a retrospective cohort study from 68 NICUs in Germany, that analyzed the role of erythrocyte transfusion as a risk factor in preterm infants from 22 weeks to 28 weeks + 6 days. Out of 12565

newborns, 49.2% developed any type of ROP, decreasing from stage one to four. The correlation between RBC transfusion and not only ROP incidence, but progression and increased treatment requirements was clearly seen in this study. Higher GA however could reduce ROP incidence, progression and treatment requirement by 0,2-0,3-fold. (61).

RBCT in preterm infants are linked to an increased risk of ROP due to changes in Hb composition and oxygen delivery. Transfusions contain Hb A (HbA), thus raise the HbA levels, which have a lower affinity for oxygen compared to fetal Hb (HbF). As a result, immature tissues, such as the developing retina, are exposed to more oxygen because HbA releases oxygen to the tissues more easily. Oxidative stress and possible endothelial damage can result from the production of oxygen free radicals by this increased oxygen availability. ROP is characterized by aberrant blood vessel growth that may be triggered by these consequences, which may interfere with normal retinal vascular development.

ROP risk is further increased by the presence of pro- and anti-inflammatory mediators in stored RBC's, which can exacerbate oxidative stress and vascular instability. This mechanism could explain the association between higher RBC transfusion volumes and the increased likelihood of developing ROP in preterm infants (60,61)

Other conditions, including sepsis, IVH, hypotension and hypothermia may also be risk factors for developing ROP, emphasizing the complex, multifactorial nature of the condition (58). In conclusion, ROP poses a considerable risk to preterm newborns, with RBCT emerging as a key contributor. Early diagnosis and treatment are crucial in preventing visual damage or blindness. Close monitoring of transfusion techniques is critical for reducing the risk of ROP onset and progression in this sensitive group.

5.5.2.2 NECROTIZING ENTEROCOLITIS (NEC):

NEC is an inflammatory bowel necrosis, commonly seen in preterm infants. One to three out of 1000 live births suffer from this life-threatening disease, out of which more than 90% are preterm infants (62). Around 10 % of NICU deaths can be connected to NEC, an "inflammatory cellular death of the bowel in the neonatal period" (63). It is a complex disorder with multiple causes, including ischemia, mechanical injury, infection, iatrogenic factors (e.g.: excessive enteral feeding, catheterization), and immune barrier dysfunction. Currently, there is no one unified consensus on causality (63,64). NEC complications include sepsis, disseminated intravascular coagulation, intestinal obstruction, fistulae shock, multi-organ failure, visual impairment, deafness, developmental as well as psychomotor impairment (63).

In general, the risk for NEC increases by decreasing GA and birth weight, but the exact etiology is still unclear (62). However, there is research linking the likelihood of NEC in VLBW newborns with severe anemia and RBCT (65). The correlation between NEC and RBCT was initially described by researchers in 1987, who looked into an outbreak of 33 NEC cases, many of them occurring after RBCT (66). The widely used name 'transfusion-associated necrotizing enterocolitis' (TA-NEC) describes this correlation.

A retrospective study from a Lithuanian University Hospital (Kauno Klinikos) from 2005 to 2014 investigated the correlation between RBCT and NEC. VLBW infants with NEC (Bell stage 2a/ above) were compared with a control group, consisting of newborns with comparable GA and birth weight. Prior to the onset of NEC and during the hospital stay, the study group had significantly higher rates of RBC transfusion and transfusion volume (67).

Additionally, as mentioned in the chapter about RBCT, a higher volume of blood transfusion has been linked to transfusion-associated NEC (TANEC) in VLBW infants (68).

However, despite these study results, many infants that undergo blood transfusions do not develop NEC. This suggests that in cases where a connection is observed, there may be additional underlying factors that predispose certain infants to develop NEC following a transfusion (59).

One such underlying potential factor is feeding during transfusion. Some studies link NEC to feeding during transfusion, whereas others suggest that restricting meals has no effect on the risk of NEC (69). French specialists advocate suspending feedings for VLBW newborns during transfusion (47,70,71). According to a 2021 literature review, 4 studies analyzing this relation came to the conclusion that there was no reduction of TANEC after the introduction of feeding protocols (72). Further research on the basis of evidence-based feeding protocols are required to investigate this question in the future.

Another factor potentially influencing the NEC development is timing. Many studies report an association between RBCT and development of NEC in a specific range of hours. In a study from Christensen and colleagues from 2010, including 118 patients suffering from stage three NEC, 38% received an erythrocyte transfusion 18 +/- 12 hours before symptom onset (73).

To further analyze this correlation, a retrospective study conducted from 2004 to 2007 including a matching control infant (according to weight, GA and admission date) to each infant suffering from NEC born before/ at 34 weeks of gestation. Of the patients, 38% had a history of receiving RBC transfusions within 48 hours before the beginning of NEC, though it is important to mention that these patients were mostly ELBW neonates. Important to point out is that the RBC transfusion rates

were comparable in NEC and control patients. To conclude, in this study RBCT were not associated with NEC in the majority of patients, and they could just be a sign of a more severe disease overall (62). Aligning with these results, an observational cohort study from January 2010 to February 2014 in Atlanta (USA) found severe anemia, not RBC transfusion to be a risk factor for NEC (74).

There are vague explanations for the potential mechanism between RBC transfusion and NEC development. Changes in mesenteric artery blood flow, reoxygenation and reperfusion causing oxidative stress injury is the most commonly described mechanism (75,76).

To conclude, RBCT may affect NEC, a serious bowel illness in premature newborns. While the specific reasons are unknown, preterm and low birth weight increase the risk. Nevertheless, there are several conflicting findings among research, ranging from findings suggesting transfusions may be risk factors for NEC to suggestions that anemia itself, rather than transfusion, is the true risk factor, and even conclusions indicating no association at all (65). There are many concerns about how the volume and other factors surrounding the transfusion could potentially increase or decrease the risk of NEC. Concerns about feeding safety often revolve around the danger of developing NEC (9).

Further research comparing liberal vs constrictive transfusion practices is needed to have a better understanding of this link and to better manage this complication in the future. The influence of different transfusion thresholds, different baseline patient characteristics and unclear definition of TANEC led to different study results so far and must be managed adequately in the future.

5.5.2.3 INTRAVENTRICULAR HEMORRHAGE (IVH):

Intraventricular hemorrhage is a common condition found in newborns, especially in preterm newborns. Preterm infants with IVH are at high risk for brain damage and poor neurodevelopmental outcomes. IVH in preterm neonates often occurs during the first 72 hours after delivery, and in most cases, between 6-24 hours. This makes the time frame critical for prevention of this problem and its long-term effects. Improvements in prenatal and neonatal care have significantly improved the survival rate of preterm newborns, especially those delivered before 25 weeks GA. There is a 25-30% incidence in these neonates. (77,78).

Based on Papile Grading, IVH Grade 1 is in the periventricular matrix, Grade 2 IVH extends into the ventricles, Grade 3 has dilated ventricles, and in Grade 4 IVH the parenchyma is involved (79).

Severe IVH (Grade 3/4) occurs in 35% of preterm infants born before 26 weeks of gestation, while severe IVH in newborns with 28 to 29 weeks of gestation is seen in 15% (80), making low GA an important risk factor. Further risk factors for IVH include intrauterine infection, low birth weight, low

Apgar score, vaginal delivery, sepsis, acidosis, early usage of vasopressors, as well as male gender (78).

Research suggests a link between early postnatal RBCT and IVH, although the mechanisms causing the relation remain unclear. It is thought that large variations in blood pressure and blood flow in immature capillary beds can more likely lead to rupture and bleeding (81). Since IVH can cause anemia and necessitate RBCT itself, it makes the causative relationship with RBCT even more unclear (82).

Again, not only incidence, but also progression is thought to be related to RBCT. Another study found that out of 417 neonates with Grade 1 IVH, 24 developed a Grade 2 and 22 a Grade 4 IVH. Results showed that RBCT was the most important factor to IVH progression in this study, however there is no clear explanation to that, making it another complication where additional studies are necessary (83).

Christensen and colleagues found that 59% of newborns with IVH received transfusions during the first 24 hours after birth (80), while a Leiden University study found a lower rate of 14%. These results may be explained with stricter transfusion protocols patient selection, making it hard to compare studies. In the study from the Netherlands, the majority of the very preterm infants had IVH at the time of transfusion. Infants who had transfusions prior to IVH diagnosis were often younger and had additional risk factors (82). On the other hand, the American study from Christensen showed successful reduction of RBC transfusions by a new transfusion protocol (from 58% prior to 25% after protocol change), along with a reduction of IVH cases from 17% prior to 8% (80). There are some studies suggesting that a more liberal RBC transfusion method leads to reduced IVH in neonates, indicating a close relationship between transfusion and IVH incidence. However, there are limitations to the study results listed above: The link between transfusion and IVH is very likely due to the clinical reasons, rather than the transfusion itself. In this case any intervention that decreases or avoids the need for early RBC transfusions is linked to lower rates of IVH incidence (84).

In summary, RBCT may be a risk factor for IVH incidence and progression, but the exact mechanism is still unclear and many studies have different results ranging from a close association to no association.

5.5.2.4 TRANSFUSION RELATED ACUTE LUNG INJURY (TRALI):

TRALI has been identified as a clinical condition since the 1950s and is a life-threatening complication of blood transfusion, however there are only very few cases reports (85). It was first documented in adult patients but is now recognized as an uncommon but significant complication of

transfusion in children (86). According to the United States Food and Drug Administration (FDA), TRALI is the leading cause of transfusion related death, with an incidence of 1 in 5000 units of RBCT (87). Unfortunately, it is often overlooked, especially in premature newborns. TRALI is a syndrome with acute, noncardiogenic pulmonary edema with hypoxia within 6 hours of transfusion. Furthermore, to diagnose a real TRALI, there should be no risk factors for developing a lung injury (e.g aspiration, shock, sepsis). Possible TRALI is diagnosed when there are risk factors for lung injury, and delayed TRALI when signs occur after 6 to 72 hours. As for clinical signs, acute respiratory distress, bilateral lung infiltrates and hypoxemia (PaO_2/FiO_2=300 or SpO_2<90% on room air). For differential diagnosis, Transfusion-associated- circulatory overload (TACO), another complication of transfusions with similar clinical signs, that will not be further explained in this thesis, should be thought of. Increased pulmonary capillary permeability causes pulmonary edema, while proinflammatory mediators from stored blood products, as well as specific antibodies in blood products that bind to recipients antigens are also important factors in the etiology, however the precise etiology and pathophysiology are unknown (87).

Treatment of TRALI usually involves ventilation and hemodynamic support (88). The small number of TRALI reports in neonates may be owing to a lack of knowledge, lack of unanimity on the criteria, or difficulties in diagnosis due to other causes (85,86).

A 2012 retrospective study collected 108 neonates' medical data before, during and after transfusion. These 108 neonates received 330 packed RBCT in total. Post-transfusion lung injury, defined as increased highest mean airway pressure ≥ 2 cm H2O or FiO_2 of 40.15 in the 6-h after transfusion, was observed in 23 if these neonates. The neonates that were affected from lung injury had lower birth weight, as well as lower GA. Additionally, neonates with transfusion associated lung injury had a higher risk of developing NEC and dying within 24 hours following transfusion. To conclude, in neonates undergoing intensive or step-down care, around 8% of transfusions resulted in persistent deterioration of lung function and increasing requirement for respiratory assistance (72).

5.5.2.5 BRONCHOPULMONARY DYSPLASIA (BPD)

BPD is a common chronic disease requiring supplementary oxygen at 36 weeks due to disrupted alveolar development. According to reports, 80% of newborns born at the GA of 22 to 24 weeks will develop BPD. It can lead to later asthma, recurrent lower respiratory tract infections, and activity intolerance. Risk factors include low GA low birth weight, maternal smoking, male sex, chorioamnionitis, oxygen therapy and duration of ventilation, as well as sepsis, NEC and pulmonary

hemorrhage. The exact pathophysiology of BPD is unknown due to its complex nature, although RBCT is thought to be associated with BPD (89). As for the mechanism of this correlation, free radicals and iron overload from stored blood are thought to be causative. Additionally, the hyperoxic environment caused by RBC containing adult Hb with higher oxygen affinity may play a role in alveolar-capillary disruption and oxidative injury (89)

A meta-analysis supporting the association showed a significant correlation between RBC transfusion and BPD. Compared to newborns without transfusion, those with RBC transfusion had 4.01 times the risks of developing BPD (75). Furthermore, a retrospective analysis found that the time of the initial transfusion and the onset of anemia did not correlate with the risk of BPD. Only severe anemia may raise the chance of developing BPD in anemic neonates. BPD severity and volume are correlated with more RBC transfusions. Reducing RBC transfusions and preventing severe anemia should be accomplished by medical means (90).

Due to heterogeneity across studies so far, further research is needed to confirm the association and provide better understanding.

5.5.2.5 PATENT DUCTUS ARTERIOSUS (PDA):

Hardly any studies can be found that analyze the correlation of PDA und RBC transfusion. However, there was a study performed in a Korean NICU with 250 VLBW newborns that included all possible short-term outcomes such as IVH, NEC, ROP, BPD, PDA and sepsis. In the linear regression analysis from Lee et al., PDA and early-onset sepsis were the only short-term outcomes found to be not associated with RBC Transfusion (75).

5.5.2.6 LONGTERM NEURODEVELOPMENTAL OUTCOMES

RBC transfusions may produce both short- and long-term problems in the neonate population. Research of 56 preterm infants indicated that neonates with a restrictive RBC transfusion regimen had significantly improved neurocognitive outcomes, including associative verbal fluency, reading skills, and visual memory at follow-up 8-15 years later (71). On the other hand, the TOP and ETTNO trials, described in the chapter ‘Indications for red cell transfusion’, showed no neurodevelopmental impairment at 2 years age. Evaluated outcomes were cognitive deficits, hearing or vision impairments and cerebral palsy (53).

To conclude, transfusions of RBC increase the risk of inflammatory responses, morbidity, as well as neonatal mortality. Thus, reducing iatrogenic blood loss in preterm infants may not only reduce the necessity for RBCT but also the risk of complications (45).

In summary, small retrospective and observational studies provide the majority of evidence demonstrating the link between RBC transfusion and unfavorable outcomes. Although retrospective research might create ideas and reveal relationships, they hardly establish a causal relationship. Therefore, the mentioned studies for each outcome may be helpful in understanding relationships between transfusion and its complications but cannot prove these outcomes, so there is the need for much deeper research for all the possible outcomes in the future.

6. EXECUTIVE SUMMARY

Prematurity is the leading cause of neonatal mortality worldwide, with preterm and very low birth weight infants being particularly susceptible to anemia due to their underdeveloped hematopoietic systems and frequent medical interventions. Anemia in newborns is a physiological phenomenon often caused by a combination of factors, including the immaturity of their red blood cell production, a reduced red blood cell life span and the rapid postnatal growth that increases the demand for red blood cells. Maternal factors, such as iron deficiency, poor nutrition, and the mode of delivery, also significantly influence neonatal anemia, making maternal health and proper nutrition vital for reducing the risk of anemia in both the mother and the newborn.

Neonates, particularly preterm infants, experience a more severe and earlier decline in hemoglobin levels compared to full-term infants, a condition known as "anemia of prematurity." The etiologies of this type of anemia can be classified into anemia due to Hemorrhage/Blood Loss, Hemolysis and failure of red cell production, with Blood Sampling belonging to Blood Loss and being the most important cause of. This type of anemia requires medical intervention.

Hypoxia, caused by inadequate oxygen supply through anemia, can lead to common clinical signs in newborns including tachycardia, tachypnea, poor weight gain, and increased apneic episodes, and severe cases may lead to organ dysfunction such as Necrotizing Enterocolitis, Heart Failure, and neurodevelopmental impairments.

Pharmacological strategies for managing anemia in very low birth weight infants include Erythropoiesis-stimulating Agents and Iron Supplementation. These treatments aim to improve oxygen delivery and prevent the complications associated with severe anemia.

Non-pharmacological strategies such as Delayed Cord Clamping and Umbilical Cord Milking are effective in reducing anemia in very low birth weight infants by enhancing placental transfusion. Both methods improve hematocrit levels and decrease the need for subsequent Red Blood Cell Transfusion. Reducing iatrogenic blood loss through the use of point-of-care devices, small-volume blood sampling, and umbilical blood sampling has been successful in decreasing the need for Red Blood Cell Transfusion's and improving neonatal outcomes.

Despite advancements in alternative therapies, Red Blood Cell Transfusion remains the primary treatment for managing anemia in preterm infants, especially those with very low birth weight. However, Red Blood Cell Transfusions carry significant risks, including exposure to blood-borne infections, graft-versus-host disease, and metabolic and cardiovascular complications, as well as the potential for iron overload and increased oxidative stress. Oxidative stress caused by transfusions, particularly in preterm infants, is concerning as it can contribute to the development of conditions like Bronchopulmonary Dysplasia, Retinopathy of Prematurity, and Intraventricular Hemorrhage. Red Blood Cell Transfusions also increase the risk of Necrotizing Enterocolitis, though conflicting evidence suggests that severe anemia itself, rather than the transfusions, may be the primary risk factor. Transfusion-related Acute Lung Injury is a rare but serious complication of Red Blood Cell transfusions in neonates, leading to respiratory distress and requiring ventilation support. Additionally, early Red Blood Cell transfusions have been linked to an increased risk of. Patent Ductus Arteriosus on the other hand has not been strongly linked to Red Blood Cell transfusion.

While Red Blood Cell Transfusion is still the measure of choice to combat severe anemia and the resulting hypoxia, due to the many possible complications it brings along, medical treatment should aim to apply a restrictive Red Blood Transfusion strategy. As to enable such a restrictive regime for Red Blood Cell Transfusion, the causing anemia has to be prevented or kept minimal.

As iatrogenic blood loss from frequent blood sampling in preterm infants remains a major contributor to anemia, particularly in very low birth weight infants, necessitating careful evaluation of the necessity for laboratory tests to minimize unnecessary blood loss.

The numerous techniques to reducing or even preventing neonatal anemia which have been discussed in this thesis, will further evolve with technological advancements like point-of-care devices. But lastly, further improvements also rely on a lucent discussion and recognition of the effects of excessive iatrogenic blood loss, for which this thesis aims to contribute to.

7. LIST OF REFERENCES

1. Harrison MS, Goldenberg RL. Global burden of prematurity. Semin Fetal Neonatal Med. 2016 Apr;21(2):74–9.

2. Vogel JP, Chawanpaiboon S, Moller AB, Watananirun K, Bonet M, Lumbiganon P. The global epidemiology of preterm birth. Best Pract Res Clin Obstet Gynaecol. 2018 Oct;52:3–12.

3. McNelis KM, Fenton TR. Neonatal Nutrition Assessment. In: Principles of Neonatology [Internet]. Elsevier; 2024 [cited 2025 Jan 10]. p. 178–91. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780323694155000229

4. Kiatchoosakun P, Jirapradittha J, Paopongsawan P, Techasatian L, Lumbiganon P, Thepsuthammarat K, et al. Mortality and Comorbidities in Extremely Low Birth Weight Thai Infants: A Nationwide Data Analysis. Child Basel Switz. 2022 Nov 25;9(12):1825.

5. Zhao B, Sun M, Wu T, Li J, Shi H, Wei Y. The association between maternal anemia and neonatal anemia: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2024 Oct 18;24(1):677.

6. Berihun GA, Tesfaye G, Adissu W, Tadasa E, Adamu K, Kombe AT, et al. Prevalence and Associated Factors of Anemia among Newborns at Jimma Medical Center, South-west Ethiopia. J Blood Med. 2024;15:129–40.

7. Aslamzai M, Danish Y, Hakimi T, Jawadi B. Evaluation of the factors associated with anemia in neonates admitted to the Neonatal Unit of Maiwand Teaching Hospital: A cross-sectional study. Glob Pediatr. 2024 Jun;8:100164.

8. Ree IMC, Lopriore E. Updates in Neonatal Hematology: Causes, Risk Factors, and Management of Anemia and Thrombocytopenia. Hematol Oncol Clin North Am. 2019 Jun;33(3):521–32.

9. Villeneuve A, Arsenault V, Lacroix J, Tucci M. Neonatal red blood cell transfusion. Vox Sang. 2021 Apr;116(4):366–78.

10. Strauss RG. Anaemia of prematurity: pathophysiology and treatment. Blood Rev. 2010 Nov;24(6):221–5.

11. Su PC, Chung HW, Yang ST, Chen HL. Effect of Small Volume Blood Sampling on the Outcomes of Very Low Birth Weight Preterm Infants. Children. 2022 Aug 8;9(8):1190.

12. Walter AW. Perinatal Anemia [Internet]. Vol. 2022. MSD Manual; 2022. Available from: https://www.msdmanuals.com/professional/pediatrics/perinatal-hematologic-disorders/perinatal-anemia

13. M. Dionisio L, A. Dzirba T. Neonatal Anemia. In: Mauricio Barría R, editor. Topics on Critical Issues in Neonatal Care [Internet]. IntechOpen; 2022 [cited 2025 Mar 8]. Available from: https://www.intechopen.com/chapters/78720

14. Carroll PD, Widness JA. Nonpharmacological, blood conservation techniques for preventing neonatal anemia--effective and promising strategies for reducing transfusion. Semin Perinatol. 2012 Aug;36(4):232–43.

15. Lanzkowsky P. Anemia During the Neonatal Period. In: Lanzkowsky's Manual of Pediatric Hematology and Oncology. 6th ed. Elsevier; 2016. p. 51–68.

16. Kliegman RM, St Geme JW, Blum NJ, Shah SS, Tasker RC, Wilson KM. Blood Disorders. In: Nelson Textbook of Pediatrics. 21st ed. p. 961–74.

17. Jung N, Kim C, Kim H, Seo Y, Hwang J, Yang M, et al. Changes to Blood-Sampling Protocol to Reduce the Sampling Amount in Neonatal Intensive Care Units: A Quality Improvement Project. J Clin Med. 2023 Sep 1;12(17):5712.

18. Chaudhary N, Jassar R, Singh R. Neonatal Anemia [Internet]. 2022. Available from: https://www.newbornjournal.org/doi/JNB/pdf/10.5005/jp-journals-11002-0027

19. Tilahun D, Yimer MA, Zamanuel TG. High Magnitude of Neonatal Anemia Among Sick Newborns Admitted to University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. J Blood Med. 2022 Jun;Volume 13:293–302.

20. Katheria AC, Erickson-Owens DA, Mercer JS. Delayed Cord Clamping and Cord Milking. In: MacDonald's Atlas of Procedures in Neonatology. 6th ed. Wolters Kluwer; 2019.

21. Aboalqez A, Deindl P, Ebenebe CU, Singer D, Blohm ME. Iatrogenic Blood Loss in Very Low Birth Weight Infants and Transfusion of Packed Red Blood Cells in a Tertiary Care Neonatal Intensive Care Unit. Children. 2021 Sep 25;8(10):847.

22. Marrs L, Niermeyer S. Toward greater nuance in delayed cord clamping. Curr Opin Pediatr. 2022 Apr 1;34(2):170–7.

23. Song D, Jegatheesan P, DeSandre G, Govindaswami B. Duration of Cord Clamping and Neonatal Outcomes in Very Preterm Infants. Raju T, editor. PLOS ONE. 2015 Sep 21;10(9):e0138829.

24. Fogarty M, Osborn DA, Askie L, Seidler AL, Hunter K, Lui K, et al. Delayed vs early umbilical cord clamping for preterm infants: a systematic review and meta-analysis. Am J Obstet Gynecol. 2018 Jan;218(1):1–18.

25. Saito-Benz M, Flanagan P, Berry MJ. Management of anaemia in pre-term infants. Br J Haematol. 2020 Feb;188(3):354–66.

26. Katheria AC, Lakshminrusimha S, Rabe H, McAdams R, Mercer JS. Placental transfusion: a review. J Perinatol. 2017 Feb;37(2):105–11.

27. Orpak ÜS, Ergin H, Çıralı C, Özdemir ÖMA, Koşar Can Ö, Çelik Ü. Comparison of cut and intact cord milking regarding cerebral oxygenation, hemodynamic and hematological adaptation of term infants. J Matern Fetal Neonatal Med. 2021 Jul 18;34(14):2259–66.

28. Widness JA. Pathophysiology of Anemia During the Neonatal Period, Including Anemia of Prematurity. NeoReviews. 2008 Nov 1;9(11):e520.

29. Lewis AE, Kappel SS, Hussain S, Sangild PT, Zachariassen G, Aunsholt L. Trial-related blood sampling and red blood cell transfusions in preterm infants. Acta Paediatr Oslo Nor 1992. 2023 Dec;112(12):2486–92.

30. Uberos J, Fernandez-Marin E, Campos-Martínez A, Ruiz-López A, García-Serrano JL. Blood products transfusion and retinopathy of prematurity: A cohort study. Acta Ophthalmol (Copenh) [Internet]. 2023 May [cited 2025 Feb 14];101(3). Available from: https://onlinelibrary.wiley.com/doi/10.1111/aos.15269

31. Jakacka N, Snarski E, Mekuria S. Prevention of Iatrogenic Anemia in Critical and Neonatal Care. Adv Clin Exp Med Off Organ Wroclaw Med Univ. 2016;25(1):191–7.

32. Widness JA, Madan A, Grindeanu LA, Zimmerman MB, Wong DK, Stevenson DK. Reduction in red blood cell transfusions among preterm infants: results of a randomized trial with an in-line blood gas and chemistry monitor. Pediatrics. 2005 May;115(5):1299–306.

33. Choi HY, Corder W, Tefera E, Abubakar KM. Comparison of Point-of-Care versus Central Laboratory Testing of Electrolytes, Hemoglobin, and Bilirubin in Neonates. Am J Perinatol. 2022 Dec;39(16):1786–91.

34. Bahr TM, Carroll PD. Cord blood sampling for neonatal admission laboratory testing—An evidence-based blood conservation strategy. Semin Perinatol. 2023 Aug;47(5):151786.

35. Mu TS, Prescott AC, Haischer-Rollo GD, Aden JK, Shapiro JB. Umbilical Cord Blood Use for Admission Blood Tests of VLBW Preterm Neonates: A Randomized Control Trial. Am J Perinatol. 2023 Jul;40(10):1119–25.

36. Banerjee A, Bhattacharyya N, Ghosh R, Singh S, Adhikari A, Mondal S, et al. Non-invasive estimation of hemoglobin, bilirubin and oxygen saturation of neonates simultaneously using whole optical spectrum analysis at point of care. Sci Rep. 2023 Feb 9;13(1):2370.

37. Lewis AE, Kappel SS, Hussain S, Sangild PT, Zachariassen G, Aunsholt L. Trial-related blood sampling and red blood cell transfusions in preterm infants. Acta Paediatr Oslo Nor 1992. 2023 Dec;112(12):2486–92.

38. Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Neonatal Group, editor. Cochrane Database Syst Rev [Internet]. 2014 Apr 26 [cited 2025 Mar 7]; Available from: https://doi.wiley.com/10.1002/14651858.CD004863.pub4

39. Yasmeen BHN, Chowdhury M a. KA, Hoque MM, Hossain MM, Jahan R, Akhtar S. Effect of short-term recombinant human erythropoietin therapy in the prevention of anemia of prematurity in very low birth weight neonates. Bangladesh Med Res Counc Bull. 2012 Dec;38(3):119–23.

40. Mieth RA, Barrielle L, Hegemann I, Konetzny G, Rüegger C. Erythrozyten-Transfusion bei Neugeborenen [Internet]. Eds. Christoph Rüegger; 2022. Available from: https://www.neonet.ch/application/files/7816/6581/6573/EC-Transfusionen_bei_Neugeborenen.pdf

41. Steinmacher J, Pohlandt F, Bode H, Sander S, Kron M, Franz AR. Randomized trial of early versus late enteral iron supplementation in infants with a birth weight of less than 1301 grams: neurocognitive development at 5.3 years' corrected age. Pediatrics. 2007 Sep;120(3):538–46.

42. Agarwal A, Sikriwal D, Ahuja A, Mallaiah R. Reducing incidence of red cell transfusion among preterm babies in a tertiary care neonatal intensive care unit: A retrospective observational study. J Clin Neonatol. 2019;8(1):19.

43. Widness JA. Treatment and Prevention of Neonatal Anemia. NeoReviews. 2008 Nov 1;9(11):526–33.

44. Guzzetta NA. Benefits and risks of red blood cell transfusion in pediatric patients undergoing cardiac surgery. Paediatr Anaesth. 2011 May;21(5):504–11.

45. Counsilman CE, Heeger LE, Tan R, Bekker V, Zwaginga JJ, Te Pas AB, et al. Iatrogenic blood loss in extreme preterm infants due to frequent laboratory tests and procedures. J Matern-Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet. 2021 Aug;34(16):2660–5.

46. Lau W. Neonatal and pediatric transfusion. Can Blood Serv [Internet]. 2017 Aug 2;(Chapter 13). Available from: https://professionaleducation.blood.ca/en/transfusion/clinical-guide/neonatal-and-pediatric-transfusion

47. Favrais G, Wibaut B, Pladys P, Saliba E. [Blood transfusion to pre-term neonates: What is new in the French guidelines since 2002?]. Arch Pediatr Organe Off Soc Francaise Pediatr. 2017 Sep;24(9):894–901.

48. New HV, Berryman J, Bolton-Maggs PHB, Cantwell C, Chalmers EA, Davies T, et al. Guidelines on transfusion for fetuses, neonates and older children. Br J Haematol. 2016 Dec;175(5):784–828.

49. Strauss RG, Burmeister LF, Johnson K, Cress G, Cordle D. Feasibility and safety of AS-3 red blood cells for neonatal transfusions. J Pediatr. 2000 Feb;136(2):215–9.

50. Fergusson DA, Hébert P, Hogan DL, LeBel L, Rouvinez-Bouali N, Smyth JA, et al. Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: The ARIPI randomized trial [Internet]. Paediatrics Publications; 2012. Available from: https://ir.lib.uwo.ca/paedpub/1844

51. Whyte R, Kirpalani H. Low versus high haemoglobin concentration threshold for blood transfusion for preventing morbidity and mortality in very low birth weight infants. Cochrane Database Syst Rev. 2011 Nov 9;(11):CD000512.

52. Wittenmeier E, Piekarski F, Steinbicker AU. Blood product transfusions for children in the perioperative period and for critically ill children. Dtsch Ärztebl Int [Internet]. 2024 Jan 26 [cited 2024 Nov 5]; Available from: https://www.aerzteblatt.de/10.3238/arztebl.m2023.0243

53. Bell EF. Red cell transfusion thresholds for preterm infants: finally some answers. Arch Dis Child Fetal Neonatal Ed. 2022 Mar;107(2):126–30.

54. Wang YC, Chan OW, Chiang MC, Yang PH, Chu SM, Hsu JF, et al. Red Blood Cell Transfusion and Clinical Outcomes in Extremely Low Birth Weight Preterm Infants. Pediatr Neonatol. 2017 Jun;58(3):216–22.

55. Prokopchuk-Gauk O, Solh Z. Irradiated, washed and CMV seronegative blood components. Can Blood Serv [Internet]. 2021 Mar 2;(Chapter 15). Available from: https://professionaleducation.blood.ca/en/transfusion/clinical-guide/irradiated-washed-and-cmv-seronegative-blood-components

56. Josephson CD, Caliendo AM, Easley KA, Knezevic A, Shenvi N, Hinkes MT, et al. Blood transfusion and breast milk transmission of cytomegalovirus in very low-birth-weight infants: a prospective cohort study. JAMA Pediatr. 2014 Nov;168(11):1054–62.

57. dos Santos AMN, Guinsburg R, de Almeida MFB, Procianoy RS, Leone CR, Marba STM, et al. Red blood cell transfusions are independently associated with intra-hospital mortality in very low birth weight preterm infants. J Pediatr. 2011 Sep;159(3):371-376.e1-3.

58. Alajbegovic-Halimic J, Zvizdic D, Alimanovic-Halilovic E, Dodik I, Duvnjak S. Risk Factors for Retinopathy of Prematurity in Premature Born Children. Med Arch Sarajevo Bosnia Herzeg. 2015 Dec;69(6):409–13.

59. Ghirardello S, Dusi E, Cortinovis I, Villa S, Fumagalli M, Agosti M, et al. Effects of Red Blood Cell Transfusions on the Risk of Developing Complications or Death: An Observational Study of a Cohort of Very Low Birth Weight Infants. Am J Perinatol. 2017 Jan;34(1):88–95.

60. Wang X, Rao R, Li H, Lei X, Dong W. Red Blood Cell Transfusion for Incidence of Retinopathy of Prematurity: Prospective Multicenter Cohort Study. JMIR Pediatr Parent. 2024 Sep 18;7:e60330.

61. Glaser K, Härtel C, Dammann O, Herting E, Andres O, Speer CP, et al. Erythrocyte transfusions are associated with retinopathy of prematurity in extremely low gestational age newborns. Acta Paediatr Oslo Nor 1992. 2023 Dec;112(12):2507–15.

62. Josephson CD, Wesolowski A, Bao G, Sola-Visner MC, Dudell G, Castillejo MI, et al. Do red cell transfusions increase the risk of necrotizing enterocolitis in premature infants? J Pediatr. 2010 Dec;157(6):972-978.e1-3.

63. Brits E, Kruger I, Joubert G. Transfusion-associated necrotising enterocolitis in very low birth weight babies: transfusion and feeding practices in two neonatal units in Bloemfontein, Free State. South Afr J Crit Care Off J Crit Care Soc. 2024;40(2):e1108.

64. Blau J, Calo JM, Dozor D, Sutton M, Alpan G, La Gamma EF. Transfusion-related acute gut injury: necrotizing enterocolitis in very low birth weight neonates after packed red blood cell transfusion. J Pediatr. 2011 Mar;158(3):403–9.

65. Song J, Dong H, Xu F, Wang Y, Li W, Jue Z, et al. The association of severe anemia, red blood cell transfusion and necrotizing enterocolitis in neonates. PloS One. 2021;16(7):e0254810.

66. Khashu M, Dame C, Lavoie PM, De Plaen IG, Garg PM, Sampath V, et al. Current Understanding of Transfusion-associated Necrotizing Enterocolitis: Review of Clinical and Experimental Studies and a Call for More Definitive Evidence. Newborn Clarksville Md. 2022;1(1):201–8.

67. Teišerskas J, Bartašienė R, Tamelienė R. Associations between Red Blood Cell Transfusions and Necrotizing Enterocolitis in Very Low Birth Weight Infants: Ten-Year Data of a Tertiary Neonatal Unit. Med Kaunas Lith. 2019 Jan 15;55(1):16.

68. Marin T, Moore J, Kosmetatos N, Roback JD, Weiss P, Higgins M, et al. Red blood cell transfusion-related necrotizing enterocolitis in very-low-birthweight infants: a near-infrared spectroscopy investigation. Transfusion (Paris). 2013 Nov;53(11):2650–8.

69. He J, Sun X, Xu X, Luo H, Tang J, Xiong T, et al. Effects of the feeding protocol during blood transfusion on splanchnic tissue oxygenation and complications in very premature infants. Front Nutr. 2024 Jul 9;11:1408717.

70. El-Dib M, Narang S, Lee E, Massaro AN, Aly H. Red blood cell transfusion, feeding and necrotizing enterocolitis in preterm infants. J Perinatol Off J Calif Perinat Assoc. 2011 Mar;31(3):183–7.

71. Bajaj M, Lulic-Botica M, Hanson A, Natarajan G. Feeding during transfusion and the risk of necrotizing enterocolitis in preterm infants. J Perinatol Off J Calif Perinat Assoc. 2019 Apr;39(4):540–6.

72. Killion E. Feeding Practices and Effects on Transfusion-Associated Necrotizing Enterocolitis in Premature Neonates. Adv Neonatal Care Off J Natl Assoc Neonatal Nurses. 2021 Oct 1;21(5):356–64.

73. Christensen RD, Wiedmeier SE, Baer VL, Henry E, Gerday E, Lambert DK, et al. Antecedents of Bell stage III necrotizing enterocolitis. J Perinatol Off J Calif Perinat Assoc. 2010 Jan;30(1):54–7.

74. Patel RM, Knezevic A, Shenvi N, Hinkes M, Keene S, Roback JD, et al. Association of Red Blood Cell Transfusion, Anemia, and Necrotizing Enterocolitis in Very Low-Birth-Weight Infants. JAMA. 2016 Mar 1;315(9):889–97.

75. Lee EY, Kim SS, Park GY, Lee SH. Effect of red blood cell transfusion on short-term outcomes in very low birth weight infants. Clin Exp Pediatr. 2020 Feb;63(2):56–62.

76. Dang D, Gu X, Jiang S, Li W, Zhou W, Cao Y, et al. RBC transfusion and necrotizing enterocolitis in very preterm infants: a multicenter observational study. Sci Rep. 2024 Jun 21;14(1):14345.

77. Tsao PC. Pathogenesis and Prevention of Intraventricular Hemorrhage in Preterm Infants. J Korean Neurosurg Soc. 2023 May;66(3):228–38.

78. Jashni Motlagh AR, Elsagh A. Effect of Transfusion on the Extension of IVH in Preterm Neonates. Iran J Neonatol IJN [Internet]. 2020 Aug [cited 2025 Mar 8];11(3). Available from: https://doi.org/10.22038/ijn.2020.43670.1726

79. Rasuli B, Weerakkody Y. Germinal matrix haemorrhage (grading). In: Radiopaedia.org [Internet]. Radiopaedia.org; 2009 [cited 2025 Mar 8]. Available from: http://radiopaedia.org/articles/7600

80. Christensen RD, Baer VL, Lambert DK, Ilstrup SJ, Eggert LD, Henry E. Association, among very-low-birthweight neonates, between red blood cell transfusions in the week after birth and severe intraventricular hemorrhage. Transfusion (Paris). 2014 Jan;54(1):104–8.

81. Goodarzi R, Molavi MA, Moayedi AR, Sooroo AK, Nazemi A. Evaluation of Incidence of Intraventricular Hemorrhage after Blood Transfusion in Preterm Neonates. Life Sci J. 2013;10(11s):237–41.

82. Skubisz A, de Vries LS, Jansen SJ, van der Staaij H, Lopriore E, Steggerda SJ. Early red blood cell transfusion and the occurrence of intraventricular hemorrhage in very preterm infants. Early Hum Dev. 2024 Feb;189:105926.

83. Baer VL, Lambert DK, Henry E, Snow GL, Christensen RD. Red blood cell transfusion of preterm neonates with a Grade 1 intraventricular hemorrhage is associated with extension to a Grade 3 or 4 hemorrhage. Transfusion (Paris). 2011 Sep;51(9):1933–9.

84. Bell EF, Strauss RG, Widness JA, Mahoney LT, Mock DM, Seward VJ, et al. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. Pediatrics. 2005 Jun;115(6):1685–91.

85. Maria A, Agarwal S, Sharma A. Acute respiratory distress syndrome in a neonate due to possible transfusion-related acute lung injury. Asian J Transfus Sci. 2017;11(2):203–5.

86. Rashid N, Al-Sufayan F, Seshia MMK, Baier RJ. Post transfusion lung injury in the neonatal population. J Perinatol Off J Calif Perinat Assoc. 2013 Apr;33(4):292–6.

87. Cho MS, Modi P, Sharma S. Transfusion-Related Acute Lung Injury [Internet]. StatPearls; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507846/

88. Gupta S, Som T, Iyer L, Agarwal R. Transfusion Related Acute Lung Injury in a Neonate. Indian J Pediatr. 2012 Oct;79(10):1363–5.

89. Tang L, Zhu TT, Zhao J. Association between red blood cell transfusion and bronchopulmonary dysplasia: a systematic review and meta-analysis. Front Pediatr. 2023;11:1095889.

90. Zhang Z, Huang X, Lu H. Association between Red Blood Cell Transfusion and Bronchopulmonary Dysplasia in Preterm Infants. Sci Rep. 2014 Mar 11;4(1):4340.

91. Bell EF. Red cell transfusion thresholds for preterm infants: finally some answers. Arch Dis Child Fetal Neonatal Ed. 2022 Mar;107(2):126–30.

TABLES AND FIGURES