Thesis

Accuracy of Pulse Oximetry-Derived Arterial Saturation in Extremely Low Birth Weight Neonates in the First Week after Birth

A retrospective data analysis at the Division of Neonatology of the Medical University of Graz from 2017 to 2021

submitted by

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Graz, 16.01.2026 *Benjamin Christian Groß m.p.*

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Abstract (Deutsch)

Hintergrund: Die optimale Sauerstoffsättigung bei Frühgeborenen stellt nach wie vor eine große Herausforderung in der neonatologischen Intensivstation (NICU) dar. Große multizentrische randomisierte Studien haben gezeigt, dass unterschiedliche Sättigungsbereiche einen erheblichen Einfluss auf das Risiko verschiedener neonataler Komplikationen und die Mortalität haben. Da die klinische Behandlung und Entscheidungen am Krankenbett in hohem Maße von einer kontinuierlichen, nicht-invasiven Pulsoxymetrie abhängen, sind die Zuverlässigkeit und Genauigkeit dieser Messwerte von entscheidender Bedeutung. Jüngste Veröffentlichungen weisen jedoch auf eine systematische Verzerrung der Pulsoxymetrie Messwerte hin, die zu einer ungenauen Darstellung der tatsächlichen arteriellen Sauerstoffsättigung führen kann.

Zielsetzung: Das Ziel dieser Studie war es, die potenzielle Verzerrung zwischen der per Pulsoxymetrie gemessenen peripheren Sauerstoffsättigung (SpO_2) und dem Goldstandard, der arteriellen Sauerstoffsättigung (SaO_2), innerhalb der hochgradig gefährdeten Untergruppe von Säuglingen mit extrem niedrigem Geburtsgewicht (ELBW; <1000 g) in der frühen Neugeborenenphase zu untersuchen.

Methoden: Eine retrospektive Datenanalyse wurde an der Neugeborenen Intensivstation der Medizinischen Universität Graz durchgeführt. Die Studie umfasste 36 ELBW-Säuglinge (mittleres Gestationsalter 26+0 Wochen) mit insgesamt 173 gepaarten SpO_2- und SaO_2-Messungen, die in den ersten 48 Stunden nach der Geburt durchgeführt wurden. Ein lineares gemischtes Effektmodell berücksichtigte wiederholte Messungen und definierte den Messfehler als „SpO_2-SaO_2-Bias".

Ergebnisse: Es wurde eine hochsignifikante, sättigungsabhängige proportionale Verzerrung festgestellt (*p <0,0001*). Mit jedem Anstieg des SaO_2-Werts um 1% verringerte sich der Messfehler um 0,645% (Standardfehler: 0,039), was darauf hindeutet, dass sich der SpO_2-Wert bei niedrigeren Sättigungswerten zunehmend vom SaO_2-Wert unterschied.

Diskussion: Die Ergebnisse zeigen eine systematische Überschätzung des SpO_2-Werts bei niedrigeren Sättigungsbereichen (SaO_2 <90%) und eine Unterbewertung

bei hohen Werten (SaO_2 >95%). Diese Studie stimmt mit früheren Arbeiten zu Frühgeborenen überein und bestätigt, dass bei ELBW-Säuglingen in den ersten 48 Stunden nach der Geburt eine erhebliche Verzerrung vorliegt. Eine Überschätzung bei niedrigen Werten ist besonders kritisch, da sie eine relevante Hypoxämie in der hochgradig gefährdeten ELBW-Population verschleiern kann.

Schlussfolgerung: Die SpO_2-Überwachung bei ELBW in den ersten 48 Stunden nach der Geburt zeigt eine signifikante Unterschätzung von sowohl einer Hypoxämie als auch einer Hyperoxämie. Obwohl die Pulsoxymetrie für die Trendüberwachung unerlässlich ist, sollte sie nicht die einzige Grundlage für die Anpassung des Sauerstoffgehalts bei Frühgeborenen sein. Zukünftige Untersuchungen sollten sich auf die Entwicklung verfeinerter Algorithmen und verbesserter Kalibrierungsverfahren für Frühgeborene und die damit verbundenen komplexen Herausforderungen konzentrieren.

Abstract

Background: Optimal oxygen saturation targeting in premature neonates remains a critical challenge in neonatal intensive care unit (NICU). Large multicenter randomized trials have demonstrated that different saturation ranges significantly impact the risk of several neonatal complications and mortality. Since clinical management and bedside decisions depend heavily on continuous, noninvasive pulse oximetry, the reliability and accuracy of these readings are essential. However, recent literature has indicated a systematic bias in pulse oximetry readings that may result in inaccurate reflection of the true arterial oxygen saturation.

Objective: The aim of this study was to explore the potential bias between peripheral oxygen saturation (SpO_2) measured by pulse oximetry and gold standard, arterial oxygen saturation (SaO_2) within the highly vulnerable subgroup of extremely low birth weight (ELBW; <1000g) infants in the early neonatal period.

Methods: A retrospective single-center analysis was conducted at the NICU of Medical University of Graz. The study included 36 ELBW infants (mean gestational age 26+0 weeks) with a total of 173 paired SpO_2 and SaO_2 measurements obtained during the first 48 hours after birth. A linear mixed effects model accounted for repeated measurements, defining the measurement error as "SpO_2-SaO_2 bias".

Results: A highly significant, saturation-dependent proportional bias was identified ($p < 0.0001$). For every 1% increase in SaO_2, the measurement error decreased by 0.645% (standard error: 0.039), indicating that SpO_2 increasingly differed from SaO_2 at lower saturation levels.

Discussion: The findings reveal a systematic overestimation of SpO_2 at lower saturation ranges (SaO_2 <90%), reversing to an underestimation at high levels (SaO_2 >95%). While aligning with previous work in preterm infants, this study confirms that significant bias exists in ELBW infants in the first 48 hours after birth. Overestimation at low levels is particularly critical as it may mask relevant hypoxemia in highly vulnerable ELBW population.

Conclusion: SpO_2 monitoring in ELBW in the first 48 hours after birth shows a significant underestimation of both hypoxemia and hyperoxemia. Although pulse oximetry is essential for trend monitoring, it should not be the only basis for adjusting

oxygen levels in premature infants. Future investigations should focus on developing more refined algorithms and improved calibration processes for premature infants and the complex challenges they present.

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List of Abbreviations

AC	*Alternating current*
BPD	*Bronchopulmonary dysplasia*
BW	*Birth weight*
CLD	*Chronic lung disease*
COHb	*Carboxyhemoglobin*
CNS	*Central nervous system*
CP	*Cerebral palsy*
CPAP	*Continuous positive airway pressure*
COT	*Canadian Oxygen Trial*
CRP	*C-reactive protein*
CSF	*Cerebrospinal fluid*
DC	*Direct current*
ELBW	*Extremely low* birth weight
ELGAN	*Extremely low gestational age neonate*
EPO	*Erythropoietin*
EPT	*Extremely Preterm*
FiO_2	*Fraction of inspired Oxygen*
FHbF	*Fraction of fetal hemoglobin*
FO_2Hb	*Fractional oxyhemoglobin*
GA	*Gestational age*
Hb	*Hemoglobin*
HbA	*Adult Hemoglobin*
HbF	*Fetal Hemoglobin*
HFOV	*High-frequency-oscillatory ventilation*
HHb	*Deoxyhemoglobin*
IGF-1	*Insulin-like growth factor 1*
IL-1β	*Interleukin-1 beta*
IL-6	*Interleukin-6*
IQR	*Interquartile range*
IR	*Infrared*
IRDS	*Infant respiratory distress syndrome*
IVH	*Intraventricular hemorrhage*
LBW	*Low birth weight*
LED	*Light-emitting diode*
LGA	*Large for gestational age*
LISA	*Less invasive surfactant administration*
LoA	*Limits of Agreement*
MetHb	*Methemoglobin*
MRI	*Magnetic resonance imaging*
NEC	*Necrotizing enterocolitis*
NICU	*Neonatal intensive care unit*
ODC	*Oxygen dissociation curve*
O_2Hb	*Oxyhemoglobin*
OS	*Oxidative stress*
PaO_2	*Arterial oxygen pressure*
PDA	*Patent ductus arteriosus*
PEEP	*Positive end-expiratory pressure*
pO_2	*Partial pressure of oxygen*
PMA	*Postmenstrual age*
PPROM	*Preterm premature rupture of the membranes*
PVL	*Periventricular leukomalacia*
PVHI	*Periventricular hemorrhagic infarction*
PHVD	*Post-hemorrhagic ventricular dilatation*
RDS	*Respiratory distress syndrome*
ROP	*Retinopathy of prematurity*
ROS	*Reactive oxygen species*
SaO_2	*Arterial oxygen saturation (co-oximetry)*
SD	*Standard deviation*
SGA	*Small for gestational age*
SHb	*Sulfhemoglobin*
SpO_2	*Peripheral oxygen saturation (pulse oximetry)*
TNF-α	*Tumor necrosis factor alpha*
tHb	*Total Hemoglobin*
VEGF	*Vascular endothelial growth factor*
VLBW	*Very low birth weight*
WHO	*World Health Organization*

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1 Introduction

1.1 Premature Neonates

Definition

The World Health Organization (WHO) defines a preterm (premature) neonate as an infant born before 37 completed weeks of gestation. Prematurity can be categorized by gestational age as extremely preterm (<28 completed weeks), very preterm (28 to 32 completed weeks), and moderate-to-late preterm (32 to 37 completed weeks). Preterm infants can also be classified by birth weight: low birth weight (LBW <2,500 g), very low birth weight (VLBW <1,500 g), and extremely low birth weight (ELBW <1,000 g) at birth (1,2). Preterm birth occurs for two main reasons: spontaneous preterm labor with intact membranes or preterm premature rupture of membranes (PPROM). Less commonly, preterm delivery is medically indicated because of maternal or fetal complications (3).

Epidemiology

Worldwide, preterm birth occurs in about one out of every ten liveborn infants, which translates to an estimated 13.4 million preterm births in 2020. Around 15% of global preterm births from 2010 to 2020 happened before 32 gestational weeks (4). Preterm birth rates have remained largely stable (9.9% of live births in 2020 vs 9.8% in 2010), with only a slight decline in absolute numbers (13.4 million in 2020 vs 13.8 million in 2010). Rates vary substantially by geographic region and national income. Half of all worldwide preterm births (estimated 7.4 Million) occur in only six countries, Nigeria, Pakistan, Indonesia, India, China and the United States. In addition, about 90% of preterm infants are born in low- and middle-income countries, with more than 64% occurring in South Asia and sub-Saharan Africa (4,5).

Morbidity

Preterm infants face a high risk of both acute and long-term complications because multiple organ systems are structurally and functionally immature at birth. The need for intensive care increases significantly with decreasing gestational age (6). Key neonatal morbidities include difficulties with thermoregulation, infectious complications, intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, anemia of prematurity, retinopathy of prematurity and chronic lung disease, previously known as bronchopulmonary dysplasia (7).

Mortality and Outcomes

Neonatal death is defined as death occurring within the first 28 days after birth. The neonatal period is commonly divided into an early phase, covering the first seven days, and a late phase from day 7 to day 27. Deaths occurring after day 28 but before the completion of the first year of life (<365 days) are classified as infant deaths (8). There is an inverse correlation between mortality rates and both birth weight and gestational age, meaning that having lower birth weight or lower gestational age or both at the same time are linked to a higher risk of neonatal death (6) Globally, approximately 44% of all deaths in children under five years of age occur during the neonatal period. Preterm birth complications are one of the three leading death causes under the age of five (9). Although under-five mortality has declined over the past two decades, largely due to reduced deaths from infectious diseases such as pneumonia, malaria, diarrhea, and measles, preterm birth-related complications have emerged as the leading cause of death among children under five, with most deaths occurring during the neonatal period (5). Rates of survival among prematurely born babies vary drastically across the globe. Additionally, risk of adverse outcomes rises with decreasing gestational age. An infant born preterm at 24 completed weeks has a 50% chance of surviving the neonatal period, provided that the birth was in a high-income country. However, if the birth occurred in a low-income country, only one out of ten babies born at 28 completed weeks would survive. On the contrary, survival rates at 28 weeks in high income countries are up to 90% (7,10). In case of surviving these neonatal conditions, many suffer continuously throughout life from neurodevelopmental and physical health issues. Learning impairments, visual disorders, chronic lung disease of prematurity and cerebral palsy are just a few amongst a vast pattern of impairments related to prematurity and its complications. Families, society and the healthcare systems bear a huge burden due to these challenges (10). In fact in the recent estimates of global burden of disease, neonatal disorders have consistently been the number one cause for lost human capital since 1990 (11).

1.1.1 Extremely Premature and Extremely Low Birth Weight Neonates (ELBW)

Extremely low gestational age neonates (ELGANs) are born before 28 completed weeks of gestation, while extremely low birth weight (ELBW) neonates weigh less than 1,000g at birth. These terms are commonly used in the literature and frequently overlap (12). During the fetal period, growth accelerates greatly, especially in the third trimester, when weight increases from roughly 650g at 24 weeks (50^{th} percentile) to about 2,800g at 36 weeks. Lacking this crucial terminal development time due to preterm birth results in both prematurity and low birth weight (1,13). ELGANs account for approximately 5.2% of all preterm births worldwide, but contribute disproportionately to prematurity-related morbidity and mortality (14). ELGANs and ELBW neonates represent infants most severely affected by prematurity and therefore share similar complications and morbidities such as nosocomial infection, intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, retinopathy of prematurity and chronic lung disease, which reflect both neonatal and long-term complications of prematurity (1,15). In a recent retrospective study, differences in mortality and morbidity between ELBW and VLBW were investigated. The group of ELBW showed a lower mean gestational age (26.3 ± 2.3 weeks vs 29.7 ± 2.4 weeks) and a significantly higher mortality rate than VLBW newborns (26.7% vs 7.0%). In addition, the common morbidities including respiratory distress syndrome, early- and late-onset sepsis, intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis and bronchopulmonary dysplasia were significantly more common in ELBW than in VLBW neonates. Therefore, ELBW neonates represent infants with highest risk of prematurity associated complications (16).

1.2 Oxygen Saturation Targeting in Neonatal Intensive Care

Virtually all ELGANs require some form of respiratory support immediately after birth. In neonatal care, the use of supplemental oxygen is a standard practice, particularly in the management of most common lung disease, infant respiratory distress syndrome (IRDS). Targeted oxygen supplementation aims to ensure adequate tissue oxygenation while avoiding harmful effects associated with an

oversupply of oxygen. Therefore, arterial oxygen saturation is continuously monitored using pulse oximetry. However, knowledge of the percentage of oxygenated hemoglobin alone does not provide sufficient context to interpret total oxygen content and oxygen requirement. The optimal oxygen saturation target ranges to balance between excessive and deficient arterial oxygenation in premature newborns, remains unclear to date (17). Historically, the first concerns about oxygen targets appeared in the 1950s, when early randomized trials demonstrated that unrestricted oxygen use in preterm infants increased both the incidence and severity of ROP significantly (18). This new insight resulted in a time characterized by limited inspiratory oxygen concentrations, trying to prevent oxygen toxicity. While these restrictions tried to minimize the risk for ROP development, it contrarily led to an increase in mortality. As a result, the field faced the persistent challenge of determining how to deliver sufficient oxygen to avoid hypoxic injury without triggering oxygen related complications. In order to investigate this very important question, three clinical trials were conducted and their results were later analyzed by the Neonatal Oxygenation Prospective Meta-analysis (NeOProM) Collaboration (19,20).

1.2.1 Evidence from Key Trials

Three major studies have provided important insights into how different peripheral oxygen saturation (SpO_2) target ranges affect outcomes in preterm infants. These include the SUPPORT trial, BOOST II, the Canadian Oxygen Trial (COT) and the meta-analysis of the findings by NeOProM (20). The SUPPORT trial included over 1,300 ELGANs and compared two different SpO_2 target ranges: 85-89% and 91-95%. The trial found that the lower target reduced the incidence of severe ROP, a major concern in preterm infants receiving oxygen therapy. However, this benefit came with a significant increase in mortality in infants in the lower target group. These findings demonstrated the difficulty of determining optimal oxygen saturations, as more restrictive oxygenation reduced some complications but increased others (21). The BOOST II trial, which was conducted across the United Kingdom, Australia, and New Zealand, supported the conclusions from the SUPPORT trial. This study in 2108 neonates also found that lower SpO_2 targets reduced the risk of ROP but resulted in higher mortality rates. Furthermore, in this

trial infants in the group with lower SpO_2 limits showed increased rates of necrotizing enterocolitis (NEC), another severe complication in preterm infants associated with oxygen therapy. Together, these findings highlight the extreme vulnerability of preterm infants to even small changes in oxygen saturation and demonstrate that efforts to reduce one complication can unintentionally increase the risk of others. Therefore the study also indicates that more restrictive oxygen targets may not always be beneficial for overall neonatal health (19). The Canadian Oxygen Trial (COT) also compared lower and higher oxygen saturation ranges. Unlike the earlier SUPPORT and BOOST II trials, COT found no significant difference in mortality or in key secondary outcomes such as retinopathy of prematurity or necrotizing enterocolitis. However, infants in the lower-target group were weaned from oxygen slightly earlier, suggesting a modest benefit in reducing oxygen exposure (17). Overall, these results emphasized the importance of using individual approaches when it comes to supplemental oxygen therapy. Their contributions of valuable data helped create a broader evidence base that was synthesized in the later NeOProM meta-analysis (20). This Neonatal Oxygenation Prospective Meta-analysis collaboration combined individual patient data from five randomized trials, including SUPPORT, BOOST II, and COT. No significant difference between lower (85-89%) and higher (91-95%) SpO_2 targets for the composite of death or major disability at 18-24 months was found. However, lower oxygen targets were linked to higher mortality and more NEC, while ROP treatment was less frequent in the lower-target group. Overall, the NeOProM results confirm the ongoing clinical dilemma, that while lower oxygen targets can reduce some risks, they may increase others. The reduction in ROP risk comes at the cost of an escalated mortality rate and an increased incidence of NEC (20).

1.2.2 Oxygen Therapy Guidelines

Current neonatal practice has converged on mid-range oxygen saturation targets for ELGANs, translating the findings from above mentioned clinical research into practical bedside guidance. Because of the earlier mentioned trade-offs, the optimal oxygen saturation targets remain unidentified, especially for premature neonates with gestational age greater than 30 weeks. The European Consensus Guidelines on the Management of Respiratory Distress Syndrome recommend maintaining a

peripheral oxygen saturation (SpO_2) between 90% and 94%, with the low and high-alarm limits typically set at 89% and 95%. These limits are used to reduce the amount of time infants drift outside the desired range and to help avoid both hypoxemia and hyperoxemia. They also state that there is no scientific evidence supporting the exact alarming thresholds. However, using tight limits is considered a practical approach to reducing exposure to dangerous oxygen extremes. The guidelines emphasize the importance of continuous pulse oximetry and the careful adjustment of inspired oxygen to keep the infants within the desired ranges. They further note that infants with established bronchopulmonary dysplasia may require slightly higher saturation targets, as this can lower their risk of developing pulmonary hypertension. This highlights the need to tailor oxygen management to the individual infant's condition rather than applying a uniform target for all preterm neonates (22). The recommended mid-range strategy builds on the conclusions of the NeOProM Collaboration, which were outlined above (20).

1.3 Oxygen Measurement Methods

1.3.1 Pulse Oximetry

Pulse oximetry is a widely applicable, noninvasive technique for continuously monitoring arterial oxygen saturation at peripheral sites, typically on the extremities (23). This technique is used universally in critical care and other clinical situations to get immediate information on the arterial oxygen saturation and therefore recognition and prevention of hypoxia (24). In pediatric medicine many clinical decisions, such as use of supplemental oxygen or escalation of care, are often based solely on oxygen saturations measured by pulse oximetry. Thus, pulse oximetry has become a key tool in clinical assessment and clinical decision-making (25).

1.3.1.1 Principle and Methodology

The science behind pulse oximetry is based on two physical principles, which are essential to understanding the method's limitations. The principles include, first, the differentiation between oxygenated hemoglobin (O_2Hb; Oxyhemoglobin) and deoxygenated hemoglobin (HHb; Deoxyhemoglobin), and second, the calculation

of oxygen saturation exclusively on arterial blood (23). O_2Hb and HHb have different absorption spectra in the red and near-infrared (near-IR) ranges. This fact is utilized in pulse oximetry, where light-emitting diodes (LEDs) emit light at two specific wavelengths, red (660nm) and near-infrared (940nm). These wavelengths are chosen not only because they are differentially absorbed by O_2Hb and HHb, but also because they penetrate biological tissue effectively while minimizing nonspecific absorption by non-blood components. The extent of light absorption at each wavelength reflects the relative concentrations of O_2Hb and HHb. O_2Hb absorbs more near-infrared light and less red light than HHb. A photodiode positioned on the opposite side of the tissue detects the transmitted, unabsorbed light. The absorbed fractions of red and near-IR light are then used to calculate the proportion of oxygen-saturated hemoglobin (26). The other key aspect of pulse oximetry is the isolation of the arterial signal. This is due to the arterial pulsation, which leads to changes of blood volume and moreover in light absorption from diastole to systole. These absorption changes create a dynamic, alternating current (AC). Venous blood and other tissues like skin, fat or bone absorb the light in a stable manner, reflected in a constant direct current (DC). Consequently non-arterial absorption is corrected for by a microprocessor, so oxygen saturation of arterial blood can be isolated (23). The Beer-Lambert law provides the theoretical basis for pulse oximetry by describing how light absorption relates to the concentration of absorbing substances. This law consists of two components. Lambert's law states that light absorption is directly proportional to the distance light travels through a material. Beer's law states that absorption is directly proportional to the concentration of the absorbing substance. When applied together, these principles show that the concentration of hemoglobin in arterial blood has a linear relationship with the amount of light absorbed. This relationship allows the pulse oximeter to estimate oxygen saturation (26).

Calculation and Calibration

Pulse oximeters use detected absorbances to calculate a "Red to Infrared Modulation Ratio" called [R] (23). This ratio is obtained by comparing how red light and infrared light are absorbed over the arterial pulse, isolating the pulsatile component (AC) from the constant background signal (DC). At low concentration of O_2Hb, equal to low oxygen saturation, in arterial blood, the time-dependent

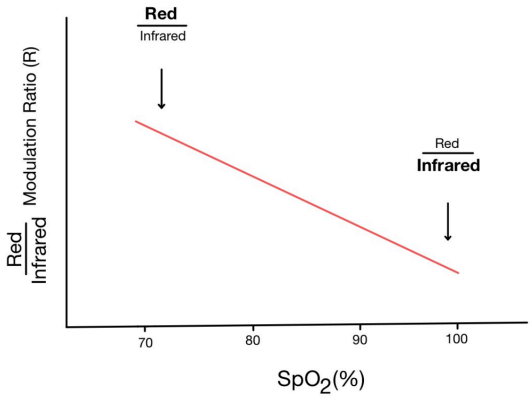


Figure 1 *- Relationship between "Red to Infrared Modulation Ratio" (R) and SpO_2 in pulse oximetry. Adapted from Chan et al. (23)*

absorbance of red light (660nm) is greater than the one of the infrared portion (940nm) [$A_{660nm,AC} > A_{940nm,AC}$; A = Absorbance]. Hence, the corresponding R value at low arterial oxygen saturations is high. On the contrary at low HHb levels, the relationship is inversed, meaning absorbance of infrared light exceeds the absorbance of red light [$A_{660nm,AC} < A_{940nm}$] in the arterial signal, leading to a smaller R value (23). Graphically the R value decreases with increasing arterial oxygen saturation, as shown in Figure 1. However, it must be emphasized that an adequate pulse signal is a fundamental prerequisite for obtaining reliable pulse oximetry measurements (26). To convert the absorbance ratio into the SpO_2 reading, manufacturers calibrate pulse oximeters against reference data gained from healthy volunteers, in whom SaO_2 is experimentally lowered and compared directly with the simultaneously measured R values. The mathematical relationship between R and SaO_2 is determined by various examinations in healthy volunteers. While taking arterial blood, the corresponding R value is assessed in parallel by pulse oximetry. SaO_2 in blood sample is determined using the gold-standard method known as co-oximetry, which will be discussed in detail in section 1.3.2 (27). SaO_2 of candidates

is altered to a minimal around 70% in order to provide R values in a SaO_2 range from 100%-70%. Thus, this equals the range in which pulse oximetry is largely reliable. Since readings below 70% cannot be compared to experimental references, they may not be quantitatively adequate (26).

1.3.1.2 Accuracy and Limitations

In neonatal intensive care, pulse oximetry provides a simple, continuous, and noninvasive method to monitor SpO_2. Yet, its accuracy in preterm infants is affected by physiological and technical factors. These include the specific characteristics of fetal hemoglobin, the complex relationship between SpO_2/SaO_2, and PaO_2, and several other potentially influential factors. Recognizing these influences is crucial, since even small measurement errors can have considerable effects on clinical management in preterm infants (28–30).

1.3.1.2.1 Fetal Hemoglobin and the Oxygen Dissociation Curve

Fetal hemoglobin (HbF) is the main oxygen carrier in the blood of fetuses and neonates. Its molecular structure differs from adult hemoglobin (HbA) in that it contains two γ-chains instead of β-chains. This structural difference reduces the interaction of HbF with 2,3-diphosphoglycerate, which results in a higher oxygen affinity. The oxygen dissociation curve (ODC) describes the relationship between the partial pressure of oxygen (PaO_2) and SaO_2. Its sigmoidal shape reflects how hemoglobin binds oxygen in the lungs and releases it in the tissues. A leftward shift of the curve means that hemoglobin holds onto oxygen more tightly, achieving higher saturation at a lower PaO_2, whereas a rightward shift indicates reduced affinity and easier oxygen release. The predominance of HbF therefore shifts the ODC to the left. At the low partial pressures *in utero* this enables oxygen to transfer across the placenta from maternal to fetal blood. Postnatally however, the same principle limits the oxygen release to the tissues. Around 20 gestational weeks, the proportion of HbA begins to increase, but at birth HbF still accounts for the majority of the total hemoglobin in term infants (60-80%) and up to 90% in extremely preterm neonates. Fraction of HbF (FHbF) declines gradually with advancing gestational age or after the transfusion with adult erythrocytes (30–32). In terms of light absorption, HbF behaves almost identically like HbA at the wavelengths used by pulse

oximeters. Therefore, HbF does not significantly distort the optical estimation of SpO_2 in pulse oximetry (30,33). However, the leftward shift in the ODC position of the HbF, affects the relationship between PaO2 and SaO2. Because of the higher oxygen affinity, a certain SpO_2 or SaO_2 corresponds to a lower PaO_2 in neonatal than in adult blood. Consequently, an SpO_2 value that appears clinically adequate on the monitor may mask lower arterial oxygen tensions and a smaller oxygen reserve, increasing the risk of unrecognized hypoxemia. Inversely, after transfusion the increased HbA fractions results in the ODC shift to the right, which elevates PaO_2 for the same SpO_2 value (31,32). In conclusion, even though the association of SpO_2 to SaO_2 remains close, the relationship between SaO_2 and PaO_2 varies considerably depending on the FHbF. This distinction has important clinical implications, as SpO_2 is a good indicator of hemoglobin saturation but does not accurately reflect arterial oxygen tension. In HbF-rich blood of preterm infants, SpO_2 should therefore be used mainly to monitor trends, and arterial blood gas analysis is required when the exact PaO_2 is needed (29–31).

1.3.1.2.2 Additional Limitations

The accuracy of pulse oximetry can be evaluated by comparing the oxygen saturation calculated by the device (SpO_2) with arterial oxygen saturation (SaO_2) obtained through co-oximetry, which remains the gold-standard method for determining hemoglobin oxygenation (27). In healthy subjects, the standard deviation of the difference between SpO_2 and SaO_2 is about 2%, matching the accuracy usually reported by manufacturers. Statistically, this implies that approximately 5% of readings may deviate by 4% or more, meaning that although the average error is small, larger errors are also possible. Methodologically, pulse oximetry depends on photoplethysmographic detection of light absorption in arterial blood and uses an empirical calibration derived from measurements in healthy adult volunteers. This calibration process assumes consistent optical path length and tissue perfusion. When applied to clinical populations, such as critically ill patients or preterm infants with different vascular and optical characteristics, the actual accuracy is often lower than predicted. These limitations introduce a systematic error for this patient population (27). Additional factors including motion artifact, low perfusion, suboptimal probe alignment, ambient light interference, and variations

between device algorithms can further contribute to measurement error (27,28). Recent neonatal data further confirmed a greater inaccuracy of pulse oximetry in daily clinical practice. Wackernagel et al. (29) analyzed more than 27,000 paired SpO_2-SaO_2 measurements and found that SpO_2 overestimated SaO_2 by an average of 2.9 ± 5.8%. The accuracy was especially poor when SpO_2 values fell below 91%. The devices even failed to meet the accuracy standards set by the International Organization for Standardization (ISO). Sometimes, even when pulse oximetry readings appeared to fall within the desired range, the actual SaO_2 was not in the intended range. In 20.9% of cases, the true SaO_2 was below the lower threshold, and in 7.6% of cases it was above the upper threshold. A similar pattern was observed when comparing SpO_2 to PaO_2. Despite SpO_2 values appearing adequate, the actual PaO_2 levels were beneath the target in over half of the patients and too high in around every fifth one. The authors concluded that pulse oximetry is valuable for continuously monitoring saturation trends, but it should not be the exclusive method used to make decisions about adjusting oxygen therapy in neonatal patient populations. Furthermore, even within the recommended saturation range for infants, most tested pulse oximeters did not meet ISO accuracy criteria (29). Further evidence supports these observations. In an earlier study focusing on very preterm infants, Rosychuk et al. (34) found, that almost 40% of SpO_2 values in the range from 85-89%, had corresponding true SaO_2 values (determined by co-oximetry) that were below 85%. Collectively, these studies demonstrate that pulse oximetry may systematically overestimate arterial oxygenation within the saturation range for preterm neonates, where precise oxygen management is crucial. Therefore, careful interpretation of SpO_2 values is essential, and arterial blood gas analysis remains necessary to accurately determine arterial oxygen pressure (29,34). Additionally, the physiological characteristics of the oxygen dissociation curve, as outlined above, creates further limitations when oxygen saturations are high. The upper part of the curve is nearly flat, which means that significant increases in arterial oxygen pressure (PaO_2) above 100 mmHg result only in minor changes in SpO_2. This makes pulse oximetry poorly suited for identifying hyperoxemia in HbF-rich patients, a particularly important limitation for preterm infants, where excessive oxygen tension contributes to retinopathy of prematurity and oxidative injury to vital organs such as the lungs or the brain (27,31).

1.3.2 Co-oximetry of Arterial Blood

Co-oximetry is an *in vitro* spectrophotometric technique that measures the relative amounts of different hemoglobin forms in a blood sample by analyzing light absorption at multiple wavelengths (35). It is widely seen as the reference method for determining true SaO_2 and provides the basis for the empirical calibration of pulse oximeters (27). In contrast to pulse oximetry, which estimates SpO_2 using only two wavelengths of light and an empirical calibration, co-oximeters directly measure the hemoglobin species using multiple wavelengths (36).

1.3.2.1 Principle and Methodology

Co-oximetry is based on multi-wavelength spectrophotometry and the Beer-Lambert law, which describes the relationship between the concentration of a substance and the amount of light it absorbs. Each type of hemoglobin has a unique light absorption spectrum. This allows the co-oximeter to calculate the fractional concentrations of the different hemoglobin forms. Therefore, co-oximeters reliably quantify O_2Hb, HHb, dyshemoglobins such as carboxyhemoglobin (COHb), methemoglobin (MetHb), sulfhemoglobin (SHb) and calculated SaO_2 (37). Modern co-oximeters measure the light absorption at more than one hundred wavelengths, which improves measurement precision, allowing the detection of fetal hemoglobin due to its unique absorption characteristics (36). When it comes to interpretation of co-oximetry, the distinction between SaO_2 and fractional oxyhemoglobin (FO_2Hb) is central. This is because co-oximeters quantify all hemoglobin species fractionally, whereas clinical oxygenation depends on the proportion of hemoglobin, that is actually able to transport oxygen. The functional value SaO_2 represents the fraction of oxygenated hemoglobin relative only to hemoglobin forms, that are capable of binding oxygen, like O_2Hb and HHb.

$$SaO_2 = \frac{c(O_2Hb)}{\big(c(O_2Hb) + c(HHb)\big)}$$

FO_2Hb, on the other hand, is the relation of oxygenated hemoglobin to the total hemoglobin (tHb), which includes dyshemoglobins that cannot transport oxygen (36).

$$FO_2Hb = \frac{c(O_2Hb)}{c(tHb)}$$

The measurement is performed *in vitro* on a hemolyzed blood sample, preferably arterial blood. Applied to an optical cuvette, the device records absorbance across the selected wavelengths and uses algorithms to calculate the partial concentrations of each hemoglobin species. This allows accurate measurement of true SaO_2, which differs from SpO_2 estimated only by two-wavelength pulse oximetry (35,36).

1.3.2.2 Accuracy and Limitations

Because co-oximetry directly measures different hemoglobin forms, it provides highly accurate saturation values and is commonly used as the reference standard to validate non-invasive oximetry methods, such as pulse oximetry. However, Law et al. (38) showed that the actual SaO_2 values can vary by 2-3% between different co-oximeters, especially when oxygen saturation is low. This finding challenges the common assumption that all co-oximeters produce identical and interchangeable measurements. This adds an important limitation, as even the reference method contains manufacturer-dependent bias (38). From a broader medical perspective measurement accuracy is also affected in cases of elevated dyshemoglobins or unstable hemoglobin variants. When MetHb levels exceed about 10%, light absorption patterns can overlap and consequently lead to false calculations of the individual hemoglobin fractions (39). In the same way hemoglobin M variants or sulfhemoglobin can change the spectral characteristics of blood, resulting in incorrect identification of hemoglobin types (36). But even when hemoglobin composition is normal, significant differences between different devices can occur because calibration methods and algorithms differ between manufacturers (38). However, when an accurate measurement of different hemoglobin types is needed, co-oximetry is essential. It becomes especially important when pulse oximetry readings and arterial blood gas results do not match, since only co-oximetry directly measures hemoglobin species (36,39). Even though the method is considered the gold standard, any variability or inaccuracy within co-oximetry itself can influence the calibration of pulse oximetry and indirectly contribute to discrepancies between SpO_2 and SaO_2 and therefore to an even higher inaccuracy of pulse oximetry (27,38).

1.4 Oxygen-related Neonatal Complications in Premature Neonates

For the purpose of this thesis, complications attributable or contributing to either inadequate arterial oxygenation (hypoxia), such as infant respiratory distress syndrome (IRDS), intraventricular hemorrhage (IVH) and necrotizing enterocolitis (NEC) or to excessive oxygen exposure (hyperoxia), including periventricular leukomalacia (PVL), retinopathy of prematurity (ROP) and bronchopulmonary dysplasia (BPD), are addressed. These conditions are further categorized according to the timing of their clinical manifestation into neonatal complications (occurring within the first 28 days of life) and long-term complications (potentially resulting in lifelong chronic morbidity). Across all discussed conditions, appropriate oxygen targeting and the avoidance of both hypoxia and hyperoxia are essential to reduce the risk of these morbidities.

1.4.1 Infant Respiratory Distress Syndrome (IRDS)

IRDS is a critical condition, typically observed in preterm infants. It arises due to a lack of sufficient endogenous surfactant and the immaturity of the lungs (22). Clinically, IRDS presents as respiratory insufficiency developing directly after delivery or within the first few hours (40).

Epidemiology

IRDS is the most frequent complication associated with prematurity and represents also the leading respiratory disease among premature infants. Thus, its contribution to neonatal morbidity and mortality is substantial. Caused primarily by an underdeveloped pulmonary system including surfactant deficiency, incidence rates increase with decreasing gestational age as well as birth weight (40). Infants who survive a respiratory distress syndrome are at increased risk of developing severe long-term complications (41). IRDS occurs in approximately 30-50% of preterm infants born at less than 32 weeks' gestation, with the incidence exceeding 90% among those born before 24 gestational weeks (1,42). Additionally, IRDS prevalence may be estimated using surfactant administration rates, as roughly 50% of neonates born between 22+0 and 32+6 weeks' gestation in Europe are treated

with exogenous surfactant (22). Risk factors for RDS include male sex, low gestational age and birth weight, as well as maternal factors such as diabetes mellitus, chorioamnionitis and multiple pregnancy. Recent evidence has demonstrated that maternal diabetes mellitus, including both gestational and pregestational forms, is an important risk factor for the development of neonatal respiratory distress syndrome (41).

Etiology and Pathogenesis

The respiratory distress syndrome in preterm infants is essentially characterized by an immature fetal lung and lack of surfactant. Therefore, understanding the embryological development of the lung is crucial in comprehending the causes and mechanisms of IRDS. Lung development can be sorted into stages, embryonic, pseudoglandular, canalicular, saccular and the alveolar stage. Throughout these stages airways progress from bigger towards the next smaller division until around 34-36 weeks when alveoli start to form and mature. Consequently, gas exchange in preterm neonates, who may have not yet reached alveolar stage, takes place within the saccular structures consisting of terminal bronchioles. Thus factors influencing gas exchange, such as diffusion distance and surface area of the lung, are inadequate at the canalicular or saccular stages. Additionally, by lowering the surface tension of alveoli and avoid end-expiratory airway collapse, surfactant plays another critical role in maintaining a normal postnatal respiratory function. IRDS results from surfactant deficiency. Surfactant production begins at approximately 22 weeks' gestation in type II pneumocytes but remains minimal until it increases markedly at around 34 to 35 weeks' gestation (43,44). Once released, surfactant creates a thin film at the alveolar air-liquid interface, where it improves lung compliance and enables the reopening of previously collapsed airways. Part of the used surfactant is then reabsorbed by type II pneumocytes to sustain a surfactant cycle and maintain necessary levels within the air spaces. This cycle is also immature in premature infants, which further aggravates their surfactant deficiency (44). Due to immaturity as well as epithelial and endothelial damage, increased permeability of the alveolar-capillary barrier allows plasma proteins such as albumin and fibrinogen to enter the alveolar space. These proteins inhibit surfactant function, and their accumulation in the alveolar space was responsible for the former term "hyaline membrane disease". These molecules act as strong surfactant inhibitors,

further reducing overall surfactant activity. Taken together, primary surfactant deficiency in the structurally immature, terminal saccular lung is further exacerbated by secondary inactivation of existing surfactant due to inhibitory proteins (1,45).

Pathophysiology

In a state of pulmonary surfactant deficiency, either by insufficient production or discharge, alveoli become destabilized. This leads to diffuse alveolar collapse, known as atelectasis, and impaired alveolar ventilation shortly after birth. Although alveoli may continue to be perfused, the absence of adequate ventilation leads to a ventilation-perfusion mismatch, which causes both hypoxemia and hypercapnia (high blood CO_2 levels). As a result, tidal volumes are reduced and pulmonary compliance declines. The lungs become stiff, requiring more effort to breathe, which further impairs gas exchange and intensifies hypoxemia and hypercapnia leading to acidosis. This metabolic complication promotes systemic hypotension, pulmonary vasoconstriction and reduces blood flow through the lungs, enhancing right-to-left shunting. Right-to-left shunting is a process, in which deoxygenated venous blood bypasses gas exchange and enters the arterial circulation. This can occur intrapulmonary, by perfusing collapsed lung regions and extrapulmonary *via* foramen ovale and ductus arteriosus. In combination with the present blood gas abnormalities (hypoxemia, hypercapnia) and acidosis, the physiological initiation of surfactant production after birth is hindered. These processes create a self-sustaining cycle (“circulus vitiosus”) of respiratory failure in neonatal IRDS (44,45).

Clinical Presentation and Diagnosis

As previously mentioned, a typical patient with IRDS is a preterm infant with low birth weight, showing first signs and symptoms directly after birth or in the next 3 to 4 hours post-partum. The main clinical signs are increased respiratory rate or tachypnea (>60/min), which often appears combined with increased work of breathing (dyspnea), and expiratory “grunting”. Characteristic signs of dyspnea in these neonates are jugular, inter- and subcostal retractions of the chest wall and nasal flaring. IRDS patients may have decreased breath sounds in auscultation and also present with signs of insufficient peripheral circulation, like cyanosis and faded peripheral pulses. Although clinical signs might be subtle in the beginning, the absence of adequate therapy can rapidly escalate into severe, life-threatening

respiratory failure within 1 to 2 days after birth (1,40,45). In the past, the diagnosis heavily relied on radiographic chest imaging, with typical findings including reticulogranular “ground glass” appearance with visible air bronchograms. However, recent consensus guidelines have de-emphasized the role of radiographic diagnosis and grading of IRDS and have highlighted the importance of the clinical presentation, typically being a premature neonate with postnatal onset of ongoing respiratory failure, characterized by increased breathing effort and supplemental oxygen requirement (22). In addition, lung ultrasound has become an important bedside tool in the assessment of neonatal respiratory distress. Sandig et al. (46) recently proposed a structured lung ultrasound protocol for neonates, allowing evaluation of pleural features, lung artifacts, consolidations, and effusions in a standardized way. This approach supports the differentiation of neonatal respiratory disorders, including IRDS, while integrating sonographic findings with the clinical presentation. In IRDS, typical sonographic findings include an irregular or thickened pleural line, diffuse confluent B-lines (“white lung”), reduced or absent A-lines, and subpleural consolidations in more severe cases. Therefore, lung ultrasound is a rapid, repeatable and radiation-free assessment that may support early clinical decision-making in preterm infants with respiratory failure (46). This aligns with the growing tendency for preemptive therapy based primarily on clinical signs and symptoms and ultrasound findings, aiming to prevent the progression to more severe forms of IRDS (22).

Management

Given the complexity of the topic, this section aims to provide rather a structured overview. Since IRDS is closely linked to preterm birth, management begins already with prenatal care and follows strategies, aiming to reduce preterm birth in general. As a first step, pregnant women at high risk of delivering before 30 weeks’ gestation should be transferred to specialized perinatal centers to provide optimal care, including RDS management (22). If delivery is expected before 34 weeks, a single course of antenatal corticosteroids is recommended to promote lung maturation and reduce the incidence and severity of IRDS (22,47). Prenatal care may also involve administration of magnesium sulphate, to protect the central nervous system of the preterm infant, and if necessary short-term use of tocolytic drugs, in order to gain essential time (22,48). The next aspect of treatment deals with direct postnatal

stabilization of the newborn in the delivery room. Core recommendations in this setting include delayed cord clamping for at least 60 seconds, as this is associated with improved neonatal outcomes (22,49). Additional recommendations include the administration of continuous positive airway pressure (CPAP) ventilation in spontaneously breathing preterm neonates, using T-piece devices and considering intubation only in cases where infants remain unresponsive to positive pressure ventilation. When initial supplemental oxygen is required during stabilization, a blender delivering controlled air-oxygen mixtures should be used to ensure specific FiO_2 levels for different patient groups. For infants <32 weeks, initial FiO_2 of ≤ 0.30 is advised and from ≥32 weeks room air (0.21) is recommended for initial stabilization (22,50,51). Apart from these initial starting points, FiO_2 levels should be fine-tuned in both directions, as indicated by pulse oximetry readings. Concerning oxygen saturation, it is advised to aim for levels from 90% to 94% in premature infants and therefore set alarm thresholds to 89% and 95% in order to minimize fluctuations. Careful monitoring is essential to reduce the risk of complications due to hyperoxemia and hypoxemia, such as ROP and NEC (22,52). A stepwise approach to respiratory support is central in the treatment of IRDS, beginning with the least invasive methods. For spontaneously breathing infants CPAP remains first-line therapy along with synchronized nasal intermittent positive pressure ventilation (sNIPPV), a more advanced ventilation technique, which may also help reduce BPD. One of the most critical interventions in IRDS management is the surfactant replacement therapy. Guidelines suggest, in very preterm infants who need intubation soon after delivery, surfactant administration should be part of the initial stabilization strategy. Evidence shows that natural, animal-derived surfactant preparations are more effective than synthetic ones. Less invasive surfactant administration (LISA) is now viewed as the standard approach for spontaneously breathing infants supported with CPAP. The recommended strategy combines non-invasive respiratory support with early surfactant delivery using the LISA technique, which has become the preferred approach in modern IRDS care. Early administration of surfactant produces the best outcomes, and higher initial doses have generally been shown to provide greater benefit than lower ones. Treatment is typically initiated when oxygen requirements increase, for example, when FiO_2 surpasses 0.30 on sufficient CPAP support, or when lung ultrasound points towards surfactant deficiency. In larger infants (>1,000 g), surfactant can also be delivered

via endotracheal tubus (22,53). In neonates with established IRDS, where non-invasive methods reach their limits, mechanical ventilation should be used, although in a lung-protective manner. This involves the initial use of methods like volume-targeted ventilation or high-frequency oscillation ventilation, while also aiming to maintain the shortest possible duration of mechanical ventilation. In cases where signs of respiratory distress continue despite treatment and other possible causes have been eliminated, a second or even third dose of surfactant may be necessary (22). As the patients suffer from other prematurity related conditions, the supportive care management should focus on maintaining adequate thermoregulation, fluid and electrolyte balances and the nutritional support. Continuous monitoring, frequent clinical evaluations and following treatment protocols allow early detection and reducing complications. Overall, IRDS management emphasizes individualized care fitted to the severity of disease, while aiming to prevent complications and support optimal neurodevelopment in preterm infants (1,22).

1.4.2 Intraventricular Hemorrhage (IVH)

Definition and Epidemiology

Intraventricular hemorrhage (IVH), particularly from germinal matrix, is the most frequent type of intracranial bleeding seen in preterm infants. The condition originates primarily in the subependymal germinal matrix, a region of the premature brain characterized by numerous but still delicate vessels, making it particularly vulnerable to bleeding (1).

Epidemiology and Prognosis

The occurrence of IVH decreases with advancing gestational age. The condition affects about 30-40% of very preterm infants born before 32 weeks and approximately one in four infants with a birth weight below 1,500g (54,55). Large cohort studies and meta-analyses report that around one in three infants born before 28 weeks' gestation develop any degree of IVH, while severe hemorrhage occurs only in roughly 10-15% of cases. Most events appear soon after birth, with about half developing within the first 24-48 hours. Only a small proportion are detected as early as 6 hours after birth, and around 10% within the first 12 hours (55,56). Although advances in perinatal care have improved survival of preterm infants, the

overall incidence of intraventricular hemorrhage has not significantly declined in the past two decades (55). Outcomes are strongly related to the severity of IVH. Higher grades are linked to increased mortality, but even low-grade hemorrhages can affect development of the brain in a negative manner. While milder forms may be associated with minor cognitive or visual damages, severe IVH often results in cerebral palsy, cognitive and sensory deficits, or epilepsy (54,55). A major complication is post-hemorrhagic ventricular dilatation (PHVD), a process potentially following intraventricular hemorrhage, which is linked to adverse outcomes and requires long-term cerebrospinal fluid shunting in approximately 20% of affected infants (57,58).

Pathogenesis

The development of germinal matrix-intraventricular hemorrhage (GM-IVH) in preterm infants is complex and influenced by multiple factors. It can be described in three main stages: an initial phase of vulnerability, followed by the injury itself and its later consequences. In preterm infants, the germinal matrix contains densely packed, immature vessels that lack stable connective-tissue support and effective autoregulation, making them highly susceptible to fluctuations in cerebral blood flow. This fragility arises from the rapid formation of new vessels within the germinal matrix, a process stimulated by growth factors including VEGF, which makes the region extremely sensitive to fluctuations in cerebral blood flow and pressure (54,58). During the first days of life, events such as respiratory distress, hypoxia, hypercapnia, acidosis or sudden shifts in blood pressure can easily damage these vessels and cause bleeding. Additionally, the delicate vessels of the germinal matrix can become even more unstable under the influence of inflammation, coagulation problems, or damage caused by restored blood flow, which may contribute to hemorrhage (54,55,59). Following the rupture, the release of blood and its breakdown products trigger secondary injury, characterized by progenitor cell loss and white matter damage mediated by oxidative stress and inflammation. Furthermore, compression of terminal veins can produce the most severe form of IVH. What was traditionally termed grade IV hemorrhage is now recognized as periventricular hemorrhagic infarction (PVHI), a venous injury of the periventricular white matter rather than an extension of bleeding into the ventricles (54,55,57). As blood enters the ventricular system, it can obstruct the normal reabsorption of

cerebrospinal fluid (CSF), which causes PHVD or hydrocephalus, typically appearing within 7 to 14 days after IVH (55). The germinal matrix slowly regresses and has nearly disappeared by 34-36 weeks of gestational age, making the preterm period, especially before 32 weeks, the time of the highest risk (59).

Clinical Presentation and Diagnosis

The clinical presentation of GMH-IVH varies significantly among preterm infants. It can range from completely asymptomatic cases to severe neurological decline. A major proportion of infants with IVH show no clinical symptoms, particularly those with milder grades. These silent cases account for approximately 25% to 50% of all IVH occurrences. Therefore they are detected only through routine cranial ultrasound screening rather than by observation of clinical signs (54,59). When IVH does produce symptoms, the severity and progression can differ significantly. The most frequent pattern is a slow and fluctuating development over several hours or days. Affected infants may become less active and responsive, have decreased muscle tone and sometimes showing subtle changes in their eye movements or breathing rhythm (55,58). Because very preterm infants often appear unstable, these symptoms can blend in without pointing towards the diagnosis. Only a small amount of infants experience a sudden and severe decline, sometimes within minutes or hours. These drastic cases may show signs like seizures, coma, abnormal breathing, or even heart and circulation failure. This rapid form can also come with other features, such as a bulging fontanelle, changes in cranial nerve function, or a sudden drop in hematocrit levels (54,55). Since the clinical appearance is mostly nonspecific, the diagnosis of intraventricular hemorrhage (IVH) in preterm infants is mainly based on cranial ultrasound. Therefore, it represents the standard tool for both screening and monitoring. Current guidelines recommend performing an initial scan in the first 4 to 7 days of life in all infants born before 32 weeks' gestation, as most hemorrhages occur during this early period (56,59). Afterwards, follow up scans are used to detect delayed lesions or complications such as PHVD (57). A well-known classification for IVH is the Papile grading system, with an adjusted form for cranial ultrasound and MRI. It grades hemorrhage severity according to how far the bleeding extends and whether it affects the ventricles or brain tissue. An IVH that remains limited to the germinal matrix is categorized Grade I. Blood in the ventricles but without enlargement is

Grade II, whereas an expansion of the ventricles by blood equals Grade III. Although often labeled as Grade IV in the original Papile system, parenchymal lesions in this category are now better described as PVHI, showing one of the limitations of the traditional grading system (59).

Management and Prevention

The management focuses mainly on supportive care and prevention of secondary brain injury, as there is no specific therapy that can reverse an existing hemorrhage. The first step is to maintain a stable circulation and respiratory system, avoiding rapid fluctuations in oxygenation, blood pressure, or carbon dioxide, since these can worsen cerebral perfusion. Essential measures include a careful handling, sensitive and gentle ventilation and if necessary the correction of coagulation or metabolic instabilities (54,58). Infants with moderate or severe IVH require close neurological and ultrasound monitoring to detect complications such as post-hemorrhagic ventricular dilation or hydrocephalus. These conditions may need a spectrum of different neurosurgical management strategies, ranging from repeated lumbar punctures to surgical treatments, including cerebrospinal fluid drainage or shunting (57). Prevention on the other hand plays a central role in reducing both the risk and the severity of IVH. Antenatal corticosteroids given to mothers at risk of preterm delivery remain one of the most effective strategies, as they promote vascular maturation and therefore reduce the incidence of severe IVH. Important measures during and immediately after delivery are the previously mentioned supportive care, delayed cord clamping (DCC) and delicate resuscitation techniques, aiming to maintain stable cerebral blood flow. In neonatal intensive care preventive management strategies involve minimal handling, a midline head positioning, and ensuring a stable blood pressure and ventilation. Additionally, strict infection control methods and carefully managing oxygen and CO_2 levels help reducing the risk of new or worsening bleeding (54,55,58). Optimal management of oxygenation depends largely on accurate, continuous SpO_2 monitoring.

1.4.3 Periventricular Leukomalacia (PVL)

Definition and Epidemiology

In premature infants, injury to the periventricular white matter, known as periventricular leukomalacia (PVL), is the most frequent pattern of white matter damage and the leading underlying reason of both cerebral palsy and cognitive impairment. The condition develops when ischemic and inflammatory processes damage the periventricular region, causing necrosis and injuring a population of oligodendrocytes, whose maturation is essential for later myelination. PVL typically affects preterm neonates born before 32 gestational weeks, when the periventricular regions lack adequate vascularization and are particularly sensitive to hypoxia, metabolic stress and inflammation. Around 28 weeks the risk for white matter injury reaches its maximum (55,60,61). With advances in neonatal intensive care and neuroimaging, PVL is now understood as a spectrum of white matter dysmaturation rather than exclusively a necrotic lesion. Classical cystic PVL, which is characterized by focal tissue loss and cystic cavitation, has become relatively uncommon in modern populations. Diffuse, non-cystic injury is now the predominant form of PVL (55). Recent research indicates that severe cystic lesions appear in only a small number of cases. However, diffuse white-matter abnormalities are found a great proportion of very preterm infants. These diffuse white matter injuries appear to be strongly associated with subtle but persistent long term neurocognitive and behavioral problems (55,62).

Pathogenesis

Pathogenesis of PVL involves a combination of factors. These include hypoxic-ischemic injury, inflammatory processes, oxidative stress and all acting during a critical stage of white matter development. In the period of extremely and very preterm birth (<32 gestational weeks) periventricular white matter consists largely of preoligodendrocytes, a particularly vulnerable cell type. Because these immature cells do not yet have the protective features of mature oligodendrocytes, they are highly vulnerable to hypoxia-ischemia and inflammatory injury, increasing their likelihood of entering programmed cell death. The surviving ones attempt to regenerate by increasing in number. However, they fail to mature into fully functional

oligodendrocytes, resulting in poor myelination and contributing to long-term white matter damage (62). The periventricular white matter of preterm infants is predisposed to ischemia because of its immature vascular structure and limited autoregulation. Before 32 weeks' gestation, the deep white matter lies at the border between long and short penetrating arteries, forming a watershed zone with limited collateral circulations. Even minor decreases in blood pressure or oxygenation can therefore cause localized ischemia (60). Besides this structural weakness, preterm infants also have immature blood flow regulation in the brain. In healthy term infants, the brain can maintain stable blood flow even when blood pressure changes. However, preterm infants lack this protective mechanism. As a result, their cerebral blood flow depends directly on blood pressure. This makes the preterm brain very vulnerable to problems like hypotension, infections, or respiratory problems that may change carbon dioxide levels. Another important influence is inflammation, which further increases cellular injury. When cytokines are released and microglia become activated, reactive oxygen species and free radicals build up leading to damage of preoligodendrocytes and axonal disorganization (61). Most importantly this leads not only to areas of tissue loss but also to widespread disturbances in white matter development and reduced maturation of the overlying cortex. For this reason, PVL is now viewed less as a purely ischemic injury and more as a disorder of overall brain development (62,63).

Clinical Presentation and Diagnosis

Most infants with PVL do not show clear clinical signs during the neonatal period. Diffuse, non-cystic forms are usually asymptomatic, whereas cystic PVL may later lead to increased muscle tone, delayed motor milestones, or spastic paralysis (60,62). The primary imaging technique for screening is cranial ultrasound. Initially, it may show increased periventricular echogenicity that later transform into fluid filled cysts within two to six weeks. When echogenicity persists or cysts develop, the risk of future motor impairment is considerably higher. When cystic injury involves the parieto-occipital areas, motor impairment in later childhood is more likely, highlighting the importance of lesion location for long-term prognosis (60,61). Magnetic resonance imaging (MRI) is more sensitive than ultrasound for detecting diffuse, non-cystic white matter injury and is best performed at calculated term age. It frequently shows reduced white matter volume and delayed myelination, both

predictors of later neurodevelopmental deficits. Given the unreliability and absence of early clinical signs, routine imaging is advised for all very preterm patients or those who are clinically unstable (55,63).

Management and Prevention

There is no curative treatment once periventricular leukomalacia (PVL) or white matter injury has developed. Management therefore focuses on prevention and neuroprotection. Comprehensive neuroprotective measures, recommended for all forms of preterm brain injury including PVL, cover the antenatal, perinatal, and postnatal period. Antenatal interventions remain the most effective preventive measures. Administration of corticosteroids to mothers at risk of preterm delivery improves tissue maturation and reduces severe white matter injury, while magnesium sulfate provides additional neuroprotection and lowers the risk of cerebral palsy, although its specific effect on PVL is less clear (55,64). Practicing gentle stabilization methods during delivery and delayed cord clamping also serve a neuroprotective purpose. Postnatal prevention focuses on maintaining stable cerebral perfusion and limiting inflammatory and oxidative stress. Avoiding hypotension, low carbon dioxide levels, and large swings in blood glucose level and oxygen saturation helps diminish ischemic-hypoxic injury in the periventricular watershed areas. Monitoring cerebral circulation helps preserve a steady brain perfusion. In neonatal intensive care settings, keeping painful procedures to a minimum, providing good nutrition including maternal breast milk, and encouraging early skin-to-skin and family integrated care all contribute to improved brain health and white matter maturation. Ongoing rehabilitation therapy at home remains essential, as continued rehabilitation helps the brain recover and rebuild after injury (55). Precise infection control and prompt sepsis treatment are also crucial, as inflammatory cytokines and oxidative stress can aggravate neurological damage (60,61). Current experimental work suggests that stem cell therapies and treatments aimed at modulating the immune response or reducing inflammation, with agents like erythropoietin, melatonin and caffeine have shown improved outcomes in preclinical studies and remain active areas of research (64).

1.4.4 Necrotizing Enterocolitis (NEC)

Necrotizing enterocolitis remains one of the most common emergencies in neonatal care and is almost exclusively seen in premature infants. The hallmark of NEC is an ischemic injury of the intestinal mucosa accompanied by severe transmural inflammation. Gas-producing bacteria contribute to the disease process, and the resulting intramural gas may advance into the bowel wall and portal venous system (1,65).

Epidemiology

Almost all cases of necrotizing enterocolitis occur in premature infants, and approximately 90% affect very low birth weight (VLBW, <1,500 g) or extremely premature neonates. The risk of NEC shows a clear inverse relationship with both gestational age and birth weight. Among extremely low birth weight (ELBW, <1,000g) and VLBW infants, the incidence is around 10%, whereas term or near-term infants account for only about 10% of all NEC cases. The timing of disease onset is also influenced by maturity. In very preterm infants, NEC typically presents around the fourth postnatal week, while infants born closer to term more often develop NEC within the first week of life (65). Mortality remains substantial ranging from 20% to 30%, with the highest mortality observed among infants who require surgical interventions. But also a lower gestational age and lower birth weight are consistently associated with a higher risk of death (66).

Etiology and Pathogenesis

The exact pathophysiological mechanism is not fully understood. Epidemiological findings suggest that several factors may contribute to the pathogenesis of NEC , making it a multifactorial disease. NEC appears to develop when the premature intestine, with its immature barrier and intensified inflammatory reactivity, is exposed to abnormal or dysregulated microbial colonization (65). Impaired intestinal perfusion with subsequent ischemia is thought to play an important role, as reduced blood flow can weaken mucosal integrity and therefore harm the intestinal barrier function. A number of other factors have been identified as being associated with the development of NEC. Both antenatal complications that compromise fetal stability and postnatal conditions can further increase intestinal vulnerability. As

previously stated, a significant determining factor for NEC is prematurity, with gestational age and birth weight being the primary indicators. The introduction of enteral feeding is thought to be a risk factor, whereas in unfed newborns the condition is rare (66). A rapid escalation in enteral feedings has been shown to increase the probability of NEC. Nevertheless, the complete withholding of enteral feedings can also be detrimental. This is due to the fact that it can cause intestinal atrophy, a prolonged parenteral feeding period, increased permeability and inflammation, and also late onset sepsis. A promising approach in this regard is to initiate the feeding process with small amounts of mother's expressed breast milk and gradually increasing the volume over time. Altered or delayed establishment of a healthy intestinal microbiome is considered an important contributor to NEC risk given that necrotizing enterocolitis does not manifest until at least 8 to 10 days postpartum, which is the time required for anaerobic bacteria to colonize the intestinal tract. Despite the absence of a specific pathogen associated to NEC, the administration of prolonged antibiotic therapy has been demonstrated to diminish diversity within the gut microbiota. This can lead to decreased colonization resistance, which may consequently increase the risk for NEC (65).

Clinical presentation and Diagnosis

Many affected preterm infants appear clinically stable beforehand, often feeding well and gaining weight until the disease begins. Early manifestations can look like neonatal sepsis, making the initial distinction challenging (67). This is due to the fact that the initial presentation of NEC can include nonspecific symptoms like temperature fluctuations, apnoea, lethargy, bradycardia, inadequate glucose regulation and low systemic blood pressure. Furthermore, laboratory findings are common but also ambiguous. Such findings may include thrombocytopenia, hyponatremia, metabolic acidosis (including lactate acidosis), neutropenia and high amounts of leukocytes (66). The suspicion of necrotizing enterocolitis is raised when gastrointestinal signs and symptoms occur in a predominant manner. The intestinal disturbances associated with this condition include abdominal distension and tenderness and commonly seen in the beginning, the sudden change towards refusal to feed. Confirmation relies on characteristic imaging findings, most notably pneumatosis intestinalis and, in some cases, portal venous gas (67). Persistently dilated, gas-filled intestinal loops on repeated imaging may support the diagnosis,

while free intraperitoneal air typically indicates a disease progression. It is important to acknowledge that there are many necrotizing enterocolitis-like conditions with various clinical presentations, differing also in pathogenesis as well as preventive and therapeutic strategies. Common early gastrointestinal signs of a “classic” NEC include progressive abdominal distension, feeding intolerance, and occasionally the passage of stools containing blood. The clinical course may worsen rapidly, with initial subtle symptoms sometimes progressing within hours to perforation, peritonitis, or bowel ischemia. Presentations in premature infants and children born at term vary in time of onset and association to other hemodynamic problems. The condition manifests at an earlier stage and is more often related to intestinal abnormalities, congenital heart disease and perinatal stress in mature infants. These factors have been identified as potential contributors to alterations in mesenteric blood flow (65).

Therapy

In instances where the diagnosis of NEC is highly suspected or has already been established, the initiation of medical treatment is imperative. The treatment of necrotizing enterocolitis involves a complete cessation of all enteral feedings, intravenous fluid resuscitation, the establishment of central intravenous access, the insertion of an orogastric tube to achieve gastric decompression and the initiation of parenteral nutrition to maintain metabolic stability. Empiric broad-spectrum antibiotics are started to cover gram-positive, gram-negative, and anaerobic organisms, and the regimen is adjusted once culture results are available. Antifungal therapy may be added if fungal organisms are isolated or if clinical deterioration occurs despite antibacterial treatment (66). Symptomatic medical management is utilized for the treatment of specific clinical conditions, including transfusions of erythrocyte products, platelets and fresh frozen plasma in situations of symptomatic anemia, thrombocytopenia and coagulopathy. Hemodynamic instability may require vasoactive support, and respiratory assistance is often necessary due to restricted pulmonary reserve from abdominal distension and systemic inflammation (66). The ongoing assessment includes serial abdominal examinations and imaging to monitor for pneumoperitoneum or worsening intestinal injury (65). Because approximately one-third to one-half of infants ultimately require surgery, early involvement of pediatric surgeons is recommended. Free air in the abdomen

remains the definitive indication for operative intervention, while persistent acidosis, thrombocytopenia, fixed bowel loops, abdominal wall discoloration or hemodynamic instability despite maximal medical therapy are considered relative indications (65,66). Surgical strategies include peritoneal drainage or laparotomy, with evidence showing that many infants initially treated with drainage ultimately require laparotomy and that outcomes may vary depending on disease severity (65).

1.4.4.1 Oxidative Stress and NEC

Oxidative stress (OS) is defined as excess of reactive oxygen species (ROS) in combination with lack of antioxidants. This imbalance occurs either due to excessive production of ROS or a deficiency in the body's antioxidant defense mechanisms. ROS are physiologically produced in living organisms, acting as mediators in numerous cellular processes, including the immune response. High concentrations of ROS induce oxidative stress (OS), damaging cells and tissues by peroxidation of lipid membranes and causing protein and DNA damage. The abrupt shift from the low-oxygen intrauterine environment to extrauterine life places newborns under substantial oxidative pressure. In preterm infants this postnatal oxidative load is intensified because their antioxidant systems are immature and rise only slowly after birth, making them even more vulnerable to OS (68). Premature neonates show a reduced ability to enhance antioxidant production in response to postnatal oxygen exposure. Additionally prematurity is associated with increased ROS generation, which further aggravates the imbalance. Preterm delivery often requires postnatal interventions such as resuscitation and the use of supplemental oxygen, resulting in increased exposure to high oxygen concentrations that can markedly increase ROS formation. Moreover, oxidative stress, as evidence suggests, contributes to pathogenesis of NEC. Recent works have observed patients, diagnosed with necrotizing enterocolitis, exhibit elevated levels of total oxidative stress (TOS) and a higher oxidative stress index (OSI) and have demonstrated an association between oxidative stress markers in cord blood and later NEC development (68). As previously stated, the etiology of NEC is multifactorial, resulting from a convergence of several factors rather than a single cause. Therefore oxidative stress is one among many contributing aspects in its pathogenesis. Multiple processes, such as hypoxemia, hyperoxemia, prostaglandin pathways, damage to endothelial cells and reperfusion of ischemic tissues, ultimately lead to the

production of ROS, which exert oxidative effects. A key mechanistic feature involves cycles of reduced intestinal perfusion followed by reperfusion, with both phases contribute to oxidative stress, the latter producing a rush of ROS when oxygen reenters previously hypoxic tissue. Through these mechanisms, both excessively low and excessively high oxygen saturation levels promote the development of NEC (68).

1.5 Oxygen-related Long Term Complications of Premature Neonates

1.5.1 Retinopathy of Prematurity (ROP)

Definition

ROP is a multifactorial vasoproliferative disease of the retina in premature infants. Its occurrence and severity tend to rise with decreasing gestational age and birthweight, meaning that the smallest and most premature infants are most affected (1). Because infants born at term usually already have a fully matured retinal vasculature, the condition is almost limited to those born preterm. In normal development, retinal vessels grow outward during gestation, beginning centrally at the optic nerve head and gradually extending toward the periphery ending at the ora serrata. In preterm infants the retina remains incompletely developed, and the degree of immaturity differs depending on the timing of birth. If ROP remains undiagnosed or treatment is delayed, it can progress to permanent loss of vision. Therefore, infants at risk must receive well-timed screening to identify and treat ROP before complications develop, although not all need treatment (69).

Epidemiology

As earlier mentioned the incidence of retinopathy of prematurity (ROP) increases with the degree of prematurity. Extremely low birth weight infants show clearly higher rates of ROP and of treatment-requiring disease compared with very low birth weight infants (16). As the survival of extremely premature infants continued to improve, a larger number of children now live with long-term visual consequences, including those linked to ROP as well as associated conditions such as myopia or strabismus (70). In a multicenter, observational registry study by Pfeil et al. (71) a

ROP registry from Germany was used, among other purposes, to examine changes in demographic parameters. In total 353 infants treated for ROP were recorded between 2011 and 2020, representing about 15% of all infants treated for ROP nationwide. Across all screened newborns, 3.5% required treatment, with annual rates ranging between 1.9% and 5.1%. In the United States, an analysis from 2003 to 2019 found that the overall fraction of premature infants diagnosed with ROP increased from 4.4% to 8.1% (72). ROP epidemiology also differs significantly worldwide. Differences in neonatal care resources, survival rates between low-, middle- and high-income countries, and population-level risk profiles have led to substantial geographic variation. Countries with intermediate infant mortality rates have the biggest problem with severe ROP and ROP-related blindness, highlighting the complex relationship between national mortality indicators and ROP incidence (73).

Classification

The International Classification of Retinopathy of Prematurity, updated in its third version, built upon the original 1984 classification system that has remained the standard framework ever since (74). This classification provides a unified way to define different manifestations of ROP, making it easier to establish clear treatment criteria and support research in the field. The ICROP system describes ROP using four elements: the retinal zone affected, the disease stage, the extent measured in clock hours, and the presence or absence of plus disease. Zones indicate how far the disease lies from the center of the retina, with Zone I closest to the optic disc and Zones II and III extending progressively outward. In the recent third version zone II was split into posterior zone II and peripheral zone II, due to the potentially worse disease in the posterior region. Stages reflect the severity of vascular abnormalities, progressing from a demarcation line in Stage 1 to total retinal detachment in Stage 5. To examine the extent of the condition, the retina is separated into 12 segments, analogous to hours of a clock. Plus disease is defined by dilation and increased curvature of the posterior retinal vessels, whereas preplus disease also shows abnormal vascular dilation or twisting of blood vessels or even both but does not meet the criteria for plus disease. These features are crucial in defining a certain level of ROP (74). As it can be seen in the final results of the Early Treatment for Retinopathy of Prematurity (ETROP) study by Good et al. (75) descriptions for ROP treatment threshold were conceived. The characteristics were

meant to predict whether an eye is most likely to profit from early therapy or regress spontaneously and therefore should be left untreated. Type 1 ROP, defined as any stage in Zone I with plus disease, Stage 3 in Zone I without plus disease, or Stage 2 or 3 in Zone II when plus disease is present, requires treatment. Thus treatment is commonly not recommended if type 1 ROP conditions are not fulfilled (75).

Pathogenesis

Retinopathy of prematurity results from an interruption of normal retinal development both neuronal and vascular. The severity is due to the level of immaturity of the retina itself as well as the duration of impaired development. This interruption is driven by a combination of losing the protective intrauterine environment and being exposed to postnatal stressors, while the central pathological mechanism involves compensatory but abnormal blood vessel growth (76). In the context of normal fetal retinal development, initial vasculogenesis begins at the optic nerve head around 12 gestational weeks and advances from the posterior to the peripheral retina until roughly 22 weeks. Beyond this stage, the formation of new blood vessels occurs via the mechanism of angiogenesis, a process primarily influenced by the vascular endothelial growth factor (VEGF). Around gestational weeks 40 to 44 postmenstrual age the retinal progress is finished, consequently being born extremely prematurely disrupts this crucial phase at vulnerable times (71). It is evident, that ROP contains two phases. The first phase, starting right after birth, is characterized by delayed physiological retinal vascularization as well as loss of previously formed retinal vessels. These phenomena have been shown to be associated with premature neonatal stressors, including extrauterine hyperoxia, low levels of insulin-like growth factor 1 (IGF-1) and lack of VEGF receptors. IGF-1 has been demonstrated to play an essential role in the healthy growth and development of multiple tissues, including those found in the brain and blood vessels. It is hypothesized that another contributing factor to the pathogenesis in phase 1 is the deficiency of maternally derived long-chain polyunsaturated fatty acids in an extrauterine setting. While in phase 1 the stop of vessel growth is the predominant problem, phase 2 is characterized by abnormal retinal neovascularization. This process starts around four to eight weeks after birth when the retina starts becoming metabolically hypoxic. The hypoxia-derived metabolic state leads to increased levels of VEGF and erythropoietin (EPO), which in turn were too low in phase 1. New poorly functioning blood vessels start

proliferating mainly due to these VEGF and EPO levels. The retinal perfusion remains inadequate through these fragile and leaky vessels, which causes further detachment and appearing of fibrous scar tissue (73,76).

Management

As seen in the International Classification of Retinopathy of Prematurity (ICROP) therapy is based on the level of disease (74). First line therapies include laser photocoagulation of the peripheral avascular retina and intravitreal application of anti-VEGF substances. Treatment ambitions cover the avoidance of vision loss and blindness as well as protection of the retinal structure. Both therapy options have advantages and disadvantages and should thus be considered individually. Laser therapy ablates the peripheral avascular retina, which consequently lowers the risk for further angiogenesis and relapse of disease. Because VEGF expression is tightly linked to retinal vascular development, intravitreal anti-VEGF therapy aims to preserve retinal structure, limit pathological neovascularization, and may be the superior option for aggressive and type 1 ROP. Further, after completing pharmacological treatment, physiological vascular growth of retinal vessels may be restored (73). Treatment criteria as shown above in section on classification were established in the multicentric ETROP study. In summary, treatment is recommended for infants with type I ROP, whereas patients with type II ROP are typically monitored and treated only if signs of progression toward threshold disease appear (75).

1.5.1.1 The Role of Oxygen in ROP

Since 1952, it is known by work of Patz et al. (77) that retinopathy of prematurity is linked to exposure of high oxygen levels. Hyperoxemia and oxygen toxicity contribute to the arrest of normal retinal vascular growth, which represents phase 1. The arrest is mediated by suppression of key growth factors such as VEGF and erythropoietin when oxygen saturations are unusually high. A normal environment for the infant in utero after 20 weeks of pregnancy provides a mean oxygen pressure typically less than 50 mmHg. Prematurely born infants are therefore exposed to unfamiliar high levels of oxygen, even at room air, but especially when given additional oxygen in medically required situations (76). Thus using 100% oxygen during resuscitation drastically increases risk for developing ROP and therefore sustaining appropriate oxygen saturations is a major factor in preventing ROP in

neonatal intensive care units (NICU) (73). Finding the right amount of oxygen saturations between too high levels, to prevent death in critical postnatal situations, and too low levels, to prevent phase 1 ROP, is still not commonly known. Nevertheless it has a key role in neonatal care (76). Evidence from one early study evaluating different oxygen saturation targets in infants born before 28 weeks showed a lower need for ROP treatment in groups managed with lower saturation targets compared to higher ranges, and these saturation values were assessed using pulse oximetry (78).

1.5.2 Bronchopulmonary Dysplasia (BPD)

Definition and Epidemiology

Bronchopulmonary dysplasia (BPD) is the most frequent chronic lung disease of prematurity and one of the main causes of long term respiratory morbidity among preterm infants (79–81). The condition was first recognized in infants who developed chronic respiratory insufficiency after receiving mechanical ventilation and oxygen therapy for the respiratory distress syndrome. With advances in perinatal care, such as antenatal corticosteroid administration, surfactant replacement therapy, and less invasive ventilation strategies, the disease pattern has evolved. The modern form of BPD is now defined less by inflammation and fibrosis, due to ventilator and oxygen injury, but more by impaired alveolar and vascular growth, representing a disorder of development of the immature lung (79,82,83). As neonatal care evolved, so did the diagnostic criteria for BPD. Traditional oxygen based definitions, such as the need for supplemental oxygen at a specified postnatal age, showed limitations in predicting outcomes as the modalities of respiratory support expanded. A more recent definition of BPD is based on the level of respiratory support needed close to the infants term age, rather than oxygen use alone. Data from large population studies indicate that infants needing both supplemental oxygen and positive-pressure support at 36-40 weeks postmenstrual age (PMA) are at highest risk for long term respiratory morbidity (80,84). Therefore, this support-based approach has become the most clinically relevant definition and supports the understanding of BPD as a disease of disrupted lung growth rather than purely ventilator-induced injury. Epidemiologically, BPD remains a major problem although survival of extremely preterm infants has improved. Reported incidence varies with gestational

age, birth weight, and the applied definition. It affects roughly 20% of infants weighing less than 1,500g and up to 45% of those born before 29 weeks of gestation (79,81). The improvements in survival of extremely preterm neonates, contributes to a stable or slightly increased overall incidence of BPD, reflecting the group at the highest risk (80,81,83).

Pathogenesis and Pathophysiology

Bronchopulmonary dysplasia (BPD) develops through multiple interacting factors. Inflammation, mechanical injury from ventilation, altered pulmonary blood flow, genetic or epigenetic influences and oxidative stress all affect the immature preterm lung. The main problem occurs when normal lung development is disrupted. In preterm infants, the lungs are still in the late canalicular or saccular stage of development. When growth is interrupted at this critical stage, the alveoli and blood vessels cannot develop properly, which results in a simplified lung structure that may remain permanently abnormal (79,80,85). Several factors that play a key initiating role have been investigated. The risk of BPD increases when mothers experience complications such as chorioamnionitis, premature rupture of membranes, or elevated blood pressure during pregnancy. Infant characteristics also play a role, with lower gestational age, male sex, and growth restriction contributing to higher vulnerability (79,86). These early injuries expose the immature lung to inflammation and insufficient oxygen supply. Inflammatory mediators such as interleukin-1ß (IL-1ß), tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6) are upregulated in the airways and circulation of affected infants, increasing tissue injury and interfering with surfactant function (82). This disturbs the signaling pathways that control blood vessel growth and the coordination between epithelial and endothelial tissue. Consequently, the arrest of normal alveolar development is predisposed after birth (80). After birth, oxygen exposure, mechanical ventilation, infection, and patent ductus arteriosus (PDA) become the main drivers of injury. High oxygen levels and mechanical stretch cause oxidative stress and inflammation that damage pulmonary structures, while sepsis and abnormal pulmonary blood flow worsen the inflammatory response (81,83,85). Data from a recent meta-analysis confirm that prolonged ventilation, oxygen therapy, sepsis, and PDA are independent risk factors for BPD (86). As previously mentioned, the altered communication between epithelial and endothelial cells together with reduced expression of angiogenic mediators such as VEGF lead to impaired alveolar and

capillary formation (80). Therefore, the modern form of BPD shows enlarged and simplified air spaces, thicker walls between air sacs, and reduced number of blood vessels in the lungs, in contrast to the older "classic" form of BPD, which was marked by extensive fibrosis (79). These structural changes of the pulmonary tissue contribute to the long-term reduction in lung function and the increased risk of pulmonary hypertension observed in survivors (80,82).

Clinical presentation and Diagnosis

The clinical presentation of bronchopulmonary dysplasia (BPD) varies in severity but is primarily characterized by persistent respiratory insufficiency in preterm infants who fail to wean from oxygen or ventilatory support. Clinical symptoms often include tachypnea, retractions, wheezing, and oxygen dependency beyond the neonatal period, sometimes accompanied by feeding difficulties and poor weight gain (81,82). Among infants with moderate to severe BPD, pulmonary hypertension is the most critical complication, with an incidence of approximately 24% (82). Diagnosis is based on the sustained need for supplemental oxygen or respiratory support at a defined postmenstrual age. The classic 2001 NIH definition grades BPD as mild, moderate, or severe according to inspiratory oxygen concentration and mode of ventilation at 36 weeks postmenstrual age. However, this classification does not match modern clinical practice, where non-invasive ventilation methods are commonly used. Jensen et al. (87) suggested a newer definition that categorizes BPD severity according to the type of respiratory support at 36 weeks postmenstrual age, independent of oxygen supplementation. In a large multicenter study with very preterm infants, this approach was the most accurate in predicting death or serious respiratory problems in early childhood. According to this system, infants breathing room air are considered unaffected, meaning no BPD. Infants receiving low-flow nasal cannula have mild BPD, the patients needing non-invasive ventilation have moderate BPD, and those who still require mechanical ventilation have severe BPD (87). Although the diagnosis remains primarily clinical, additional diagnostic tools such as echocardiography may help detect complications like pulmonary hypertension and are essential for monitoring potential progression (82).

Management and Prevention

The management and prevention of bronchopulmonary dysplasia (BPD) aim to minimize early lung injury, ensure normal lung development, and prevent secondary

complications. Because BPD has multifactorial origins, no single therapy is curative. Optimal care combines preventive strategies, supportive management, and selected pharmacologic interventions. The basis of prevention is minimizing exposure to factors that trigger inflammation and oxidative stress in the immature lung (81,88). One of the most important interventions before birth is the use of antenatal corticosteroids. This step accelerates the surfactant production and overall lung tissue maturation, which reduces the severity of respiratory distress and following risk of BPD (85). After delivery, using gentle ventilation techniques is essential. Starting with continuous positive airway pressure (CPAP) soon after delivery rather than early intubation can largely reduce lung damage caused by mechanical ventilation (88). Several pharmacological therapies have been shown to reduce BPD risk or support lung development in preterm infants. When caffeine therapy is initiated within the first ten days after birth, it helps minimize the duration that infants need mechanical ventilation and also lowers the occurrence of BPD without causing additional complications. Furthermore, vitamin A supplementation has shown to offer a small but meaningful benefit for extremely low birth weight infants by strengthening the epithelial tissue and improving antioxidant protection (89). In difficult cases, when infants cannot be weaned from mechanical ventilation, systemic corticosteroids are recommended. The administration of low-dose dexamethasone for a brief period after the first week has been demonstrated to ease extubation and enhance lung function. However, this treatment is limited to high-risk infants, as its potential impact on brain development remains a concern (90). Supportive measures are equally important. Optimized nutrition supports lung growth, while careful fluid management reduces pulmonary edema (80). In cases of patent ductus arteriosus (PDA) leading to excessive blood flow to the lungs, pharmacological closure with ibuprofen or indomethacin can be considered. Nevertheless, prophylactic usage has not been proven to reduce the risk of developing BPD. Also central to both prevention and treatment of BPD is the oxygen management. High concentrations of inspiratory oxygen produce reactive oxygen species (ROS) that damage the immature lung tissue. Therefore, maintaining oxygen saturation within a controlled range, avoiding both too little and too much oxygen, reduces damage via oxidative stress and lowers BPD incidence. Regarding the saturation ranges, clinical trials have shown that targeting lower oxygen saturation levels (91-94%) compared to higher levels (95-98%) results in lower BPD

rates and less need for home oxygen therapy (88). Long-term management includes regular follow-up visits to check for adequate growth, respiratory problems, and pulmonary hypertension. Some infants need continued oxygen therapy at home, and ongoing monitoring of nutrition and development is crucial for achieving the best possible outcomes (91). New emerging therapies, such as mesenchymal stem cell transplantation, insulin-like growth factor-1 (IGF-1) supplementation, and IL-1 receptor antagonists aim to repair rather than only support the developing lung. By reducing inflammation and promoting lung repair, these strategies try to restore alveolarization and vascular development. Although they still remain experimental, they may represent potential future therapies to modify the course of BPD (89,90).

1.6 Aim and Central Research Question

The aim of this study was to retrospectively evaluate the accuracy of oxygen saturation values measured by pulse oximetry in extremely low birth weight neonates treated at the Department of Neonatology of the Medical University of Graz between 2017 and 2021. Existing research evaluating the accuracy of pulse oximetry relative to arterial oxygen saturation has largely focused on preterm infants of varying gestational ages, including all neonates born before 37 weeks of gestation. In contrast, this work observes accuracy in a specifically vulnerable subgroup of preterm infants with birth weight <1,000g. Moreover, this investigation uses the latest pulse-oximetry sensors and unlike earlier studies, that often included all available measurements during the NICU stay, it analyzes only the data obtained during the most vulnerable first 48 hours after birth. The central research question of the study is to what extent pulse oximetry derived SpO_2 values reflect SaO_2 measured by arterial blood gas co-oximetry in routine clinical practice in ELBW during the first 48 hours after birth. In addition, the study examines whether the magnitude of any systematic bias varies across different oxygen saturation ranges, with particular emphasis on lower target ranges where accurate oxygen monitoring is most critical. Finally, the study tests the hypothesis that pulse oximetry systematically overestimates arterial oxygen saturation, including the measurements during the highly vulnerable immediate postnatal period.

2 Material and Methods

2.1 Study Design

This thesis is based on a retrospective single-center study carried out at the Division of Neonatology, Department of Pediatrics and Adolescent Medicine of the Medical University of Graz. The analysis includes data from preterm infants with a birth weight below 1,000g who were born between October 1st in 2017 and November 1st in 2021. The data were collected from patients' records and electronic database of COGOD III trial, a multicentric randomized controlled trial, after obtaining an additional approval of the Ethics Committee of the Medical University of Graz (Vote EK 1321/2025). Neonatal intensive care unit (NICU) ventilation protocol forms were used to extract physiological parameters concerning ventilation, including SaO_2 and SpO_2. These forms were digitalized into a pseudonymized database using Microsoft Excel. Further calculation and statistical analysis was conducted with IBM SPSS Statistics 30 and Python. The conclusions of this study are derived from the retrospective quantitative data analysis.

2.2 Hypotheses

Primary Hypothesis

The oxygen saturation levels obtained through pulse oximetry (SpO_2) differ significantly from the corresponding values determined by arterial blood gas analysis (SaO_2).

Null Hypothesis

There is no systematic difference between pulse oximetry derived arterial oxygen saturation (SpO_2) and invasively measured arterial oxygen saturation (SaO_2).

Secondary Hypotheses

1. There is a systematic difference between SpO_2 and arterial SaO_2 measurements within the individual SaO_2 subgroups (100-95%; 95-90%; 90-85%; 85-80%; <80%).
2. The magnitude of the difference between SpO_2 and the arterial SaO_2 measurements varies significantly across different arterial SO_2 saturation ranges.

3. In low arterial oxygen saturation ranges (defined as SaO_2<90%), the deviations are significantly greater than in other ranges (defined as SaO_2>90%).
4. The pulse oximetry readings systematically overestimate the corresponding arterial oxygen saturation measurements (SpO_2>SaO_2).

2.3 Study Population

Inclusion criteria

The study population included preterm infants with a gestational age below 32 weeks (≤ 31+6) and a birth weight under 1,000g who were born and treated at the Division of Neonatology of the Department of Pediatrics and Adolescent Medicine at the Medical University of Graz during the study period. This period ranged from 01.10.2017 until 01.11.2021. Only infants for whom a decision for full life support had been made at birth were eligible. Furthermore, inclusion required the availability of both pulse oximetry measurements and arterial blood samples obtained through an arterial catheter for determining arterial oxygen saturation.

Exclusion criteria

Infants were excluded if they presented with severe congenital anomalies of the brain, heart, or lungs, or with documented prenatal cerebral injury. Cases were also excluded if no decision for full life support had been made at birth. Additionally, infants without arterial blood samples obtained via an arterial catheter or with missing data that prevented valid comparison between SpO_2 and SaO_2 were not used in the analysis.

2.4 Data Collection

During the study period from October 2017 to November 2021, SpO_2 and SaO_2 data were continuously recorded manually in standardized ventilation protocol sheets. Additional parameters obtained from blood gas analyses and corresponding ventilator settings at the same time points were included in the analysis. Demographic and perinatal data, including birth weight, gestational age, sex, umbilical artery pH, Apgar scores at 1, 5, and 10 minutes, mode of delivery, exposure to antenatal corticosteroids, and postnatal respiratory support and

medications, were extracted from the patients' medical records. All personal data were pseudonymized, and strict data protection and privacy measures were applied.

2.5 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (Version 30) and Python. Continuous variables were assessed for distributional properties and are presented as mean ± standard deviation or median with interquartile range, as appropriate for each variable. Categorical variables are reported as absolute numbers and percentages. To evaluate the bias between pulse oximetry derived-SpO_2 and gold standard defined as co-oximetry measured-SaO_2, the measurement error was defined as the difference between the methods. To account for repeated paired measurements within individual patients, a linear mixed effects model was applied with patients included as a random effect and SaO_2 as a fixed effect. The agreement between SpO_2 and SaO_2 was evaluated using a Bland-Altman analysis. The measurement error (SpO_2 – SaO_2) was plotted against the gold standard (SaO_2). Proportional bias was examined using a linear mixed-effects model with SaO_2 as a fixed effect and patient ID as a random effect to account for repeated measurements within individuals. The results are reported as the proportional bias and the proportional 95% limits of agreement (LoA: mean bias ± 1.96 × SD). In addition, the paired measurements were sorted into predefined arterial oxygen saturation ranges (SaO_2: <80%, 80-85%, 85-90%, 90-95%, and 95-100%). Within each saturation range, descriptive agreement parameters were calculated, including average error as well as minimum and maximum observed errors. Pearson correlation coefficients were calculated to describe the linear association between SpO_2 and SaO_2 within the individual saturation ranges. All statistical tests were two-sided, and a *p value <0.05* was considered statistically significant.

3 Results

3.1 Screening/Exclusion

Figure 2 presents the flow diagram of the study population, showing the number of eligible patients assessed during the study period, reasons for exclusion, and the derivation of the final analytical cohort. During the study period, a total of 112 patients were eligible for inclusion. Of these, 17 were excluded due to a birth weight greater than 1,000 g, 54 due to the absence of an arterial blood sample, and 5 due to missing SaO_2 values. Consequently, 36 patients were included in the final analysis (n = 36). In most patients, multiple paired measurements were available, resulting in a total of 173 measurements included in the analysis (m = 173).

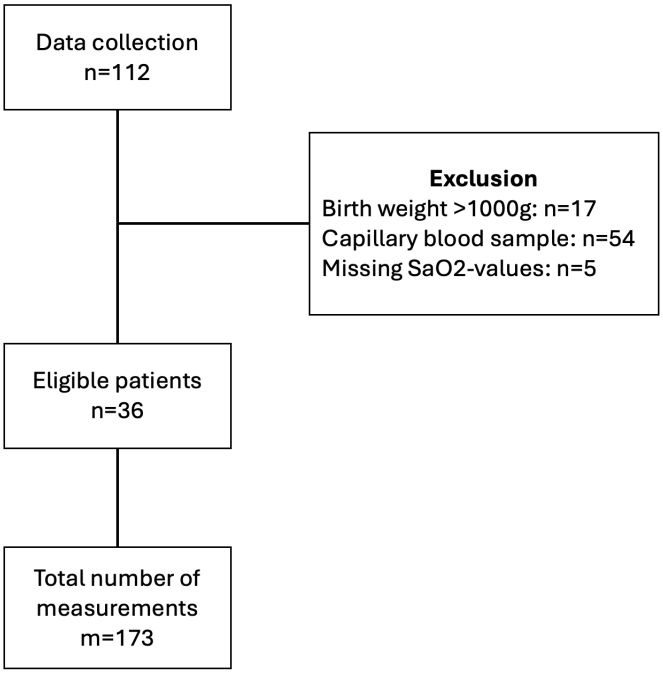


Figure 2 *- Flow diagram of the study population*

3.2 Patient Demographics

The baseline characteristics of the patients who were included in the study are presented in Table 1 and in Figures 3 and 4. The cohort consists of 22 male and 14 female ELBW neonates with an overall mean gestational age of 26+0 weeks. All patients received antenatal steroids as well as supplemental oxygen during postnatal stabilization. Delivery mode was vaginal in only one case, while the others underwent cesarean delivery. During the immediate fetal to neonatal transition only three patients underwent endotracheal intubation, and no patient required the administration of catecholamines. In two cases, the application of surfactant was performed.

The Figures 3 and 4 show the birth weight and gestational age of patients grouped by sex. The mean birthweight for all 36 patients was 782g with half of all patients ranging from 628g to 910g. The lowest birthweight in the group was 273g, and the highest was 996g. The average gestational age across all patients was 26+0 weeks. The minimum gestational age was 23+5, while the most mature included patient was born at 30+1.

Table 2 and Figure 5 summarize respiratory support mode and fraction of inspired oxygen (FiO_2) in the included patients. The distinction was made only for the type of respiratory support (invasive = via endotracheal tube vs non-invasive = via nasal mask/prongs) for simplicity reasons and in order to avoid many different abbreviations in the published literature. In 61.3% of the included measurements patients received a non-invasive respiratory support. The remaining almost 39% of cases involved the administration of invasive ventilation. In FiO_2 comparison between these two groups, the invasive ventilation group received slightly higher FiO_2 levels in average and showed a maximum FiO_2 of 100%, while among non-invasive respiratory support the highest FiO_2 was 64%. The distribution of given FiO_2 levels in relation to the SaO_2 is presented in Figure 6. The blood gas parameters collected throughout all 173 measurements are reported in a descriptive manner in Table 3.

Table 1 *- Baseline characteristics of the patients*

Variables	Total (n=36)
Neonatal parameters	
Gestational age	26+0 [24+6 – 27+3]
Birth weight [g]	782 [628 – 910]
Sex	
male	22 (61.1%)
female	14 (38.9%)
Umbilical artery pH	7.3 ± 0.087[a]
APGAR-Score – 1 min	6 [5 – 8]
APGAR-Score – 5 min	8 [7 – 9]
APGAR-Score – 10 min	9 [8 – 9]
Obstetric and maternal parameters	
Cesarean delivery	35 (97.2%)
Antenatal steroids	36 (100%)
Support during immediate fetal to neonatal transition	
Supplemental oxygen	36 (100%)
Endotracheal Intubation	3 (8.3%)
Caffeine	22 (61.1%)
Catecholamines	0 (0%)
Surfactant application	2 (5.6%)

a: Missing data of 7 patients

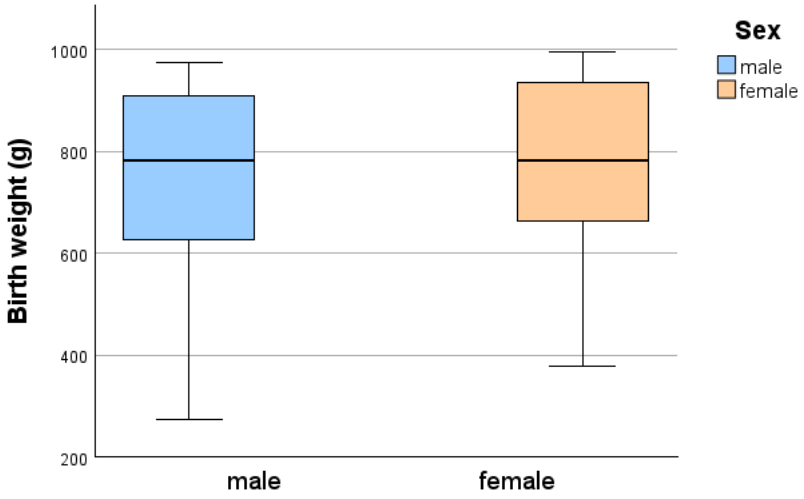


Figure 3 *- Birth weight stratified by sex*

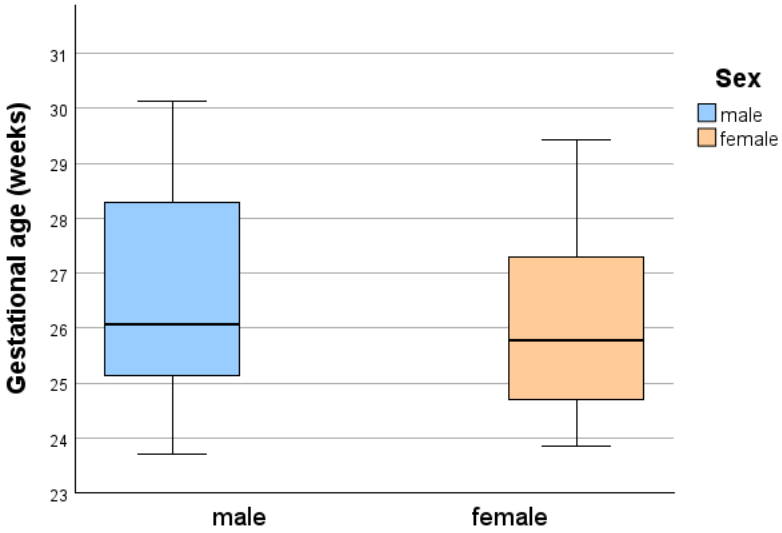
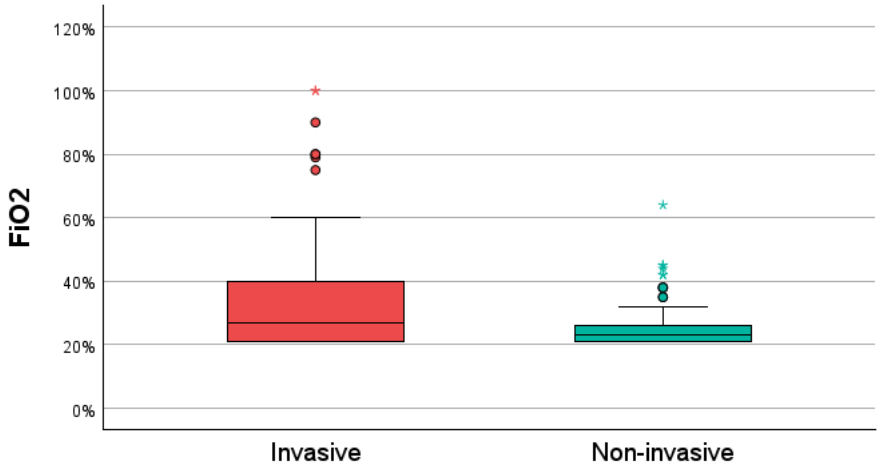


Figure 4 *- Gestational age stratified by sex*

Table 2 *- Fractional inspiratory oxygen concentration (FiO_2) between respiratory support modes*

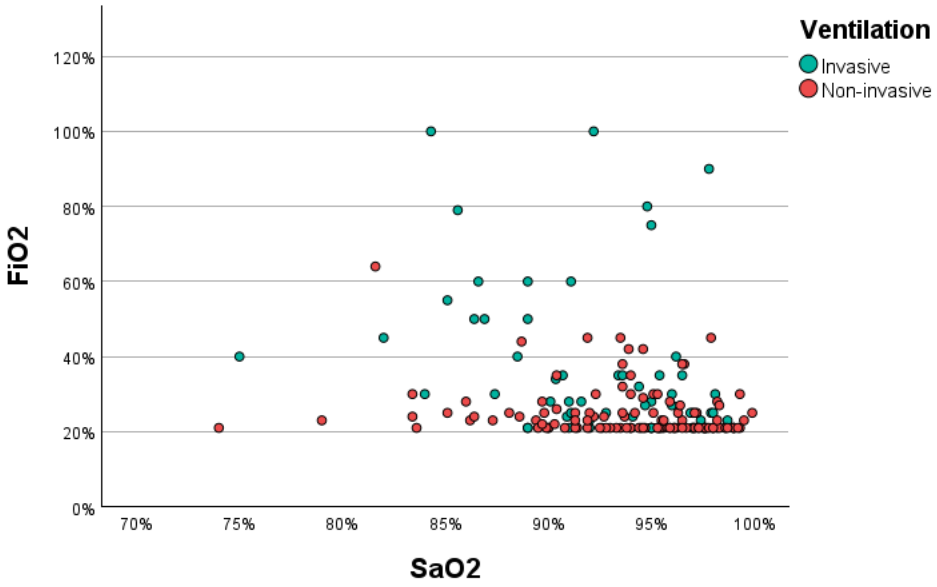
	Invasive	*Non-invasive*
Total (m=173)	67 (38.7%)	106 (61.3%)
FiO_2 (%)	27 [21 – 40]	23 [21 – 26]
Maximum FiO_2 (%)	100	64

FiO_2: Fractional inspiratory oxygen concentration



FiO_2: Fractional inspiratory oxygen concentration

Figure 5 *- Boxplot of FiO_2 by ventilation mode*



FiO_2: Fractional inspiratory oxygen concentration; SaO_2: Arterial oxygen saturation

Figure 6 *- Scatterplot of FiO_2 and the corresponding SaO_2*

Table 3 *- Descriptive characteristics of measurements*

Variables	*Total (m=173)*	*Minimum*	*Maximum*
pH	7.28 ± 0.098	6.95	7.49
$PaCO_2$	41.25 [35.50 – 51.18][a]	19.3	90.7
PaO_2	58.9 [49.6 – 68.5][b]	28.20	127.0
Standard Bicarbonate	19.45 ± 3.26[a]	8.3	30.2
Lactate	2.75 [1.80 – 5.35][c]	0.6	23.0
Base Excess	-5.10 [-7.38 – 2.80][a]	-21.3	4.3
SaO_2	93.04 ± 5.22	63.3	99.9
SpO_2	93.44 ± 3.15	76.0	100.0
FiO_2	24.0 [21.0 – 30.0]	21.0	100.0

a: Missing data of 1; **b**: Missing data of 2; **c**: Missing data of 3
$PaCO_2$: Arterial partial pressure of carbon dioxide; **PaO_2**: Arterial oxygen tension; **SaO_2**: Arterial oxygen saturation; **SpO_2**: Pulse oximetry-derived oxygen saturation; **FiO_2**: Fractional inspiratory oxygen concentration

3.3 Primary outcome

The results of the primary outcome measure: bias between pulse oximetry derived-SpO_2 and co-oximetry derived SaO_2 are presented in Table 4 and Figure 7. In 173 paired SpO_2-SaO_2 measurements, derived from 36 ELBW infants, a statistically significant systematic difference between SpO_2 and SaO_2 was detected. The difference varied in size and direction across the arterial oxygen saturation range. Across all paired measurements, an increase in arterial oxygen saturation was associated with a decrease in measurement error, defined as SpO_2-SaO_2 bias. The linear mixed effects model revealed a highly significant proportional bias of -0.645 per 1% (*p <0.0001*). This shows a negative association between SaO_2 and the measurement error (β= -0.645; standard error: 0.039; *p <0.0001*). In other words, for each 1% increase in arterial oxygen saturation, the difference between SpO_2 and SaO_2 decreases by approximately 0.65 percentage points. The intercept calculated by the model was 60.566 (standard error: 3.670, p <0.0001), representing the mathematically expected difference between SpO_2 and SaO_2 at an arterial oxygen saturation of 0%. Figure 7 illustrates the Bland-Altman plot, which visually demonstrated the observed saturation-dependent bias. At low arterial oxygen saturation levels, the results were primarily positive, reflecting an overestimation of SpO_2 by pulse oximetry. As arterial oxygen saturation levels increased, the magnitude of the differences decreased, and at higher saturation levels, the differences became negative. The limits of agreement (LoA) were calculated to be ± 5.096%. As indicated by the mean bias of the specific oxygen saturation, the upper limit of agreement is approximately 5.1% above the mean bias, while the lower LoA is, respectively, below the mean bias. These LoA revealed substantial fluctuations across the entire saturation range, with maximum deviations of +20.00% at low saturation levels and -11.20% at intermediate saturation levels.

The measurement error was also examined within the predefined groups of arterial oxygen saturation ranges. For SaO_2 values below 80% (paired measurements: m = 4), the mean bias was the highest among all saturation ranges at +14.175%, with individual errors ranging from +11% to +20%. In the 80-85% range (m = 7), the mean bias decreased to +6.671%, with individual errors between +5.00% and +8.59%. Overestimation of SpO_2 values persisted in the 85-90% saturation range (m = 25), where the mean bias was +4.324% and individual deviations ranged from

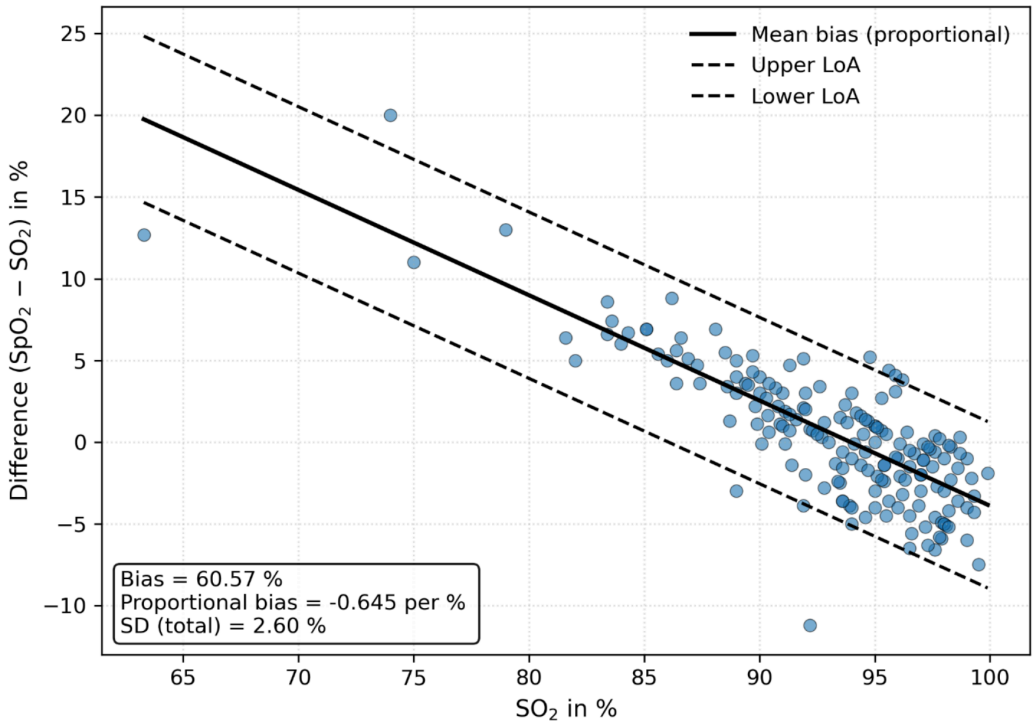
-3.00% to +8.80%. With increasing SaO_2, the magnitude of measurement error progressively decreased. In the 90-95% range (m=60), the mean bias was +0.267%, indicating near agreement between SpO_2 and SaO_2 on average. However, despite this small mean difference, individual measurement errors within this range remained considerable, ranging from -11.20% to +5.20%. At higher arterial oxygen saturation levels between 95% and 100% (m=77), the direction of the bias reversed. In this range, pulse oximetry underestimated the SaO_2, with a mean bias of -2.069%. The individual errors ranged from -7.50% to +4.40% in this group. Pearson correlation coefficients calculated between SpO_2 and SaO_2 values within individual saturation ranges were weak in saturation ranges 85% to 90%, 90%-95% and 95-100% with p-values >0.150. At SaO_2 values below 80% correlations were high r>0.810 but statistical significance persisted only for SaO_2 range of 80 to 85% r=0.811 (p=0.027).

These results can be used to evaluate the predefined hypotheses. The primary hypothesis, which states that the oxygen saturation derived by pulse oximetry -SpO_2 differs systematically from the corresponding arterial oxygen saturation measurement- SaO_2, was confirmed. A highly significant, saturation-dependent, systematic difference was observed between SpO_2 and SaO_2, consequently rejecting the null hypothesis of no systematic difference. Regarding the secondary hypotheses, mean differences between SpO_2 and SaO_2 were observed within almost all predefined arterial oxygen saturation subgroups. The magnitude of the difference between SpO_2 and SaO_2 varied across arterial oxygen saturation ranges. There were larger average errors at lower saturations and smaller, or even reversed, errors at higher saturations. This finding is consistent with the overall saturation-dependent trend observed in the linear mixed effects model. In accordance with this pattern, mean differences below 90% arterial oxygen saturation were greater than those above 90%. However, the subgroup analyses were only descriptive. No formal statistical testing of bias within individual saturation ranges or direct comparisons between low and high saturation ranges was performed. Although the difference between the two methods increases at lower oxygen saturations, a systematic overestimation was not observed across the entire saturation range.

Table 4 *- Results of the linear mixed effects model of all paired measurements*

	β	***Standard error***	**p *value***	***IQR***
Intercept	60.566	3.670	<0.0001	[53.373, 67.758]
SaO_2	-0.645	0.039	<0.0001	[-0.722, -0.567]

SaO_2: Arterial oxygen saturation



SpO_2: Pulse oximetry-derived oxygen saturation, SO_2 (SaO_2): Arterial oxygen saturation

Figure 7 *- Bland-Altman plot of differences between SpO_2 and SaO_2 across arterial oxygen saturation levels*

Table 5 *- Results within predefined SaO_2 ranges*

SaO_2 Range	**m**	***Pearson correlation coefficient; (*p value*)***	***Bias (average error)*** (%)	***Min. error***	***Max. error***
<80%	4	0.871; *(0.129)*	14.175	11.00	20.00
80% to 85%	7	0.811; *(0.027)*	6.671	5.00	8.59
85% to 90%	25	0.114; *(0.587)*	4.324	-3.00	8.80
90% to 95%	60	0.185; *(0.157)*	0.267	-11.20	5.20
95% to 100%	77	0.135; *(0.242)*	-2.069	-7.50	4.40

SaO_2: Arterial oxygen saturation

4 Discussion

In this retrospective single-center study of extremely low birth weight infants, we examined the accuracy of pulse oximetry by assessing the agreement between pulse oximetry-derived oxygen saturation (SpO_2) and arterial oxygen saturation measured by co-oximetry (SaO_2) during routine clinical care in the period of postnatal transition. Our study found a highly significant, proportional, saturation-dependent discrepancy between SpO_2 and SaO_2. Pulse oximetry overestimated the oxygen saturation at lower saturation levels, whereas the measurement error decreased with increasing saturation and shifted towards underestimation at higher saturation levels.

This proportional discrepancy between SpO_2 and SaO_2 from our cohort aligns with previous investigations that have demonstrated limited agreement between SpO_2 and SaO_2 in neonatal patients, especially in preterm neonates and infants with lower birth weight. Specifically, while our data show a consistent tendency towards higher SpO_2 values at lower SaO_2 levels, Wackernagel et al. (29) quantified this effect in a large retrospective analysis of more than 27,000 paired measurements, demonstrating a mean SpO_2-SaO_2 difference of approximately +2.9% with increasing differences towards lower saturation levels. Although the magnitude of bias observed in our study differed from that reported by Wackernagel et al.(29), the direction and saturation-dependent nature of the bias in our ELBW cohort align closely with their findings, suggesting a shared underlying limitation of pulse oximetry rather than a cohort-specific random effect. The different size of the detected bias compared to that reported by Wackernagel et al. (29) is likely due to the enormous difference in the number of measurements.

In addition, our observation that the discrepancy between SpO_2 and SaO_2 was more pronounced in lower saturation ranges mirrors the results reported by Wackernagel et al. (29), who showed that pulse oximetry failed to meet ISO accuracy criteria below SpO_2 values of 91%, a range that is particularly relevant for oxygen titration decisions in ELBW infants. The observed disagreement between SpO_2 and SaO_2, especially at lower saturation levels, is also consistent with the findings of Rosychuk

et al. (34), who reported that a significant proportion of SpO_2 measurements in the 85-89% range actually corresponded to SaO_2 values lower than 85%. This indicates a comparable saturation-dependent tendency in pulse oximetry, in which lower saturation ranges are especially susceptible to overestimation. Whereas Rosychuk et al. (34) focused on very preterm infants, their observation of the bias in a larger neonatal cohort strengthens the clinical relevance of our findings in the context of ELBW infants. While Wackernagel et al. (29) and Rosychuk et al. (34) observed a similar overestimation in lower saturation ranges, our study adds new insight by demonstrating that this bias persists even in ELBW infants, while highlighting the specific vulnerability of this group.

The variability of SpO_2-SaO_2 bias detected in our study can further be interpreted in light of the systematic review by Pritišanac et al. (30), which demonstrated a wide range of SpO_2-SaO_2 bias across neonatal studies, with reported mean differences ranging from -3.6% to +4.2%. When considered together with our results, this variability suggests that the presence or absence of statistically significant mean differences does not imply equivalence between SpO_2 and SaO_2, but rather reflects substantial physiological and technical variability fundamental to neonatal oxygenation monitoring. This is important when interpreting the 90-95% SaO_2 range, where the mean bias was near zero and seemed to suggest agreement. However, discrepancies remained substantial at the level of individual measurements, indicating that agreement may still be limited.

Taken together, our findings are consistent with previous literature demonstrating a tendency toward overestimation of arterial oxygen saturation by pulse oximetry in preterm infants, particularly at lower saturation levels. By reproducing this saturation-dependent directional bias in a highly vulnerable ELBW cohort already in the early neonatal period of the first 48 hours after birth, our study extends existing evidence and underscores that clinically relevant discrepancies between SpO_2 and SaO_2 may persist even when average differences appear small.

Clinical interpretation and implications

The clinical implications of our findings are particularly relevant in the context of contemporary oxygen targeting strategies in neonatology, which are largely based on strictly defined SpO_2 target ranges. While both Wackernagel et al. (29) and Rosychuk et al. (34) included a substantially larger number of paired measurements, their observation periods extended across the entire NICU stay. In contrast, our study focused specifically on the first 48 hours after birth. With regard to the risk of neonatal morbidities, particularly IVH, this interval represents one of the most vulnerable phases of neonatal life, following the fetal-to-neonatal transition. Accurate SpO_2 measurements are fundamental for guiding respiratory support settings, oxygen supplementation, and surfactant administration during this period. Consequently, any SpO_2-SaO_2 bias occurring within this early postnatal window may directly influence clinical decision-making in the immediate neonatal period.

We observed SpO_2 values tending to be higher than the corresponding SaO_2 at lower SaO_2 levels. This message is crucial as this indicates that seeming adherence to SpO_2 targets does not necessarily reflect adequate arterial oxygenation, particularly in clinically relevant low saturation ranges. It is highly relevant in light of the results of large randomized oxygen targeting trials. In the Neonatal Oxygenation Prospective Meta-analysis (NeOProM), Askie et al. (20) demonstrated that targeting different SpO_2 ranges (85-89% vs. 91-95%) resulted in clinically meaningful differences in mortality and incidence rates of NEC and ROP. Even though many infants were assigned to different SpO_2 target ranges they often had very similar SpO_2 values, implying a substantial overlap between the groups. Nevertheless, clinically relevant differences in the outcomes were observed. These findings highlight that even small differences in effective oxygenation, which may not be fully captured by pulse oximetry alone, can be associated with relevant clinical outcomes.

Our results suggest that discrepancies between SpO_2 and arterial oxygen saturation may contribute to accidental exposure to hypoxemia or hyperoxemia, even when pulse oximetry values remain within recommended target ranges. The observed saturation-dependent bias, characterized by pulse oximetry overestimating the arterial oxygen saturation at lower saturation levels, supports concerns that reliance

on pulse oximetry alone may provide an incomplete representation of effective oxygenation in ELBW as well as other premature infants. This interpretation is further supported by the physiological basis of fetal hemoglobin (HbF), which shifts the ODC to the left and therefore increases its affinity for oxygen. While Pritišanac et al. (30) did not find direct evidence that HbF alters the SpO_2-SaO_2 relationship, they observed that HbF changes the relationship between SpO_2 and PaO_2. This makes the ODC steeper at low saturation ranges and as a result increases the vulnerability of neonates to undetected hypoxemia at lower SpO_2 levels. Conversely, the flat upper part of the curve becomes flatter over a wider PaO_2 range. This may lead to a lower risk for undetected hyperoxemia than hypoxemia. Both HbF and the dissociation between SaO_2 and PaO_2 are important physiological concepts for interpreting pulse oximetry-based targeting in vulnerable premature infants. They support concerns that SpO_2 values alone may not reliably reflect arterial oxygenation in all clinical situations. In line with this, Wackernagel et al. (29) showed that clinically relevant PaO_2 values outside target ranges can occur despite SpO_2 readings within commonly used saturation targets, highlighting the limits of pulse oximetry as a representation for PaO_2, which may result in incomplete detection of hypoxemia or hyperoxemia. However, it should be noted that our study did not investigate any relationship to the PaO_2. Therefore, direct conclusions regarding arterial oxygen tension cannot be drawn. Nevertheless, when interpreted in the context of previous work, our findings align with the conclusions of Wackernagel et al. (29), who also used arterial blood gas analysis as their reference method and concluded that decisions concerning oxygen supplementation in neonates should not be based exclusively on pulse oximetry. Our findings support this conclusion. Taken together the need for critical and cautious interpretation of SpO_2 values is emphasized, particularly in extremely low birth weight infants with a high FHbF during clinically unstable phases.

Limitations

Several limitations of this study must be acknowledged. First, the retrospective single-center design limits generalizability, particularly given the small study population, which consisted exclusively of ELBW infants with a high neonatal vulnerability. Second, the retrospective design did not allow active control over measurement conditions. Variability in clinical status, monitoring practices, and the timing of arterial blood gas sampling may have contributed to increased measurement variability. Although repeated measurements within individual patients were appropriately accounted for using a mixed effects model approach, the number of included patients was limited to 36, from whom 173 paired measurements were obtained. While this allowed assessment of saturation-dependent trends across repeated observations, the study may have been underpowered to detect small subgroup-specific differences or to formally test differences within individual saturation ranges. In addition, PaO_2 and its relations to the topic were not specifically analyzed. Consequently, direct conclusions regarding the relationship between pulse oximetry and PaO_2 cannot be drawn from these data, and physiological interpretations must rely on indirect inference and existing literature. Finally, potential confounders such as sensor type, perfusion status, motion artifacts, and changes in clinical management could also not be controlled in this retrospective setting. These factors may have influenced performance of pulse oximetry and contributed to variability in the observed measurements. As our study highlights important discrepancies between SpO_2 and SaO_2 in ELBW infants, future research in this specific field is needed and should aim to further explore the potential underlying factors, including the influence of HbF, device-specific variations and evaluate oximeter calibration standards in pulse oximetry. To confirm our findings, studies with larger patient groups are needed to provide more robust data and increase statistical power. A new calibration approach should be considered, ideally using FHbF rich blood and ELBW controls.

5 Conclusion

This study identifies a critical, saturation-dependent proportional disagreement between SpO_2 and SaO_2 in extremely low birth weight infants. Pulse oximetry overestimates arterial oxygenation at lower saturations and underestimates it at higher levels compared to the gold standard co-oximetry from an arterial blood sample. This overestimation and underestimation phenomenon poses a risk for misguiding clinical interventions. A more critical interpretation of pulse oximetry data in this patient population is necessary. In future, developing advanced algorithms tailored to technical factors and the complex physiology of preterm neonates could improve the accuracy and ensure more reliable oxygen monitoring for these patients.

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