# PRECISION MEDICINE IN NEONATES

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# PRECISION MEDICINE IN NEONATES

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EDITORIAL
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# Editorial: Precision Medicine in Neonates

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**Keywords: newborn, precision medicine, individualized medicine, neonatal intensive care, empirical medicine**

**Editorial on the Research Topic**

**Precision Medicine in Neonates**

## INTRODUCTION

Precision medicine can be defined as a structured approach to treat or prevent specific diseases based on inter-individual variability in genes (like polymorphisms), diseases (like gender, co-morbidity), environment (like drug exposure, nutrition), or lifestyle (like stress) (1). This concept also holds the promise to improve management (prevention or treatment) and subsequent outcome in critically ill newborns. Precision medicine (*subgroup approaches*) hereby serves as go between empirical (*one treatment fits all*) or stratified medicine (e.g., *disease state or sex*), and individualized (*every newborn is unique*) medicine (**Figure 1**).

With this Frontiers special issue, we intended to increase awareness among caregivers that precision medicine has a proven track record in e.g., oncology, and is likewise, also very promising to improve outcome in (pre)term neonates. Unfortunately, neonatal medicine is lagging behind in implementing this concept. To further quantify this, a PubMed for "precision medicine" on April, 23 2021 resulted in 64,080 hits, with an exponential increase from 2010 (>1,000 hits/year) onwards, while "precision medicine + newborn" only resulted in 826 hits, with a delayed increase from 2015 onwards.

This reflects the relevance of this focused Research Topic on precision medicine in neonates, as the concepts related to precision medicine and the anticipated gains in clinical outcome matter to (pre)term neonates: the neonatal community (care providers, clinical researchers, and parents) should move from one "uniform" protocol for every newborn toward precision driven, patient tailored treatments and support (2). Its feasibility and relevance are illustrated in the papers included, clustered in papers on respiratory diseases, neurology, colonization and infectious diseases, or macro- and microcirculation related (hemodynamics, renal, and retinal) diseases.

## RESPIRATORY DISEASES

Bronchopulmonary dysplasia (BPD) remains the most common complication of preterm birth with long lasting sequelae. Most intervention studies to prevent BPD were conducted on a population level, without "precision concepts" like variation in clinical and biological diversity, genetic predisposition or environmental factors. In a review paper, Onland et al. explored potential approaches to implement precision concepts in neonatal BPD care, summarizing the available evidence on electrical impedance tomography and electromyography of the diaphragm, genomic variation in caffeine metabolism, and volatile organic compound analysis in exhaled breath (Onland et al.).

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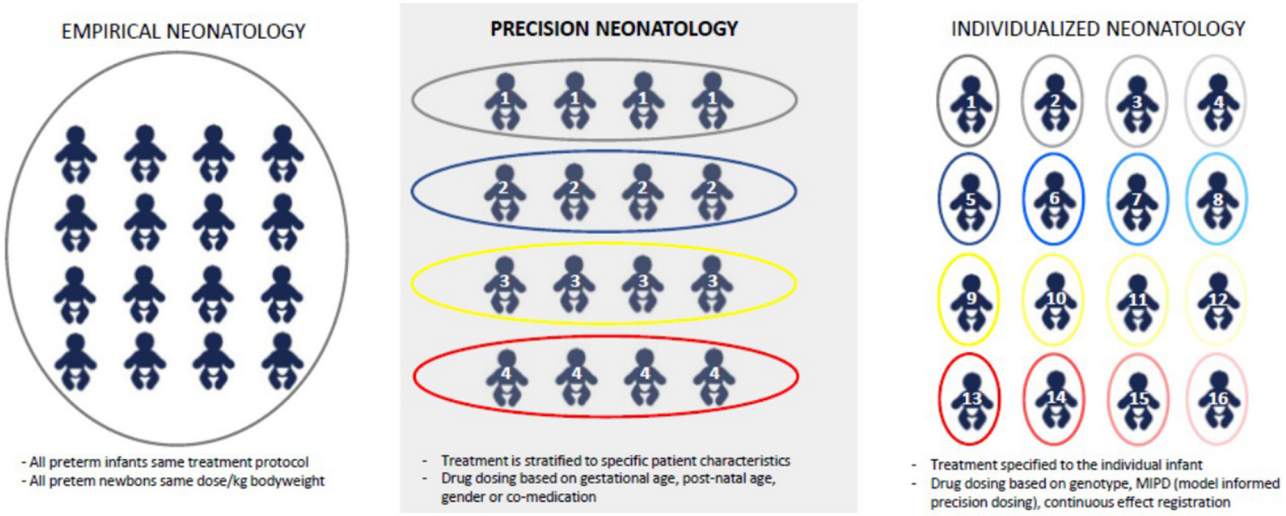


**FIGURE 1 |** Different concepts related to empirical medicine, precision medicine, or individualized medicine.

Two other original studies focused on outcome and risk stratification for two medical interventions for respiratory distress syndrome (RDS), i.e., the need for a second dose of surfactant or nitric oxide. Preterms in need of a second dose of surfactant for RDS had identifiable risk factors (growth restricted, outborn, no antenatal steroids). Interestingly, this more severe RDS (reflecting the need for a second dose), phenotype was associated with lower survival, but with similar outcome in survivors (Greiner et al.). While a second dose of surfactant (up to 300 mg/kg) is still standard of care, nitric oxide remains much more controversial based on the available evidence on benefit/(long term) outcome data (Vieira et al.). The current analysis hereby paved the way for a precision medicine approach within this population by providing evidence that a specific subgroup of preterm neonates (pulmonary hypertension present, prolonged rupture of membranes, and antenatal steroid exposure) had better outcome.

## NEUROLOGY

Despite a machinery of tools (ultrasound, Magnetic Resonance Imaging, electro-encephalography, Near Infrared spectroscopy, general movements) to assess neurological (dys)function during neonatal stay, predictive performance for the subsequent individual neurodevelopmental, long-term outcome remains poor. Machine learning approaches were suggested as powerful approach to change performance of prediction and prognosis in the field of neonatal neurology (Tataranno et al.).

## COLONIZATION AND INFECTIOUS DISEASES

The evidence of lung microbiota dysbiosis and the applications of probiotics to prevent BPD were examined and discussed (Yang and Dong). Based on the same gut-lung axis concept and BPD risk, the correlation between intestinal and pharyngeal microbiota was explored in 13 premature neonates. While intestines and pharynx shared some microbiota (like streptococcus), both sites also had unique profiles, so that linking of data on microbiota to gut-lung crosstalk outcome data (like BPD) should also consider the site of collection (Yang et al.).

Colonization patterns are obviously also affected by our practices and antibiotic prescription (when, what and how) (3). Keij et al. therefore called for a stratified management of bacterial infections in late preterm and term neonates. Differences in disease susceptibility, disease severity, immune response and pharmacokinetics and -dynamics should be considered to develop treatment algorithms for (suspected) sepsis, to assist clinicians in their decisions on to initiative or continue antimicrobial therapy in specific subpopulations (Keij et al.).

## MACRO- AND MICROCIRCULATORY DISEASES

Individualized hemodynamic management, tailored to the cardiovascular (patho)-physiology, and clinical characteristics of each individual patient or subgroups is another promising application of precision medicine. De Boode applied this concept to three clinical syndromes commonly observed in the neonatal unit: patent ductus arteriosus, shock, and hypotension and persistent pulmonary hypertension of the newborn (De Boode).

Renal precision medicine also holds the promise to support decision making on pharmacotherapy, signal detection of adverse (drug) events and to improve accuracy prediction of short- and long-term prognosis. Recent advances in this field (acute kidney definition development, specific findings in extreme low birth weight infants, and in neonates undergoing whole body hypothermia following asphyxia) and the potential clinical

impact (secondary prevention and feasibility of nephrotoxicity risk mitigation) were discussed (Allegaert et al.). Along the same line, but integrating preterm birth with asphyxia, renal function profiles in preterm neonates with birth asphyxia within the first 24 h of life were described. Different profiles between preterms with or without asphyxia were identified. This facilitates identification of relative risks to develop renal impairment, and brings this population closer to precision medicine, and subsequent needed studies on long-term cardiovascular and renal outcome (Zhang and Zeng).

Finally, the retinal circulation and the related retinopathy of prematurity (ROP) forms another relevant morbidity in former preterms. The extent of temporal avascular area of the retina (disc diameter) and duration of mechanical ventilation at first examination of the newborn (4–6 weeks postnatal life) predicted the subsequent need for an ROP-related intervention during neonatal stay. Such indicators can be used in a risk calculator to predict the need for treatment or to power preventive studies (Chaves-Samaniego et al.).

Overall the different papers added ideas and some provided small pieces of the puzzle that we need for precision medicine. In most areas stratified medicine is still work in progress and it still quite far away from where we aim to be in neonatology. We are confident that the illustrations provided in this Research Topic will further boost the clinical research on precision medicine concepts as a powerful tool to further improve neonatal management, quality of care, and outcome.

## AUTHOR CONTRIBUTIONS

All authors contributed to the Editorial and agreed on the final version.

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REVIEW
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# Renal Precision Medicine in Neonates and Acute Kidney Injury: How to Convert a Cloud of Creatinine Observations to Support Clinical Decisions

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Renal precision medicine in neonates is useful to support decision making on pharmacotherapy, signal detection of adverse (drug) events, and individual prediction of short- and long-term prognosis. To estimate kidney function or glomerular filtration rate (GFR), the most commonly measured and readily accessible biomarker is serum creatinine ($S_{cr}$). However, there is extensive variability in $S_{cr}$ observations and GFR estimates within the neonatal population, because of developmental physiology and superimposed pathology. Furthermore, assay related differences still matter for $S_{cr}$, but also exist for Cystatin C. Observations in extreme low birth weight (ELBW) and term asphyxiated neonates will illustrate how renal precision medicine contributes to neonatal precision medicine. When the Kidney Disease Improving Global Outcome (KDIGO) definition of acute kidney injury (AKI) is used, this results in an incidence up to 50% in ELBW neonates, associated with increased mortality and morbidity. However, urine output criteria needed adaptations to broader time intervals or weight trends, while $S_{cr}$ and its trends do not provide sufficient detail on kidney function between ELBW neonates. Instead, we suggest to use assay-specific centile $S_{cr}$ values to better describe postnatal trends and have illustrated its relevance by quantifying an adverse drug event (ibuprofen) and by explaining individual amikacin clearance. Term asphyxiated neonates also commonly display AKI. While oliguria is a specific AKI indicator, the majority of term asphyxiated cases are non-oliguric. Asphyxia results in a clinical significant—commonly transient—mean GFR decrease (−50%) with a lower renal drug elimination. But there is still major (unexplained) inter-individual variability in GFR and subsequent renal drug elimination between these asphyxiated neonates. Recently, the Baby-NINJA (nephrotoxic injury negated by just-in-time action) study provided evidence on the concept that a focus on nephrotoxic injury negation has a significant impact on AKI incidence and severity. It

is hereby important to realize that follow-up should not be discontinued at discharge, as there are concerns about long-term renal outcome. These illustrations suggest that integration of renal (patho)physiology into neonatal precision medicine are an important tool to improve contemporary neonatal care, not only for the short-term but also with a positive health impact throughout life.

**Keywords: creatinine, Cystatin C, precision medicine, acute kidney injury, newborn, nephron number**

## INTRODUCTION

Precision medicine is defined as a structured approach to treat or prevent specific diseases based on the inter-individual variability in genes, physiology, and environment. This includes exploration of novel research approaches to improve the use of available information to support decision making about pharmacotherapy, signal detection of adverse (drug) events, or to improve individual prediction of short- and long-term prognosis. Neonatal renal precision medicine depends on the availability of reference intervals for any renal biomarker to support clinical decision making, tailor therapy, or support prognosis. This is still a major limitation, highlighted in the International Neonatal Consortium (INC) paper on safety, dosing, and pharmaceutical quality of medical products in neonates and during development of the neonatal adverse event severity scale. Severity grading for lab values—including kidney function—was omitted until reference values became available (1, 2).

Since maturational physiological changes are most prominent in early infancy, variability is their key feature. This is reflected in extensive inter- and intra-individual variability in serum creatinine ($S_{cr}$), resulting in a cloud instead of extractable and interpretable information for clinicians. This "cloud" is illustrated in **Figure 1**, after plotting $S_{cr}$ observations (enzymatic assay) in (pre)term neonates collected in one Neonatal Intensive Care Unit (NICU) in the first 42 postnatal days of life (3). This reflects a pattern with an initial increase and subsequent decrease during postnatal life. As relevant, there is about a 4-fold difference in $S_{cr}$ observed for all consecutive days, so that improved understanding on reference values is needed to attain precision medicine.

This $S_{cr}$ variability is partly explained by maturational changes (e.g., birth weight, gestational age [GA], postnatal age) and non-maturational changes related to pathophysiology, e.g., perinatal asphyxia, co-medication, congenital anomalies of the kidney and urinary track (CAKUT), cardiac surgery with bypass, or extra-corporeal membrane oxygenation (4, 5). Postnatal kidney adaptation is proportional to the nephron number (GA driven) and renal perfusion (postnatal adaptation, mean arterial blood pressure) (6, 7). Nephrogenesis evolves as branching morphogenesis, similar to lung, pancreas, vascular tree, or retina. Neonates <36 GA weeks are still in active nephrogenesis and thus have an increased risk for decreased nephron endowment with lifelong impact (8). The main determinants of the increase in renal function in early life are circulatory changes, driven by the increase in proportional renal blood flow to cardiac output, from 2 to 25%. This increased renal blood flow is combined with dilation of the efferent and constriction of the afferent arterioli. This explains the significant impact of non-steroidal anti-inflammatory drugs (NSAIDs, −20 up to −40% of the glomerular filtration rate [GFR] in ELBW neonates, depending on the type and dose) or asphyxia (up to −40 to −50% of GFR).

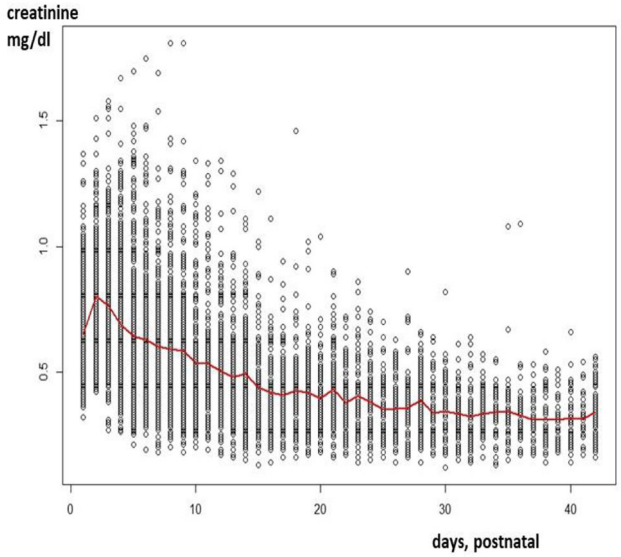


**FIGURE 1 |** Creatinine values (enzymatic assay) as observed in a cohort of 1,140 neonates (gestational age 23–42 weeks) in the first 42 days of postnatal life (3).

## CREATININE AND CYSTATIN C AS BIOMARKERS OF GFR IN NEWBORNS

$S_{cr}$ is the most commonly measured biomarker to estimate kidney function or GFR (creatinine clearance). However, before $S_{cr}$ can be used to estimate renal elimination capacity in neonates, issues that should be considered relate to physiology (renal tubular transport, hydration, muscle mass) and measurement (assay validity).

Creatinine at birth does not yet reflect neonatal but maternal $S_{cr}$ levels. Because of passive tubular back leak instead of active secretion, creatinine clearance does not yet fully reflect GFR. In contrast to later life, where creatinine clearance somewhat overestimates GFR due to active tubular secretion mediated by Organic Cation Transporter 2 (OCT-2), passive back leak occurs in early neonatal life (9, 10). Hydration is another issue, as early

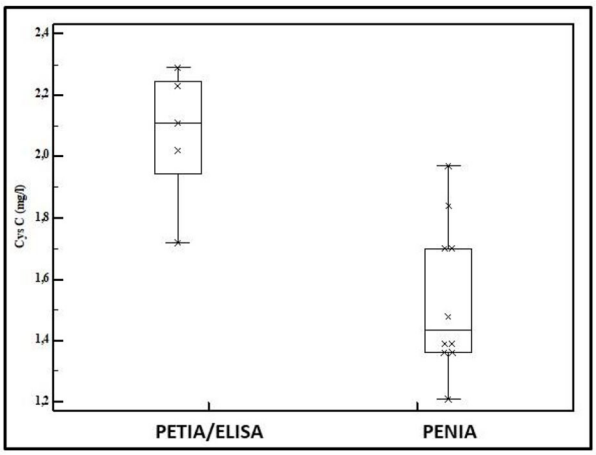


**FIGURE 2 |** Mean Cystatin C values in umbilical cord blood as reported in 15 cohorts of healthy term neonates (2 enzymatic immuno-assay [ELISA], three particle enhanced turbimetric immuno-assay [PETIA], and 10 particle enhanced nephelometric immuno-assay [PENIA]) (16–22).

neonatal life is associated with weight reduction due to free water loss, usually associated with a sodium increase. Creatinine is a low molecular weight (133 g/mol) molecule produced by muscle catabolism (creatine to creatinine), reflecting muscle mass. As estimated by creatinine excretion in urine, muscle mass increased from 12% of birth weight at 25 weeks to 19% at 34 GA weeks and 24% at term (11). In contrast, the proportional muscle mass was estimated to remain stable (22–30%) without trend related to GA in autopsy findings (12).

$S_{cr}$ values also depend on the assay, as the Jaffe assay is affected by specific constituents of neonatal serum like bilirubin or albumin concentrations. Harmonization through isotope dilution mass spectrometry (IDMS) traceability has reduced, but not eliminated, this inter-assay variability (9, 13). $S_{cr}$ measurements can subsequently be converted to estimated GFR, using the Schwartz formula (eGFR $= k$ [$L/S_{cr}$], $S_{cr}$ = μmol/l; $k$ = 0.34 in preterm, 0.45 in term infants, $L$ = length, cm). One should hereby be aware that this Schwartz formula has initially been validated (to inulin clearance) with the original, non-compensated Jaffe assay (9). Furthermore, length measurement has limitations in neonates (14). While lower $k$-values have been suggested when enzymatic assays are used, these studies have not included (pre)term neonates and infants (9). Recently, an eGFR specific to (pre)term neonates (median age 3 days postnatal age, compensated Jaffe) was suggested (eGFR= 2.32 × [weight (g)$^{0.64}$/$S_{cr}$ (μmol/l)$^{0.62}$]) following validation with inulin clearance (21.54 [SD 10.09] ml/min/1.73 m$^2$). This formula performed somewhat better compared to the original neonatal Schwartz formula (15).

Cystatin C is an alternative to $S_{cr}$ to assess eGFR and is considered to be a more sensitive indicator for minor GFR changes. Cystatin C is a 130 amino acids containing small protein, generated by any cell with elimination by GFR. Reference values have been suggested, but Cystatin C also has assay-related issues like $S_{cr}$. **Figure 2** reflects assay-specific differences in mean umbilical cord blood Cystatin C values reported in 15 cohorts of healthy term neonates. In these 15 cohorts, Cystatin C was quantified by enzymatic immuno-assays (ELISA, $n = 2$), particle enhanced turbimetric immuno-assays (PETIA, $n = 3$), or particle enhanced nephelometric immuno-assays (PENIA, $n = 10$) (16–22). Since 2010, certified reference material for Cystatin C assay standardization (IDMS) has been available, but measurement bias still exists (23). This is relevant, since the majority of studies with Cystatin C in neonates were conducted in single units or with one assay. Within this setting, Cystatin C values are associated with maturational covariates (age, weight) or perinatal diseases, like congenital renal anomalies, sepsis, and septic shock, respiratory distress, hypotension, transient tachypnea of the newborn, or perinatal asphyxia, serving as a more sensitive indicator for renal dysfunction (16, 24, 25). However, clinicians should be cautious in extracting absolute values as reported for subsequent use in their specific setting as assay-related issues may exist. Of specific relevance to neonates, steroid administration or hypothyroidism may also affect Cystatin C (16, 26). Finally, Cystatin C was not retained in the recently published eGFR formula specific to (pre)term neonates (15).

We therefore suggest development of age-dependent, assay specific $S_{cr}$ centiles to support clinical decisions and precision pharmacotherapy. We will focus on ELBW (<1,000 g) infants and on term neonates with perinatal asphyxia undergoing whole body hypothermia (WBH) to illustrate how a "$S_{cr}$ cloud" can be converted into a clinical decision tool. With these examples, we will illustrate how renal precision medicine is a crucial part of modern neonatal care. A similar approach can be considered for other subcategories like CAKUT newborns or neonates in need of cardiac bypass or extracorporeal membrane oxygenation.

## Key Messages

- There is extensive variability in $S_{cr}$ and eGFR (Schwartz formula, Wilhelm-Bals formula) within the neonatal population, driven by (patho)physiology.
- Assay related differences exist for $S_{cr}$ and Cystatin C.
- We suggest to develop age-dependent, assay specific $S_{cr}$ centiles to support neonatal precision medicine.

# RENAL FUNCTION, AKI, AND PRECISION MEDICINE IN ELBW INFANTS

Almost a decade ago, Jetton and Askenazi (27) suggested an AKI definition (neonatal modified KDIGO) specific for use in neonates. In essence, the definition is based on trends (increase) *in* $S_{cr}$ and *in urine output* to result in staging (stage 0–3) (**Table 1**). This definition was endorsed by a National Institute of Diabetes, Digestive, and Kidney (NIDDK) Diseases workshop. The attendees hereby concluded that this definition offered a reasonable starting point, but that further evaluation was needed (30, 31). In this context, a study protocol (Assessment of Worldwide Acute Kidney Injury Epidemiology in Neonates, AWAKEN) was put forward to assess its applicability and to report on neonatal AKI epidemiology (28).

We highlight some results of this AWAKEN study specific to ELBW neonates to emphasize the limitations of the KDIGO definition: (*i*) the overall AKI incidence was 29.9%, but was 47.9% in preterm neonates (<29 weeks GA) (*limited discriminating power*); (*ii*) 8/24 of the contributing units still used a Jaffe assay (*relevant differences in absolute values not considered in the definition*); (*iii*) the median number of $S_{cr}$ counts was ≤3 and ≤5/patient respectively in 10 and 15/24 of these units (*suggesting that there is not yet sufficient focus on renal function, even in AWAKEN units*) (*iv*) urine output has been quantified in 24 h intervals with 1 ml/kg/h as pivotal finding (*pragmatic, but not as suggested in the definition,* **Table 1**), (*v*) despite these limitations, the AKI stage predicted mortality (adjusted Odds Ratio 3.7) but not length of stay in <29 weeks neonates (29). In secondary analyses specific in the most immature cohorts, an increased risk for bronchopulmonary dysplasia was observed in 29–32 GA cases (adjusted Odds Ratio 4.2), but not in <29 GA neonates (32). Early (*within the first week of life*) caffeine administration was associated with reduced AKI incidence (number needed to treat = 4.3) and severity (33). This can be explained by the adenosine related effects on the glomerular vascular tone. Finally, AKI was not limited to early neonatal life but also occurred beyond day 7 (9%). Risk factors were the presence of a patent ductus arteriosus with or without NSAIDs exposure, necrotizing enterocolitis, and sepsis (34).

Although the current AKI definition may assist clinicians to recognize renal issues, there are still limitations to using this AKI tool for precision medicine in ELBW cases. As 47.9% of cases <29 weeks were classified as having AKI, the granularity needed for precision medicine is somewhat lost. The issues relate to both urine output and $S_{cr}$ as biomarkers of kidney function and AKI.

Continuous quantification of urine output is a technical burden and is even more difficult in the most immature neonates, as catheterization is invasive while sequential diaper weight is hampered by evaporation (up to 80% weight losses after 2 h of a 5 ml portion added to a diaper exposed in an incubator or under a radiant warmer) (35). This was already acknowledged by the AWAKEN study, since urine output was quantified by 24 h increments with 12% missing observations for urine output (29). Fluid overload and daily weight balance (change% = current weight – birthweight/current weight) in the 1st week of life were used as alternative markers. Based on these markers, a higher positive peak in the 1st week of life and a positive fluid balance on day 7 were associated with mechanical ventilation (36).

$S_{cr}$ itself is not an AKI biomarker, but rather an indicator of kidney function. As mentioned earlier, absolute values are affected by the assay (dependent on IDMS traceability), since Jaffe results are affected by some drugs and—more relevant to ELBW neonates—by bilirubin so that the median difference between the original Jaffe and an enzymatic assay is 0.12 to 0.27 mg/l, with always higher values for the Jaffe assay (37). Furthermore,

**TABLE 1 |** Definition of neonatal Acute Kidney Injury (AKI) by serum creatinine and urine output (28, 29).

| Stage | Serum creatinine ($S_{cr}$) | Urine output |
|---|---|---|
| 0 | No change in $S_{cr}$ or ↑ <0.3 mg/dl | >1 ml/kg/h |
| 1 | $S_{cr}$ ↑≥ 0.3 mg/dl within 48 h or $S_{cr}$ ↑≥1.5–1.9 X vs. $S_{cr}$* within 7 days | >0.5 and ≤1 ml/kg/h |
| 2 | $S_{cr}$ ↑≥ 2 to 2.9 vs. reference $S_{cr}$* | >0.3 and ≤0.5 ml/kg/h |
| 3 | $S_{cr}$ ↑≥ 3 X reference $S_{cr}$ * or $S_{cr}$ ≥2·5 mg/dl** *or* dialysis | ≤0.3 ml/kg/h |

**Reference $S_{cr}$ is the lowest prior $S_{cr}$ measurement; **a $S_{cr}$ of 2.5 mg/dl in neonates reflects an estimated glomerular filtration rate <10 ml/min/1.73 $m^2$.*

**TABLE 2 |** Centiles (10th−95th) of serum creatinine values (enzymatic assay) in a cohort of 217 extremely low birth weight (ELBW) infants in the first 28 days of postnatal age (38).

| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Samples** | 206 | 190 | 205 | 198 | 182 | 171 | 145 | 157 | 140 | 128 | 117 | 112 | 92 | 133 |
| 10th | 0.45 | 0.64 | 0.74 | 0.64 | 0.58 | 0.55 | 0.54 | 0.50 | 0.50 | 0.44 | 0.42 | 0.41 | 0.41 | 0.41 |
| 25th | 0.52 | 0.75 | 0.81 | 0.76 | 0.7 | 0.67 | 0.64 | 0.60 | 0.57 | 0.55 | 0.51 | 0.50 | 0.47 | 0.48 |
| 50th | ***0.605*** | ***0.86*** | ***0.91*** | ***0.88*** | ***0.84*** | ***0.80*** | ***0.75*** | ***0.74*** | ***0.70*** | ***0.65*** | ***0.64*** | ***0.60*** | ***0.59*** | ***0.57*** |
| 75th | 0.74 | 0.95 | 1.03 | 1.03 | 0.97 | 0.94 | 0.89 | 0.88 | 0.83 | 0.79 | 0.79 | 0.76 | 0.70 | 0.66 |
| 90th | 0.91 | 1.065 | 1.18 | 1.18 | 1.14 | 1.12 | 1.11 | 1.04 | 1.01 | 0.93 | 0.96 | 0.87 | 0.86 | 0.8 |
| 95th | 0.98 | 1.16 | 1.22 | 1.29 | 1.25 | 1.28 | 1.17 | 1.12 | 1.15 | 1.05 | 1,03 | 0.92 | 0.96 | 0.85 |
| | **Day 15** | **Day 16** | **Day 17** | **Day 18** | **Day 19** | **Day 20** | **Day 21** | **Day 22** | **Day 23** | **Day 24** | **Day 25** | **Day 26** | **Day 27** | **Day 28** |
| **Samples** | 101 | 96 | 99 | 79 | 85 | 62 | 123 | 72 | 66 | 86 | 64 | 50 | 54 | 111 |
| 10th | 0.36 | 0.36 | 0.37 | 0.35 | 0.38 | 0.37 | 0.36 | 0.37 | 0.36 | 0.35 | 0.33 | 0.34 | 0.32 | 0.32 |
| 25th | 0.44 | 0.44 | 0.43 | 0.42 | 0.42 | 0.40 | 0.39 | 0.40 | 0.40 | 0.41 | 0.38 | 0.37 | 0.37 | 0.36 |
| 50th | ***0.55*** | ***0.52*** | ***0.51*** | ***0.49*** | ***0.49*** | ***0.50*** | ***0.47*** | ***0.46*** | ***0.47*** | ***0.47*** | ***0.43*** | ***0.45*** | ***0.44*** | ***0.42*** |
| 75th | 0.63 | 0.61 | 0.60 | 0.61 | 0.57 | 0.62 | 0.54 | 0.54 | 0.54 | 0.52 | 0.52 | 0.51 | 0.50 | 0.48 |
| 90th | 0.81 | 0.69 | 0.71 | 0.73 | 0.66 | 0.68 | 0.62 | 0.68 | 0.64 | 0.64 | 0.58 | 0.59 | 0.70 | 0.55 |
| 95th | 0.87 | 0.73 | 0.78 | 0.81 | 0.73 | n.a. | 0.68 | 0.72 | n.a. | 0.67 | n.a. | n.a. | n.a. | 0.59 |

*The numbers in bold and italic represent the 50th centile, or the median.*

the maturational $S_{cr}$ changes over postnatal age are extensive in ELBW neonates. There is an initial increase to peak on day 3, with a subsequent slow decrease over postnatal age (38, 39). To further illustrate this, we have summarized the postnatal $S_{cr}$ trends (10th, 25th, median, 75th, 90th, and 95th centile) over the first 28 days of life in an cohort of 217 ELBW cases (single unit, enzymatic assay, all exposed to caffeine) in **Table 2** (38). If we focus on the median estimates, there is a clear increase from day 1 to 3 by 0.3 mg/dl, so that this median "normal" trend already qualifies for an AKI stage 1 classification. From a physiological point of view, it may even be reasonable to classify a relevant portion of ELBW as having AKI. However, by using a centile approach, more "granularity" in the data is provided to facilitate precision medicine.

Plotting individual observations or $S_{cr}$ trends over time in a single ELBW infant may facilitate recognition and quantification of an adverse drug event or may even facilitate precision pharmacotherapy, a concept somewhat similar to growth charts. To illustrate this, we compared mean $S_{cr}$ in ibuprofen-exposed ELBWs in the earlier mentioned cohort to these centiles (38). **Figure 3** (visual presentation of **Table 2** data) illustrates the reference $S_{cr}$ (gray lines) over postnatal age, with the plotted trend (black line) of median $S_{cr}$ observed in ELBW neonates exposed to ibuprofen. A shift of about 1 standard deviation in $S_{cr}$ 133 ibuprofen-exposed neonates is hereby observed (38, 39). On the other hand, we investigated how individual amikacin clearances are linked to $S_{cr}$ centiles (38). For aminoglycosides like amikacin, there is a strong correlation between clearance and GFR, also in neonates (40). Consequently, GA and ibuprofen affect amikacin clearance in early neonatal life (41). Integration of $S_{cr}$ centiles (<25th centile, 25–75th centile, or >75th centile) in this dataset further explained the individual amikacin clearance estimates (**Figure 4**) (38, 39). Along the same line, Cystatin C reference values (type of assay unclear, Modular Analytics ISE900 Analyzer, Germany) for ELBW (integrated in a cohort of very low birth weight, < 1.5 kg) infants on day 1 and 3 [mean (SD) 1.77 (0.38) and 1.61 (0.37 mg/L, respectively)] have been suggested in the literature. In contrast to $S_{cr}$ values, observations were independent of the GA (42).

## Key Messages

- Using the KDIGO-AKI definition results in an AKI incidence of about 50% in ELBW neonates. Similar to other populations, AKI is associated with increased mortality and morbidity.
- For pragmatic reasons, the criteria on urine output were converted to more extensive time intervals or weight trends.
- We suggest to use assay-specific $S_{cr}$ centiles to better describe the normal postnatal trend and its variability. We illustrated that this approach facilitates recognition and quantification of adverse drug events (ibuprofen, **Figure 3**) or to explain individual amikacin clearance (**Figure 4**).

# RENAL INJURY RELATED TO ASPHYXIA AND NEONATAL ENCEPHALOPATHY

Perinatal asphyxia is a multi-organ disease, with moderate to severe encephalopathy as pivotal finding to initiate WBH in term neonates (4). This also includes AKI. As part of precision medicine, quantifying the incidence, extent, and variability of AKI and its covariates is relevant to tailor fluid administration and pharmacotherapy (4, 43). AKI has also been identified as prognostic factor for adverse neurological outcome and death (44, 45).

AKI occurs in neonates following perinatal asphyxia, but the incidence varies and in part depends on the case mix. In 36 neonates with asphyxia (Apgar score 5 min <7), AKI

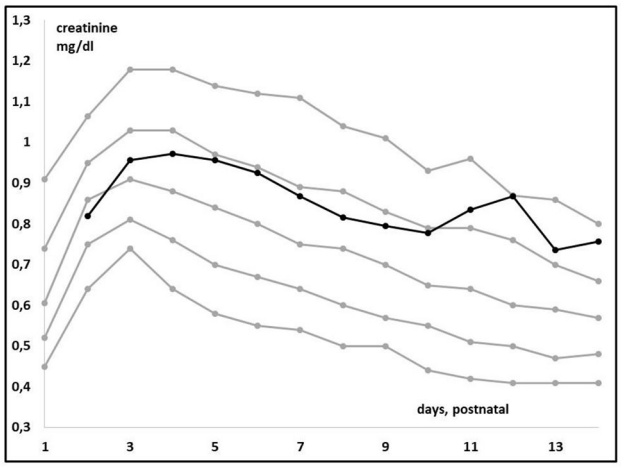


**FIGURE 3 |** Trends in median creatinine values (enzymatic assay) in extreme low birth weight (ELBW) neonates when exposed to ibuprofen ($n = 133$, black line) compared to the reference centile trends (Table, gray lines) over time in the first 14 days of postnatal life (38).

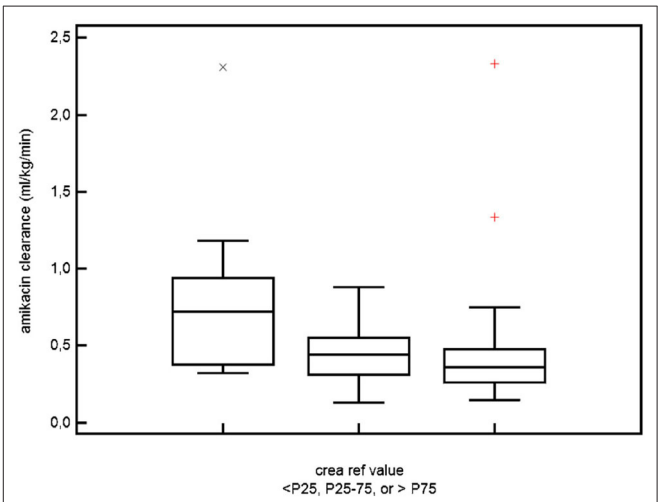


**FIGURE 4 |** The relation between serum creatinine and amikacin clearance in ELBW neonates. Differences in amikacin clearance in ELBW neonates who had a serum creatinine reference value either <25th, between 25th and 75th, or >75th centile for ELBW neonates and for the specific postnatal day (38, 39, 41) (*permission for re-use of the figure has been granted, Rightslink*).

**TABLE 3 |** Overview of serum creatinine observations (assay mentioned, mean, and standard deviation or interquartile range, all converted to mg/dl) on day 1, 2, or 3 and day 10 in term and preterm (light gray) asphyxiated neonates (criteria provided) and gestational age-matched controls (19, 44, 46, 51–54).

| Reference | Clinical features, asphyxia criteria | Assay | Day 1 asph. | Day 2/3 asph. | Day 10 asph. | Day 1 contr. | Day 2/3 contr. | Day 10 contr. |
|---|---|---|---|---|---|---|---|---|
| Gupta, 2005 (51) | Apgar score 5 min <8, 59 term cases | Jaffe | n.a. | 1.08 (0.49) | n.a. | n.a. | 0.88 (0.26) | n.a. |
| Kaur, 2011 (46) | Apgar score 1 min <7, 36/2,196 cases, ≥34 weeks | Jaffe | 0.92 | 0.95 | | | | |
| Sarafidis, 2012 (52) | Apgar score 5 min < 7 + clinical HIE (any stage) 13 cases, 24 controls | Jaffe | 1.32 (0.43) | 1.57 (1.15) | 0.73 (0.43) | 1.02 (0.26) | 0.76 (0.19) | 0.69 (0.11) |
| Hadzimuratovic, 2014 (44) | Apgar score 5 min <7 + organ dysfunction + HIE 50 cases, 50 controls | Jaffe | n.a. | 1.06 (0.40) (IQR) | n.a. | n.a. | 0.65 (0.05) (IQR) | n.a. |
| Treiber, 2014 (19) | Apgar score 5 min <7 + organ dysfunction + HIE 50 cases, 50 controls | Jaffe | 0.82 (0.17) | 0.66 (0.18) | n.a. | 0.71 (0.15) | 0.57 (0.17) | n.a. |
| Gupta, 2005 (51) | Apgar score 5 min <8, 11 preterm cases | Jaffe | n.a. | 1.34 (0.79) | n.a. | n.a. | 0.83 (0.26) | n.a. |
| Song, 2017 (53) | Definition unclear, 34–37 weeks, 48 cases and 45 controls | ? | 0.74 (0.23) | 1.07 (0.48) | n.a. | 0.7 (0.28) | 0.74 (0.29) | n.a. |
| Pan, 2018 (54) | Apgar score 5 min <4 + organ dysfunction + pH<7 (umbilical cord), <34 weeks, 71 cases, 70 controls | ? | 0.69 (0.15) | 0.72 (0.14) | n.a. | 0.72 (0.15) | 0.74 (0.13) | n.a. |

*? Stands for unknown, n.a. for not available.*

was documented in 1/11 (9%) with moderate and 12/25 (56%) with severe asphyxia (46). When we focus on WBH cases, AKI (according to the modified neonatal KDIGO criteria) was diagnosed in 39–42% (47, 48). Oliguria (<1 ml/kg/h for 12 h) was observed in 11% WBH neonates (49). In the most recent Cochrane meta-analysis, there was a trend to a lower incidence of renal impairment (urine output <0.5 ml/kg/h for ≥24 h + $S_{cr}$ >1 mg/dl) for neonates undergoing WBH (38.5 vs. 45%, risk ratio and 95% CI, 0.87, 0.74–1.02), without effect on oliguria (<1 mg/kg/h, 23 vs. 24%) (50).

**Table 3** provides an overview on $S_{cr}$ observations (including assay) from day 1 to 10 as reported in cohorts of (pre)term asphyxia neonates (including criteria) in the era before WBH. Compared to the Gupta and Kaur cohorts, the other cohorts had more restrictive inclusion criteria and likely better reflect the neonates that currently qualify for WBH (19, 44, 51–54). These data remain valuable as some neonates may miss the 6-h therapeutic window or to subsequently compare such data with WBH-related observations. In these studies, different definitions for AKI or kidney failure were used (**Table 3**).

In the Gupta cohort, 47% of asphyxia neonates were classified as having "renal failure" [blood urea >40 mg/dl, $S_{cr}$ >1 mg/dl (Jaffe) or oliguria (<0.5 ml/kg/h)] (51). Mean blood urea and $S_{cr}$ on day 3 of postnatal life were significantly higher (+60 and 30%, respectively) in asphyxiated neonates, while the standard deviation (SD) for both biochemical markers doubled, indicating an increase in patient variability. This variability was in part explained by the HIE stage (mean [SD] $S_{cr}$ for HIE stage 0, 1, 2, or 3 was 0.9 [0.2], 1.1 [0.4], 1.3 [0.8], 1.4 [0.6] mg/dl, respectively). Interestingly, the urine output was comparable between cases and controls while oliguria was rare (7/33 renal failure cases), while oliguric vs. non-oliguric renal failure was associated with higher mortality (43 vs. 8%). During follow-up, urine output normalized from day 4–6 onwards, urea and $S_{cr}$ from day 7–9 onwards (51). In the more recently reported AWAKEN cohort (113 WBH neonates, AKI incidence 42%), oliguria was more commonly observed in AKI cases (isolated oliguria in 47, 26% mixed with $S_{cr}$ thresholds, **Table 1**) (47). This confirms the complexity of AKI diagnosis, and the need to simultaneously assess both diuresis and $S_{cr}$ or Cystatin C to tailor clinical care and pharmacotherapy. Kaur et al. reported on mean $S_{cr}$ (Jaffe) values 24–36 h and 72–96 h in asphyxiated neonates (0.92 and 0.95 mg/dl), with significant differences between AKI and non-AKI neonates (1.49 vs. 0.8 mg/dl and 1.65 vs. 0.81 mg/dl) for both time intervals (46).

In the Sarafidis cohort, AKI (any $S_{cr}$ > 1.5 mg/dl, or increase >0.3 mg/dl from day 1, Jaffe) was observed in 8/13 (61%). The Apgar score at 5 min was significantly lower in subsequent AKI neonates, while other indicators of asphyxia severity (inotropics, ventilation, anti-epileptic drugs, HIE moderate/severe, mortality) also associated with AKI (52). $S_{cr}$ (day 1, 3, and 10) were significantly higher in asphyxiated neonates (1.32, 1.57, and 0.73 mg/dl) compared to controls (1.02, 0.76, and 0.69 mg/dl) with normalization on postnatal day 10. There is also a broader range (SD higher), reflecting higher inter-patient variability in renal impairment in asphyxia cases. In the Sarafidis cohort, several other biomarkers of renal GFR or tubular damage were also quantified. Serum Cystatin C (ELISA) was marginally increased only on day 1 in the asphyxiated neonates, while urine Cystatin C and Neutrophil Gelatinase-associated lipocalin were significantly increased in cases until day 10, suggesting earlier

restoration of glomerular than tubular impairment (52). In the Hadzimuratovic cohort, $S_{cr}$ (Jaffe) and Cystatin C (turbimetric assay) was significantly higher (63 and 49%, respectively) in asphyxia cases. In contrast to the findings of Sarafidis, both the absolute Cystatin C values and trends remained different, perhaps reflecting assay-related difference. The HIE stage (I vs. III) in part explained the $S_{cr}$ variability on day 3. Finally, Treiber et al. also reported on renal biomarkers ($S_{cr}$, Jaffe; Cystatin C, nephelometry) at birth (umbilical cord) and on day 3 in a cohort of asphyxia neonates. Interestingly, $S_{cr}$ and Cystatin C were significantly higher from delivery onwards. Umbilical Cystatin C was the most sensitive marker of asphyxia (receiver operating characteristic curve = 0.918) (**Table 3**).

A specific focus on preterm neonates is warranted. These patients do not qualify for WBH but can still display asphyxia-related AKI (**Table 3**). Gupta et al. (51) documented that the mean $S_{cr}$ is significantly higher (+60%) in asphyxiated (Apgar score 5 min <8) preterms. Song et al. (53) reported on $S_{cr}$ (assay unclear) values in near-term (34–37 GA) asphyxia (definition unclear, $n = 48$) and age-matched controls, and observed a significant decrease (−20%) in eGFR in asphyxia cases. Pan et al. included 71 preterms (<34 weeks) with asphyxia (pH<7 + Apgar at 5 min <4 + multi organ dysfunction) and 70 preterm controls, and collected samples at 24, 48, and 96 h. $S_{cr}$ was significantly higher at 96 h (83.5 vs. 62.9 μmol/l) and eGFR was consistently lower at 24 and 48 h (−30 and −20%, respectively) (54). Cystatin C (PENIA) had good distinguishability between asphyxiated and non-asphyxiated preterms, irrespective (<28, 28–32, or ≥32 weeks subgroups) of GA, and further discriminated between mild, moderate, and severe asphyxia. In contrast, $S_{cr}$ (assay unclear) was not discriminative (55).

AKI affects the renal and non-renal outcome in asphyxia neonates. AKI is associated with a 4.6-fold higher mortality risk in the AWAKEN study, and this higher mortality risk also holds true for post-asphyxiated neonates with AKI (29, 51). When considering the renal outcome after discharge, Hadzimuratovic et al. (44) and Gupta et al. (51) reported on normalization of renal findings at 1 and 6 months, respectively in asphyxia cases (44, 51). AKI also associates with non-renal outcome, like prolonged hospital stay (48), prolonged mechanical ventilation (47), or abnormal brain imaging findings (73 vs. 46%) at the end of the first week of life (56). Post-asphyxial renal injury (urine output + $S_{cr}$) was a prognostic factor for neurological outcome at the end of the 1st year of life (44). However, the absence of AKI neither guarantees a positive outcome (45).

Once AKI has been identified, precision medicine involves fluid and electrolyte management, drug choice (avoid nephrotoxic drugs, potential nephro-protective interventions), and for those drugs, dose selection.

In the earlier mentioned AWAKEN study, the daily %-weight change from birth weight in the 1st week of postnatal life was used to reflect the fluid balance in 645 critically ill term neonates. A higher peak fluid balance and higher fluid balance over the first week of life were independently associated with mechanical ventilation on day 7. A negative fluid balance was observed in 53% of neonates (21 vs. 41% in ventilated vs. non-ventilated neonates). Those with AKI had a consistently higher fluid balance throughout the 1st week of life (36). Suggested nephro-protective interventions include "low dose dopamine" or methylxanthines, like theophylline (57). At present, there is no robust evidence for the use of low dose dopamine to protect kidney function, while there is meta-analytic evidence that prophylactic theophylline (single intravenous dose, 5 mg/kg) results in a significant lower AKI incidence (OR 0.24) in asphyxiated neonates (58). Despite the evidence, neonatologists remain reluctant to administer theophylline as this increases metabolic activity of the brain, while hypothermia and sedation are used to reduce metabolic (cerebral) activity.

With respect to precision pharmacotherapy, it is important to realize that the mean difference in $S_{cr}$ or eGFR between asphyxiated neonates (either or not undergoing WBH) compared to term controls is clinical significant (−40 to −50%) and further adds to maturational (weight, postnatal) changes. This is reflected by the impact of asphyxia on drugs exclusively cleared by renal elimination (4). To illustrate this, amikacin clearance trends in early neonatal life based on pooling of reported datasets were plotted (**Figure 6**). There is a maturational trend in clearance, related to birth weight and postnatal age (day 1, 2, 3, 4, as indicated by different colors) compared to a subgroup of WBH neonates. These differences indicate mean differences in clearance, but do not cover the additional unexplained between-individual variability (59). Essentially, there is a shift in the Gauss curve for $S_{cr}$ or eGFR toward renal impairment, but mean differences do not fully cover the between individual variability. For this type of drugs, this means that the time interval between consecutive administrations should be extended (in general from 24 to 36 h, so compensating for the 40–50% decrease in clearance) but therapeutic drug monitoring remains compulsory as there still will be toxic trough levels in 14–25% of cases (59).

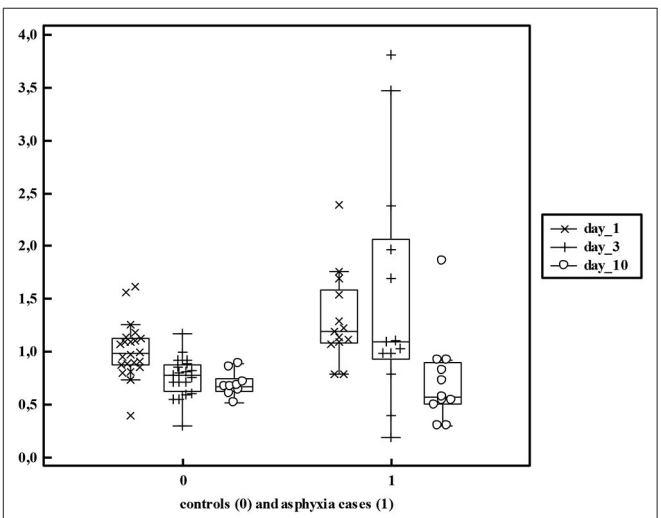


**FIGURE 5 |** Serum creatinine values in non-asphyxiated ($n = 24$) and asphyxiated neonates ($n = 13$) as reported by Sarafidis et al. (52) reflecting both the mean differences and additional variability (>5 fold) within these cohorts.

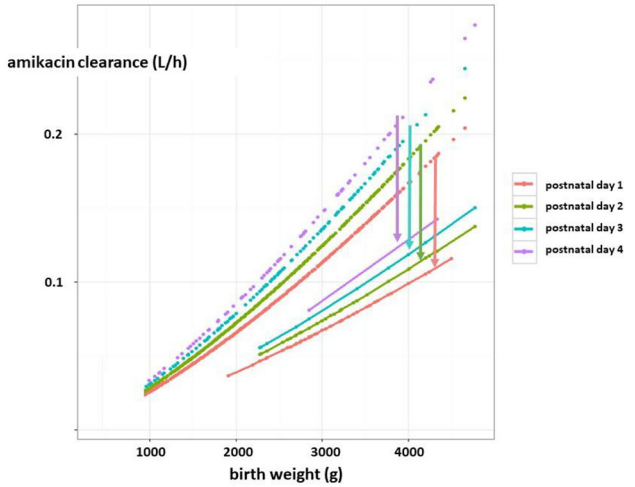


**FIGURE 6 |** Estimates of amikacin clearance (l/h) trends in early neonatal life based on pooling of reported datasets (dashed lines). There is a maturational trend in clearance, related to birth weight (g) and postnatal age (day 1, 2, 3, 4, as reflected by the colors) compared to a subgroup of term neonates undergoing therapeutic hypothermia as treatment for perinatal asphyxia (solid lines). The arrows indicate the difference in clearance between both cohorts for the respective postnatal age (in days) (4, 59) [*this figure has earlier been published in reference (4), permission for re-use has been granted, first author Anne Smits*].

## Key Messages

- Oliguria is a specific indicator of AKI, but the majority of AKI cases are non-oliguric. Assessment of the fluid balance is an alternative. This confirms the complexity of AKI diagnosis, and the need to simultaneously assess both diuresis and $S_{cr}$ or Cystatin C to tailor clinical care and pharmacotherapy.
- Asphyxia with WBH results in a clinical significant—often transient—mean decrease in eGFR (−40 to −50%), with GA and HIE stage as additional covariates (**Table 3**).
- This mean decrease in GFR affects renal drug elimination. However, there is still large (unexplained) inter-individual variability in GFR (mean vs. SD) and renal drug clearance in asphyxiated neonates (**Figures 5**, **6**).

## RENAL PRECISION AS CRUCIAL PART OF CONTEMPORARY NEONATAL PRECISION MEDICINE

Using ELBW and asphyxia as case examples, we illustrated that kidney function and AKI are relevant to contemporary neonatal care. In ELBW, AKI is associated with increased mortality and morbidity while $S_{cr}$ centiles were used to recognize and quantify adverse drug events and to explain individual amikacin clearance. Asphyxia associated AKI affects mortality and morbidity. WBH is associated with a significant mean (−40 to −50%) GFR decrease. Of relevance, there is still important variability in GFR decrease around this mean decrease, so that the mean decrease does not predict well the GFR decrease in an individual neonate. Although these data strongly suggest that better integration of renal precision is important to improve contemporary neonatal care, the AWAKEN study showed that the median number of $S_{cr}$ measurements was ≤3 and ≤5/patient in 10 and 15/24 of the units, suggesting that further improvements can be made (29). There are some elegant illustrations on how these improvements can be implemented and how a focus on renal aspect indeed improves neonatal care.

In the Baby NINJA study, 476 individual events of high-risk nephrotoxic drug exposure were observed. During these events, a daily $S_{cr}$ was obtained until 2 days after exposure or after end of AKI. Within this framework, there was a reduction in exposure (16.4 to 9.6/1,000 patient days), a reduction in drug-associated AKI (30.9 to 11%), and in AKI intensity (9.1 to 2.9/100 susceptible patient days) (60). Implementation of AKI guidelines in a single NICU resulted in improvements in recognition, diagnosis, and subsequent follow-up of AKI (61). This matters, as in ELBW infants, exposure to nephrotoxic drugs is common (87%) with gentamicin (86%), indomethacin (43%), and vancomycin (25%) as most commonly administered drugs (62). It is hereby important to highlight that follow-up of these populations remains important after hospital discharge as there are concerns on the long-term renal outcome, most pronounced in former ELBW cases (63). Research should focus on perinatal risk factors associated with impaired GFR in long-term outcome studies, but is hampered by single center cohorts, small samples sizes, and heterogeneity of GFR assessment tools (64). The diagnosis of AKI remains complex with integrated assessment both of diuresis and $S_{cr}$ or Cystatin C to tailor clinical care and pharmacotherapy. We therefore state that further integration of renal (patho)physiology into neonatal precision medicine and pharmacotherapy may not only result in better short-term outcome but also may have impact throughout pediatric life and beyond.

## AUTHOR CONTRIBUTIONS

KA initiated the project. AS and KS hereby had a specific focus on the section on acute kidney injury following perinatal asphyxia. TD and JA had a specific focus on the section of acute kidney injury. EL and DM provided crucial nephrologic expertise on these sections and on the introduction and discussion section. All authors approved the final version of the paper.

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# Risk Calculator for Retinopathy of Prematurity Requiring Treatment

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**Importance:** Vascular delay that occurs early in the development of retinopathy of prematurity (ROP) is a risk factor that can be compensated by ensuring a good rate of retinal vascularization to avoid ROP that requires treatment.

**Background:** The objective of the present study was to determine the association between ROP that requires treatment and risk factors such as the extent of the temporal avascular area of the retina and the number of days of mechanical ventilation (MV).

**Design:** Observational retrospective case-control study.

**Participants:** Two hundred and twenty-eight premature newborns included in the screening protocol for retinopathy of prematurity.

**Methods:** Subjects underwent retinal examination in the 4 and 6th postnatal weeks.

**Main Outcome Measures** : The temporal avascular area was measured in disc diameters (DD), while the MV time was measured in days of treatment.

**Results:** Patients with a longer MV time had a higher risk of treatment ($R^2$: 24.7, $p < 0.0001$; increase in risk of 8.1% for each additional day), as did those who showed greater avascular area ($R^2$: 24.7, $p < 0.0001$; increase in risk of 111% for each additional DD). An online calculator system and a table are presented for calculating the risk of ROP requiring treatment as a function of these two risk factors.

**Conclusions and Relevance:** The temporal avascular area of the retina and MV time must be taken into account in the first examination of the newborn to predict the need for ROP treatment.

**Keywords: retinopathy of prematurity, retinal vessel, oxygen-induced retinopathy, risk factors, bronchopulmonary dysplasia**

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## INTRODUCTION

Retinopathy of prematurity (ROP) is currently the world's second leading cause of preventable child blindness (1, 2). For this reason, numerous studies have been carried out to identify the main risk factors for ROP (3) and incorporate them into early ROP detection algorithms (4). Current screening algorithms are mainly based on birth weight and gestational age (3). However, several other related factors are involved, such as the avascular area of the retina, the rate of

retinal vascularization, and the time spent by the premature newborn under mechanical ventilation (MV) (3–6).

Various ROP predictive models to identify newborns requiring ophthalmologic screenings are reported in the literature. Majority show high sensitivity and medium to low specificity (7–10). Authors themselves have made clarifications regarding their own models, claiming that no predictive model will work properly at a universal level (11); in many cases newborns of ≥31 weeks or <24 weeks of pregnancy, who quite often show a wide area of non-vascularized retina after birth, are excluded (12). However, there is not a single model of practical applicability and adaptability currently available in the usual clinical practice (13) that helps physicians identify newborns with higher risk of severe ROP requiring treatment and guides the planning of checkup frequency.

The DIGIROP model concluded that one of the best variables for predicting risk of requiring ROP treatment is postnatal chronologicalage (12). Conversely, the Early Treatment for Retinopathy Of Prematurity Study showed that the wider the avascular area (zone I), the higher the risk of requiring treatment (14). The more severe a ROP is, the larger the peripheral area of retinal ischemia is, and this is associated with a greater need for revascularization and recovery time (15). Specifically, the revascularization time required to reverse ROP in zone I is significantly longer than that required in zone II, which in turn is longer than that required in zone III (16, 17). A retinal vascularization rate of <0.5 disc diameters (DD) per week (18) has been identified as an indication for ROP treatment (5). Therefore, why not study the temporal avascular area of the retina as a risk factor?

Both bronchopulmonary dysplasia and the need for MV in the premature newborn (19) are closely related to ROP. Longer MV times are associated with greater risk of ROP requiring treatment (6, 20). Recently, new oxygen therapy control measures have been implemented in neonatal intensive care units (NICUs) (21), as has early treatment by intravitreal bevacizumab or laser diode. Both these techniques have contributed to an improved prognosis for newborns with ROP (22).

The objective of the present study was to determine the risk factors for ROP requiring treatment, and to develop a system for calculating the risk of ROP requiring treatment that is based on the main risk factors and can be applied in clinical practice at the first examination of the newborn.

## MATERIALS AND METHODS

The present study was approved by the Biomedical Research Ethics Committee of Andalusia and was carried out in accordance with the ethical principles of the Declaration of Helsinki for medical research. All medical data were collected anonymously after informed consent was given and after the parents or legal guardians of the study participants gave express authorization.

**Abbreviations:** ROP, retinopathy of prematurity; MV, mechanical ventilation; DD, disc diameters; GA, gestational age; NICU, neonatal intensive care unit.

### Subjects of the Study

An observational, retrospective case-control study was conducted on 228 premature newborns who were examined between 1999 and 2019 at the NICU of the San Cecilio University Hospital in Granada, Spain. One eye of each patient was included in the study.

Inclusion criteria were: (1) birth weight < 1,500 g or GA ≤ 32 weeks, (2) birth weight between 1,501 and 2,000 g and GA ≥ 32 weeks with oxygen supply > 72 h or unstable clinical course as determined by the NICU neonatologist (apneas, neonatal acidosis, twin death, intraventricular hemorrhage, persistent ductus, sepsis, necrotizing enterocolitis, or concurrent surgical interventions), (3) preterm newborns that could be examined between the fourth and sixth postnatal weeks, and (4) preterm newborns with at least three examinations performed. Participants were excluded from the study when (1) they could not be examined between the 4 and 6th weeks after delivery, (2) they underwent fewer than three examinations, (3) they had complete retinal vascularization in the first examination, and (4) they showed medial opacity.

### Eye Examination

All patients were examined by the same pediatric ophthalmologist between the 4 and 6th postnatal weeks. The examinations used indirect ophthalmoscopy with indentation and were carried out under pharmacological mydriasis with topical anesthesia using a 20 diopter lens. This technique provides an approximate field of vision of 8 DD, which is equivalent to 45°. First, estimation of the vascular caliber is carried out and presence or absence of dilation or vascular tortuosity found in the vessels of the central retina is evaluated. Subsequently, the ROP stage and compromised area are identified. Next, eyeball indentation is performed in order to observe the peripheral retina by aligning the binocular ophthalmoscope until the raised gray line corresponding to the indentation is visible. Indentation is moved in parallel until the first temporal vessel is visualized in the periphery.

In each examination, the following data were collected: ROP stage according to the International Classification of Retinopathy of Prematurity (16), affected retinal area (zones I, II, and III), time extension, presence of preplus and plus disease, and extent of retinal avascular area (in DD) from the periphery to the center of the retina. Examinations were performed weekly or every 2 weeks until the retina was completely vascularized, except in patients with preplus or plus disease, in those with limited vascularization in zone I or posterior zone II, or in those with stage 3 ROP in any zone. In such cases, examination was carried out weekly.

Eye selection for the study (right or left) was carried out through random allocation.

The need for ROP treatment was recorded as a dependent variable, while the following parameters were recorded as independent variables: temporal avascular area of the retina in DD, GA (weeks), and MV time (days). We also collected weight gain in the first 4 weeks of postnatal life (g/day), Apgar score (23), degree of bronchopulmonary dysplasia (19), number of blood transfusions received, presence of apnea (24), persistence of arterial ductus, presence of sepsis (25),

presence of necrotizing enterocolitis, presence of intraventricular hemorrhage and presence of periventricular leukomalacia.

Disc diameters: The measurement unit used to quantify non-vascularized area of the retina was the disc diameter (DD). The horizontal diameter of the subject's optic nerve can be quickly and easily identified during fundus exploration. Once the optic disc diameter is known, extension of any retinal territory can be quantified in DDs. This measurement unit has been used by several authors to quantify the territory of retinal immaturity (26, 27).

Mechanical ventilation time (MV) was defined as the treatment time using endotracheal intubation. MV time was measured in days. However, between 2009 and 2012, the oxygen supply protocols were modified. Saturation levels were maintained at ∼95% during the years 2000–2009; from 2009–2012 they varied between 85 and 93% (6).

Postnatal weight gain was defined as the weight gained from birth up to week 4. It is expressed in g/day and is the result of the weekly weight gain average measured during routine neonate screenings.

The Apgar Scale was described by Virginia Apgar to assess the newborn's overall condition based on heart rate, breathing effort, response to stimuli, muscles tone, and skin color of the newborn. Apgar Scale at minute 1 of birth reflects the newborn's tolerance to birth, and Apgar at minute 5 reflects their adaptability level to the environment (23, 28).

Bronchopulmonary dysplasia was defined as a preterm infant chronical pulmonary disease with limited respiratory function caused by airway immaturity and exposure to high oxygen saturation due to mechanical ventilation (19). Three levels of severity (mild, moderate, and severe) were established based on gestational age (over or under 32 weeks of pregnancy) and ability to breathe room air or require oxygen at a concentration above or below 30% (29).

Presence of neonatal apnea was defined as the absence of respiratory effort for more than 20 s, or for more than 10 s with bradycardia or desaturation in newborns within the first 28 days of life (30).

Anemia and the need for blood transfusions are two conditions that have been related to ROP severity. The literature shows conflicting results. Some studies show that receiving a higher amount of blood supply has no significant impact on ROP severity (31). However, other studies show that receiving a low number of blood transfusions could result in a higher percentage of fetal hemoglobin, instead of adult hemoglobin, which could be a ROP protective factor (32).

Presence or absence of patent ductus arteriosus was confirmed in all of the cases using echocardiography. Rate of spontaneous closure of ductus arteriosus decreases considerably in newborns of <28 weeks and/or <1,000 g at birth, and its presence has been associated with several pathologies, such as ROP (33).

Sepsis was defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Organ dysfunction can be identified as an acute change in total SOFA (Sequential Organ Failure Assessment) score ≥2 points consequent to the infection (25).

Necrotizing enterocolitis was defined as an abdominal distention with pneumatosis intestinalis, portal venous gas, or both; or presence of other radiographic signs, such as fixed, dilated intestinal loops and ileus patterns (34). Most published articles show a higher risk of ROP in preterm infants with necrotizing enterocolitis. However, some authors claim that this relation could be caused by the impact of prolonged treatment with oxygen (3).

Presence of cerebral hemorrhage with intraventricular extension diagnosed by a pediatrician or neuropediatrician and confirmed through imaging was included in the study. Severity was classified using the Graeb Score (35). The presence of intraventricular hemorrhage has been associated with severe stages of ROP (36) and as one of the pathologies with higher morbidity and mortality in preterm newborns.

Presence of periventricular leukomalacia was determined using neuroimaging (primarily, ultrasonography, and/or magnetic resonance imaging) (37).

Identified cases included eyes that received treatment for threshold retinopathy of prematurity (ROP) or type 1 prethreshold ROP. Identified controls included other eyes that did not receive treatment for ROP or those presenting progression-free type 2 prethreshold ROP.

## Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 25.0; IBM Corp. Armonk, NY: IBM Corp). The variables were analyzed descriptively and expressed as means ± standard deviation. Cuantitative variables were compared using Mann-Whitney *U*-test. Qualitative variables were compared using Pearson's $\chi^2$ test. The predictive risk model was implemented using binary logistic regression between the dependent variable (ROP requiring treatment) and each of the independent variables.

# RESULTS

## Descriptive Analysis

A total of 683 premature newborns were screened, of which 455 were excluded because they did not meet the inclusion criteria. Of the 228 newborns ultimately included in the study, 38 (16.7%) received treatment, while 190 (83.3%) did not.

The mean GA of the study population was 28.83 ± 2.03 weeks; 34.6% of the newborns had a gestational age of 24–27 weeks, 47.4% of 28–30 weeks, and 18% of 31–34 weeks. The lowest GA in the study sample was 24 weeks, while the highest was 34 weeks.

The mean MV time of the entire sample was 9.4 ± 13.64 days; 28.07% of the newborns did not require MV, 42.98% required ≤ 10 days of MV, and 28.95% required > 10 days of MV.

The mean temporal avascular retinal area in the study sample was 3.46 ± 1.63 DD; 40.4% of the newborns had a temporal avascular area of < 3 DD, while 37.7% had an avascular area between 3–4 DD and 21.9% had an avascular area of ≥ 5.00 DD (**Table 1**).

**TABLE 1 |** Descriptive statistics of the study sample, average difference in continuous variables (Mann–Whitney *U*-test) and percentage difference in qualitative variables (Pearson's chi-square test) within treated and untreated ROP groups.

| Variable | Total mean ± *SD* or % | Untreated | Treated | *p* |
|---|---|---|---|---|
| Gestational age (weeks) | 28.83 ± 2.03 (Min 24.0/Max 34.0) | 29.10 ± 1.88 | 27.44 ±1.96 | <0.001 |
| Birth weight (g) | 1094 ± 268.9 (Min 537/Max 1970) | 1126.92 ± 257.67 | 935.16 ± 270.09 | <0.001 |
| Duration of mechanical ventilation (days) | 9.4 ± 13.64 (Min 0/Max 100.0) | 6.73 ± 8.62 | 22.74 ± 23.28 | <0.001 |
| Avascular area of the retina (DD) | 3.46 ± 1.63 (Min 1.0/Max 8.0) | 3.13 ± 1.44 | 5.13 ± 1.53 | <0.001 |
| Weight gain in the first 4 weeks (g/day) | 12.19 ± 6.03 (Min 0.1/Max 39.7) | 12.68 ± 6.01 | 9.70 ± 5.52 | 0.003 |
| Number of blood transfusions | 0.64 ± 0.67 (Min 0/Max 5.0) | 0.58 ± 0.60 | 0.95 ± 0.89 | 0.006 |
| Apgar score (1 min) | - (Min 0/Max 10) | - | - | 0.046 |
| Apgar score (5 min) | - (Min 0/Max 10) | - | - | 0.001 |
| Presence of bronchopulmonary dysplasia | 81.6% | 79.5% | 92.1% | 0.067 |
| Presence of apnoea | 14.5% | 7.4% | 50.0% | <0.001 |
| Presence of persistent arteriovenous ductus | 20.6% | 15.8% | 44.7% | <0.001 |
| Presence of sepsis | 40.4% | 35.8% | 63.2% | 0.002 |
| Presence of necrotizing enterocolitis (NEC) | 20.6% | 18.9% | 50.0% | <0.001 |
| Presence of intraventricular hemorrhage | 14.0% | 10.5% | 31.6% | 0.001 |
| Presence of periventricular leukomalacia | 24.1% | 19.5% | 47.4% | <0.001 |

*Min, minimum; Max, maximum.*

## Relationship Between ROP Requiring Treatment and Study Variables

**Table 2** shows the results of the binary logistic regression analysis between ROP requiring treatment and the risk factors related to retinal vascular development in ROP identified in the study.

### Relationship Between ROP Requiring Treatment Retinal Temporal Avascular Area

Greater retinal avascular area was significantly associated with greater risk of ROP requiring treatment (Nagelkerke's $R^2$: 30.1%, $p < 0.0001$). The first patients who were treated presented with 2.00 DD without vascularization. Of the patients with ≥ 6 DD of retinal avascular area, 58.83% required treatment (**Table 3**, **Figure 1**).

### Relationship Between ROP Requiring Treatment and Mechanical Ventilation Time

Longer MV times were significantly associated with greater risk of ROP requiring treatment (Nagelkerke's $R^2$ = 24.7%, $p < 0.001$), as shown in **Table 4**. After 31 days of MV, 72.7% of the newborns required treatment (**Table 4**, **Figure 2**).

## Models for Calculating the Probability of ROP Requiring Treatment

### Binary Logistic Regression Between ROP Requiring Treatment and Gestational Age at Birth

According to the logistic regression analysis, newborns with lower GA had a greater probability of requiring treatment (Nagelkerke's $R^2 = 16.1\%$, $p < 0.001$; OR: 0.631, 95% confidence interval [CI]: 0.513–0.775, $p = 0.005$]. Applying the logistic regression model between the dependent variable (ROP requiring treatment) and GA in weeks yielded the following:

$$\text{Probability of ROP requiring treatment} = 1/(1 + e^{-11.417-0.461(\text{weeks of GA})})$$

**TABLE 2 |** Analysis of the risk factors for severe ROP requiring treatment (assuming that the other variables remain constant in the sample).

| Variable | OR (CI 95%) | Nagelkerke's R2 |
|---|---|---|
| Avascular temporal retinal diameter (DD) | 2.11 (1.64, 2.70)* | 30.1%** |
| Duration of mechanical ventilation (days) | 1.08 (1.05, 1.11)* | 24.7%** |
| Weight gain in the first 4 weeks (grams/day) | 0.90 (0.84, 0.97)* | 6.5%** |
| Apgar score (1 min) | 0.95 (0.83, 1.09)* | 0.4%** |
| Apgar score (5 min) | 0.98 (0.82, 1.17)* | 0.01%** |
| Gestational age (weeks) | 0.63 (0.51, 0.77)* | 16.1%** |
| Birth weight (g) | 0.99 (0.98, 0.99)* | 13.2%** |
| Degree of bronchopulmonary dysplasia | 1.70 (1.21, 2.41)* | 7.9%** |
| Number of blood transfusions | 2.05 (1.22, 3.41)* | 6.1%** |
| Presence of apnoea | 12.57 (5.44, 29.03)* | 24.5%** |
| Presence of persistent arteriovenous ductus | 4.32 (2.04, 9.13)* | 10%** |
| Presence of sepsis | 3.07 (1.49, 6.33)* | 7%** |
| Presence of necrotizing enterocolitis (NEC) | 4.28 (2.06, 8.89)* | 10.6%** |
| Presence of intraventricular hemorrhage | 3.92 (1.72, 8.96)** | 7.0%** |
| Presence of periventricular leukomalacia | 3.72 (1.79, 7.73)* | 8.6%** |

*$p < 0.05$, **$p < 0.001$.

**TABLE 3 |** Relationship between retinal temporal avascular area and ROP requiring treatment in the sample. As avascular area increases, so did the percentage of individuals requiring treatment.

| Retinal avascular area | Untreated ROP | Treated ROP | Total | % ROP treated |
|---|---|---|---|---|
| 1-1.9 DD | 20 | 0 | 20 | 0% |
| 2-2.9 DD | 69 | 2 | 71 | 2.9% |
| 3-3.9 DD | 47 | 5 | 52 | 10.6% |
| 4-4.9 DD | 27 | 8 | 35 | 22.8% |
| 5-5.9 DD | 13 | 4 | 17 | 23.5% |
| 6-6.9 DD | 10 | 13 | 23 | 56.5% |
| 7-7.9 DD | 1 | 4 | 5 | 80% |
| 8-8.9 DD | 3 | 2 | 5 | 40% |

The probability of treatment decreased by 36.9% for each additional week of GA, if the rest of the variables remained constant.

## Binary Logistic Regression Between ROP Requiring Treatment and Duration of MV

The risk of requiring treatment for ROP is directly proportional to the days of duration of MV (Nagelkerke's $R^2$: 24.7%, $p < 0.001$) [OR:1.08 (CI: 1.049, 1.115), $p = 0.005$]. As in the previous section, following the logistic regression equations between ROP requiring treatment and the days of duration of MV:

$$\text{Probability of ROP requiring treatment} = 1/(1 + e^{-2.571+0.078(\text{days of MV})})$$

For each additional day of MV, the probability of ROP requiring treatment was increased by 8.1% provided the rest of the variables remained constant.

## Logistic Regression Between ROP Requiring Treatment and Temporal Avascular Area

A directly proportional relationship was observed between risk of ROP requiring treatment and size of retinal temporal avascular area. That is, larger avascular area was associated with greater risk of requiring treatment (Nagelkerke's $R^2 = 30.1\%$, $p < 0.001$; OR: 2.11, 95% CI: 1.648–2.701, $p = 0.005$). Again, following the logistic regression equations, we concluded that:

$$\text{Probability of ROP requiring treatment} = 1/(1 + e^{-4.640+0.746(\text{DD of avascular area})})$$

For every DD of additional retinal temporal avascular area in the premature newborn, the probability of requiring

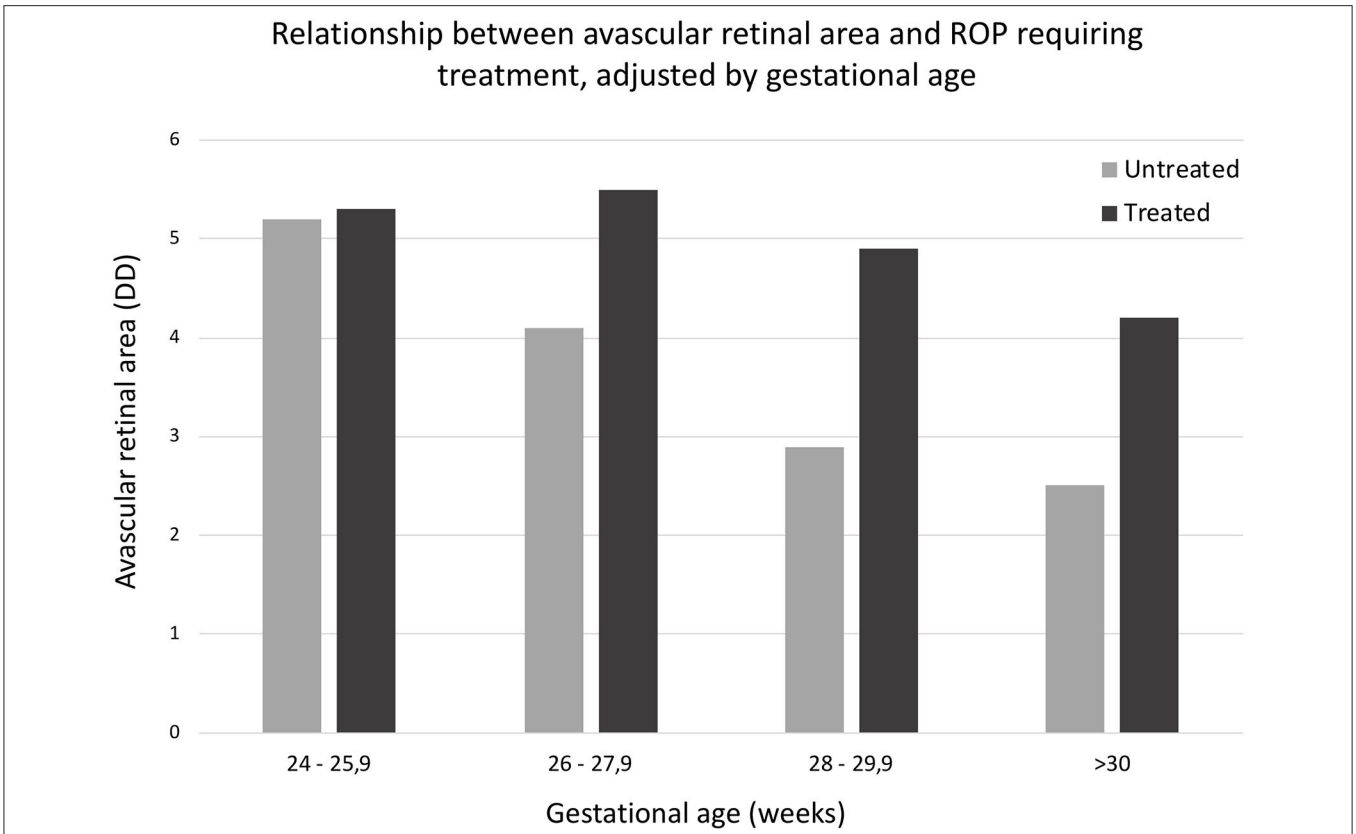


**FIGURE 1 |** Relationship between retinal avascular area and ROP requiring treatment, adjusted by gestational age. *U* Mann Whitney medium range differences: ROP no requiring treatment, 101.78; ROP requiring treatment, 178.08 ($p < 0.0001$).

**TABLE 4 |** Relationship between MV time and ROP requiring treatment. As the MV time increases, so does the percentage of individuals treated.

| Mechanical ventilation | ROP not treated | ROP treated | Total | % ROP treated |
|---|---|---|---|---|
| 0 days | 59 | 5 | 64 | 7.8% |
| 1–10 days | 89 | 9 | 98 | 9.2% |
| 11–20 days | 23 | 9 | 32 | 28.1% |
| 21–30 days | 16 | 7 | 23 | 30.4% |
| ≥ 31 days | 3 | 8 | 11 | 72.7% |

treatment increased by 111% provided the rest of the variables remained constant.

### Risk Calculator for ROP Requiring Treatment Based on the Retinal Temporal Avascular Area and MV Time

According to the multiple logistic regression model, longer MV time was significantly associated with greater avascular area and greater likelihood of ROP requiring treatment (Nagelkerke's $R^2 = 42.9\%$, $p < 0.001$; OR for avascular area in DD: 1.986, 95% CI: 1.527–2.582, $p = 0.005$; OR for MV time in days: 1.067, 95% CI: 1.033–1.101, $p = 0.005$).

$$\text{Probability of ROP requiring treatment} = 1/(1 + e^{-5.251+0.686(\text{avascularareainDD})+0.064(\text{MV time in days})})$$

On the basis of the equations of this analysis, we can conclude the results shown in **Table 5**. An automatic online calculator system for predicting ROP requiring treatment was created (available on: www.roprequiringtreatment.com).

The risk calculator sensitivity and specificity for predicting severe ROP requiring treatment, based on the levels of probability are shown in **Table 6**.

## DISCUSSION

The clinical examinations performed during the present study were carried out by a single pediatric ophthalmologist based on the guidelines of the screening program for ROP

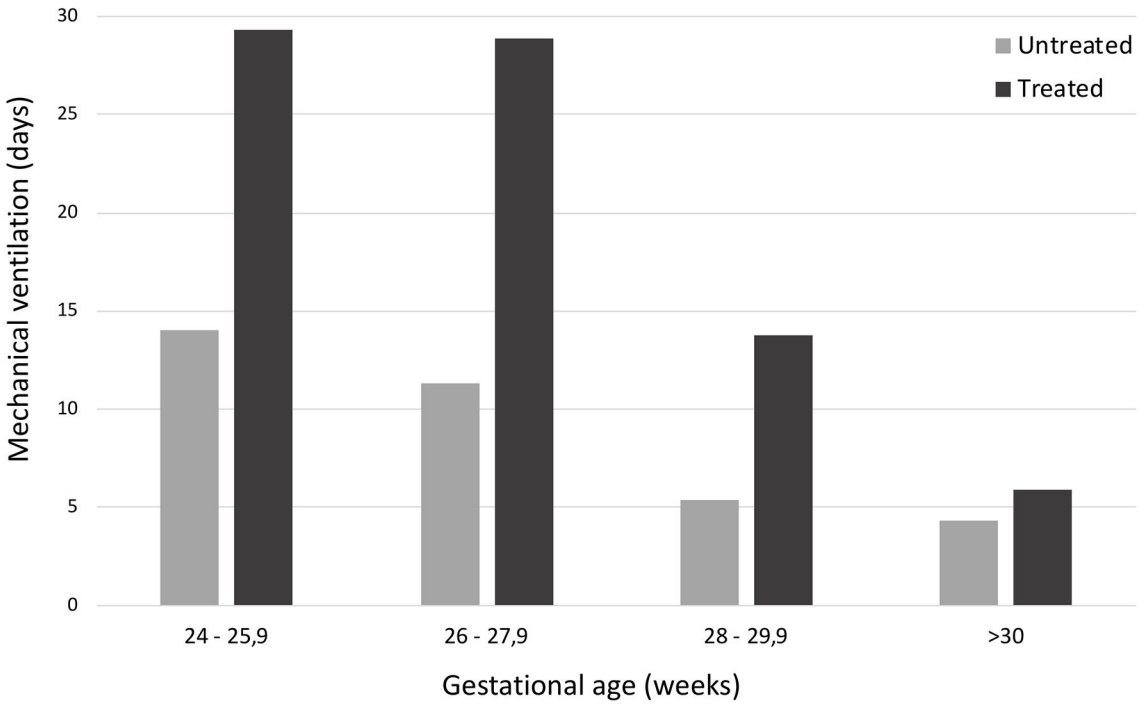


**FIGURE 2 |** Relationship between mechanical ventilation time and ROP requiring treatment, adjusted by gestational age. *U* Mann Whitney medium range differences: ROP no requiring treatment, 104.98; ROP requiring treatment, 162.09 ($p < 0.0001$).

**TABLE 5 |** Table for calculating risk of ROP requiring treatment based on the retinal temporal avascular area in DD and the MV time in days, according to the ET-ROP study (38, 39).

| Mechanical ventilation (days) | Avascular area (DD) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 0 | 0.52% | 1.03% | 2.03% | 3.94% | 7.54% | 13.93% | 24.32% | 38.96% | 55.90% | 71.57% | 83.33% |
| 5 | 0.72% | 1.41% | 2.77% | 5.35% | 10.09% | 18.23% | 30.68% | 46.78% | 63.58% | 77.61% | 87.31% |
| 10 | 0.98% | 1.94% | 3.77% | 7.22% | 13.39% | 23.49% | 37.87% | 54.76% | 70.62% | 82.68% | 90.46% |
| 15 | 1.35% | 2.65% | 5.12% | 9.68% | 17.55% | 29.71% | 45.64% | 62.50% | 76.80% | 86.80% | 92.88% |
| 20 | 1.85% | 3.61% | 6.92% | 12.86% | 22.67% | 36.80% | 53.62% | 69.66% | 82.01% | 90.05% | 94.73% |
| 25 | 2.53% | 4.90% | 9.29% | 16.90% | 28.76% | 44.50% | 61.42% | 75.97% | 86.26% | 92.57% | 96.12% |
| 30 | 3.45% | 6.63% | 12.36% | 21.87% | 35.73% | 52.47% | 68.68% | 81.32% | 89.63% | 94.50% | 97.15% |

*The percentages in green correspond to those subjects with a < 10% risk of requiring treatment, those in yellow correspond to those with a 10–15% risk, and those in red to those with a ≥ 15% risk.*

in Spain (40). Fundus examination was performed using indirect binocular ophthalmoscopy with scleral indentation, which is currently considered the gold standard technique for complete evaluation of the retina. Unlike other automated techniques such as the use of RetCam®, indirect binocular ophthalmoscopy with scleral indentation enables adequate evaluation of the peripheral retina, which is extremely important in the initial stages of ROP (41–43). However, this method does not allow photographic images to be recorded, as the RetCam® does. This limitation was partially resolved using real time video recordings of the images obtained by the binocular ophthalmoscope.

**TABLE 6 |** Model sensitivity and specificity for predicting severe ROP requiring treatment, based on the three different levels of probability in **Table 5** (low risk—green; moderate risk—yellow; high risk—red).

| Probability | Sensitivity | Specificity |
|---|---|---|
| <10% | 100% | 48% |
| 10–14.9% | 87% | 55% |
| ≥15% | 70% | 66% |

Examination instruments such as RetCam®, retinoscopy, or wide-field angiography have allowed repeated examination and analysis of retinal images in numerous studies (41–44). However, these techniques are rarely available in hospitals, so the indirect binocular ophthalmoscope continues to be used by numerous specialists. Many previous authors have measured the extent of retinal vascularization in patients with ROP to identify ROP stage and evaluate treatment efficacy (26, 45, 46).

Pediatric ophthalmology experts have a great ability to distinguish vascular dilation, ROP stages in poorly dilated eyes, and color and traction morphology of injuries. Several authors have previously measured the degree of retinal vascularization in patients with ROP to identify the ROP stage and evaluate treatment efficacy (26, 45–47). We believe that, with additional practice, indenting the temporal periphery retina and obtaining the avascular area in the DD observed during each neonatal screening should be easy. This information collected all throughout screening also allowed us to identify another progression marker: vascularization speed. Thus, insufficient vascularization speed of <0.5 DD is an indicator of ROP requiring treatment (48).

In our series, two children treated for ROP (5%) had a birth weight equal or above 1,501 g, or a gestational age of more than 30 weeks, implying that they would not have been detected by any predictive factor model, such as G-ROP (49). Two preterm infants with 2.5 DD of non-vascularized temporal retina were treated for ROP. Retinal vascularization of zone III without prior ROP of zone I or II must be accomplished to be discharged from the screening program (if screener has doubts regarding the zone, confirmatory tests may be justified) (50).

Postnatal weight gain was expressed in g/day and is the result of the weekly weight gain average measured during routine neonate screenings. After a thorough analysis of the literature (7, 10, 51–53), the measurement unit chosen for postnatal weight gain was g/day instead of g/kg/day. Newborns who are underweight at birth have been traditionally associated with a lower weight gain during neonatal period and delays in neurological development. However, a lower birth weight is not a pathogenic factor of retinopathy of prematurity (ROP) in itself, but rather, majority of cases reported in the literature are related to gestational factors (delays in fetal growth from maternal, placental or fetal causes, and prioritization of development of vital organs at the expense of other secondary structures, such as the retina) (54, 55) and newborn comorbidity, which can be the cause of lower birth weight (54, 56, 57).

Risk factors that could be related to or derived from treatment with oxygen (presence/absence of apnea and degree of bronchopulmonary dysplasia, which did not show significant differences between the groups with treated and untreated ROP) were excluded from the predictive model for calculating severe ROP requiring treatment, as these factors could be influenced by duration of mechanical ventilation (19, 29), which is indeed included in the model. It was also decided to exclude neonatal comorbidity (number of transfusions, sepsis, necrotizing enterocolitis, intraventricular hemorrhage, and periventricular leukomalacia) from the model (3, 31, 33, 37). In our study, statistically significant differences were found between the presence of those comorbidity factors and the degree of ROP. However, results described in the literature are heterogeneous, and extrapolating the prediction of this sample to other study populations that showed different results would be difficult. We believe that neonatal comorbidity must be valued individually, and that it is closely related to gestational age and birth weight.

A first model of logistic regression, including avascular area, duration of mechanical ventilation, gestational age and birth weight, was elaborated. Ninety-five percent (95%) confidence intervals deriving from this logistic regression for gestational age and birth weight were 0.74–1.37 and 0.99–1.01, respectively. As confidence intervals include the value 0, these were excluded from the calculator tool due to their low statistical significance value in the predictive model. This lack of significance could be the result of patients already being selected at the beginning of the study based on their gestational age and birth weight, according to the Screening Program for Retinopathy of Prematurity in Spain (40).

In our model, avascular area replaces gestational age given that both variables are strongly correlated, as the avascular area of the first test collects information on the pre and postnatal vascular delay. Thus, the calculator tool was developed based on two variables: avascular area and duration of mechanical ventilation. Gestational age and birth weight are the two most relevant variables considered to select newborns requiring screenings for ROP. Both variables are included in the Screening Program for Retinopathy of Prematurity in Spain (40), and most countries have their own screening criteria (40, 50, 58–60).

However, the calculator tool cannot be used to determine which neonate must be screened, but rather to customize the frequency of ophthalmological checkups. The calculation model's main use is to identify neonates that while participating in the screening program must be subject to more frequent screenings and a more intensified comorbidity control, due to a high risk of severe ROP requiring early treatment; or, neonates that can be subject to less frequent screenings due to low risk of severe ROP.

The simple design of the table based on only two variables allows for a quick and easy observation and interpretation by clinicians during their usual clinical practice, in case of unavailability of electronic device at hand. However, this calculator must be improved in the future as these variables only explain 41% of the outcome and must be validated through studies executed at other centers.

ROP has a hereditary component that ranges between 70 and 73% (61, 62). Furthermore, the vascularization of the choroid, vitreous, and retina occurs alongside the development of nutritional supply to these structures (63). In premature newborns, vascular development of the retina occurs parallel to neurological development (64). Genetics and the environment can interact during pregnancy, birth, or in the postnatal period. However, numerous factors, such as oxygen concentration, can influence vascular delay or inhibition of the retina during phase I of ROP. The resulting retinal ischemia may subsequently contribute to the development of neovessels during phase II of ROP (3).

Michaelson reported that the growth rate of the superficial retinal vessels was 100 μm/day in the human fetus (65). Factors such as low GA and other vascular development inhibitors may lead to a greater avascular area in the temporal retina (5, 48). Premature newborns who present with absence of vascularization in zone I at the first examination have a higher risk of ROP requiring treatment than those who present with absence of vascularization in zone II or III (38).

Various models of ROP screening, such as WINROP (7), CHOP-ROP (66), PINT-ROP (51), NED-ROP (67), and CO-ROP (68), have been proposed to identify newborns at risk of ROP and minimize unnecessary examinations in newborns at low risk. However, ROP cannot always be seen to its fullest extent at the first examination of the newborn (69). Therefore, once newborns are selected to undergo ophthalmological examination, clinicians must take into account the above risk factors, especially the avascular area (in DDs) and the MV time (in days). In so doing, at the first examination they will be able to identify newborns at higher risk of requiring treatment. Therefore, the risk calculation model presented in the present study may be useful for pediatric ophthalmologists during clinical practice.

The objective of this study differs from that of most studies published in the literature in a sense that it is not an ROP or severe ROP screening tool, but rather a tool to be used after the first screening, following the screening criteria of each country. By using the screening criteria of each center or country, newborns that are subjected to serial ophthalmologic screening are identified. In each country or center, screenings are carried out on a weekly or biweekly basis (40, 50), or customized to the severity of each newborn. By applying the model for predicting the risk of severe ROP requiring early treatment on the cohort of neonates, we will be able to adapt the periodicity of checkups.

Each country has its own ROP screening program based on gestational age, birth weight, and neonatal comorbidity, although almost all of them share common characteristics. Gestational age threshold is between week 30 (50, 58) and 32 (40, 59) for most countries, and birth weight is around 1,500 g (40, 50, 58, 59). However, in many developing countries, screening criteria are broader, reaching week 34 of pregnancy and 1,850 g of birth weight (60).

Previous studies carried out at our center on a preliminary patient cohort have shown the usefulness of measuring the vascular/avascular area (5, 48). Thus, vascularization speed from the temporal avascular area to the posterior pole below 0.5 DD/week has been identified as an independent risk factor for severe ROP requiring treatment (5). A study carried out at another center on a different patient cohort analyzed the impact of the risk factors for ROP and the effect of control measures of oxygen saturation levels implemented through the Oxygen With Love (OWL) program. This study revealed that the implementation of the OWL program had a significantly positive impact on the reduction of the number of days of tracheal intubation, and reduction of sepsis rates and extremely low postnatal weight gain (<7 g/day) (6).

Many severe ROP screening or early diagnosis tools have been described in the literature in the past few years. However, most of them are proposed as an initial screening tool to minimize screenings in newborns (7–10, 67, 68). whereas the model described in this study is proposed as a tool to be used on neonates who have already been selected for screenings, following the screening criteria of the country involved–in our case, Spain (40). Neonatal algorithms consider risk factors other than gestational age, postmenstrual age, birth weight, and weight gain. However, when models include ocular fundus data, other risk factors appear, such as pre-plus disease, or prolonged use of mechanical ventilator, which also contribute to a higher risk of guaranteed ROP (70).

The WINROP (Weight, IGF-I, Neonatal, ROP) study was carried out by Hellström et al. (71) to identify preterm newborns with risk of severe ROP early on and avoid unnecessary screenings on other neonates. Initially, this algorithm was based on birth weight, gestational age, and serum insulin-like growth factor-1 levels (IGF1) (7), which were later substituted by weekly weight gain (71, 72). This screening tool, like the calculator proposed by our team, can be checked online.

Another screening model, described with the purpose of identifying newborns that need ophthalmologic screenings due to risk of ROP, is the Children's Hospital of Philadelphia Retinopathy of Prematurity Risk Model. A total of 524 neonates of ≤30 weeks of pregnancy (WOP) or <1,501 g of weight were included in this retrospective study to determine, through multivariate logistic regression techniques, the cut-off point of each parameter that allowed an adequate identification of subjects with risk of type 1 or 2 ROP (8, 66).

On the other hand, the ROP Score algorithm, introduced by Eckert, Fortes Filho et al., was based on the aggregate risk of its risk factors. This algorithm was compared with the individual use of birth weight and gestational age in a cohort of 474 neonates of ≤1,500 g and/or ≤32 WOP. Risk factors included in the algorithm were the following: birth weight, gestational age, postnatal weight gain during the first 6 weeks, treatment with oxygen and need for blood transfusion in the first 6 weeks (9).

The Premature Infants in Need of Transfusion (PINT) study was created to analyze the effect of blood transfusions and level of hemoglobin on the morbidity and mortality of preterm infants (10). However, a model of early diagnosis in newborns with extremely low birth weight was developed from a patient cohort of the PINT study, based on gestational age, birth weight, and daily weight gain (51). The CO-ROP

model, developed at the University of Colorado, describes a new ROP screening algorithm based on birth weight, gestational age and postnatal weight gain. The algorithm included newborns of ≤30 WOP and ≤1,500 g at birth and a net weight gain of ≤650 g during the 1st month of postnatal life (68). The Netherlands Retinopathy of Prematurity Study Model (NEDROP) studied several ROP screening strategies and concluded that the strategy that most efficiently diagnosed ROP was the one including newborns of ≤30 WOP and ≤1,250 g of birth weight and neonates of gestational age between 30–32 WOP and 1,250–1,500 g of birth weight, with at least one risk factor (treatment with oxygen or corticosteroids, sepsis, necrotizing enterocolitis, or heart disease) (73).

Gopal suspects intuitively that the extension of the avascular retina at birth should be a predictive factor for the need for treatment of ROP (74). Meanwhile, Jayadev classifies the temporal avascular area of the retina into three degrees: (a) mild: vessels reach posterior zone III; (b) moderate: vessels enter anterior zone II; (c) severe: vessels in posterior zone I or zone II. Thus, type 1 and type 2 ROP are predicted during the first screening visit (27). Jayadev and we have determined that the avascular area of the retina is the main risk factor for ROP requiring treatment. Jayadev's multivariate analysis also includes gestational age, birth weight and sex, whereas our new model includes duration of mechanical ventilation.

### Limitations of the Study

As previously mentioned, our examination method did not allow quality photographic images to be collected. However, we were able to capture the examination images obtained. The examinations were performed by a single pediatric ophthalmologist during routine clinical practice, and the images or scans were not repeated or analyzed by multiple observers. On the other hand, over the course of the study, from 2009 to 2012, the oxygen therapy protocols underwent a drastic change, because the Oxygen with Love (OWL) program was implemented in the NICU. The application of this program significantly reduced the duration of MV, and therefore the rates of sepsis and ROP (6).

One of the strengths of our study is the wide sampling size, which provides more reliable results. However, one of the limitations is that more studies are needed to confirm the results and validate them in other populations. Additionally, this calculator model does not include every ROP risk factor, for the reasons stated above.

## CONCLUSION

Premature newborns should be evaluated in a multifactorial manner, taking into account all ROP risk factors, and the neonatal comorbidities such as presence of sepsis, apnea, bronchopulmonary dysplasia, arteriovenous ductus, and blood transfusions. The MV time and the extent of the retinal avascular area in the first examination are crucial for the development and evolution of ROP, so both should be taken into account when predicting ROP severity and the need for treatment. The risk calculator can identify the premature newborns with risk of ROP that requires treatment from their first examination in order to adapt the frequency of examinations according to the level of risk.

The calculator is not a substitute for ROP screening programs or gestational age and birth weight thresholds in each country. It is rather an additional tool to be used for neonates who are already included in the screening to identify those needing closer follow-up or special care. More studies involving a larger number of patients, different centers and a specific cohort of individuals with extremely-low birth weight are needed to confirm and validate these results.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Biomedical Research Ethics Committee of Andalusia. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

All authors have contributed significantly to the work, have read the manuscript, attest to the validity, legitimacy of the data, its interpretation, and agree to its submission to this journal.

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MINI REVIEW
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# Individualized Hemodynamic Management in Newborns

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In our aim to improve patient outcome we are transitioning from a "one-size-fits-all" protocolized approach toward an individualized hemodynamic management, that is tailored to the cardiovascular (patho-)physiology and the specific clinical characteristics of each individual patient. In this narrative review an overview is provided about an individualized approach toward various neonatal hemodynamic conditions.

**Keywords: hemodynamics, individualized medicine, neonatology, shock, hypotension, patent ductus arteriosis, persistent pulmonary hypertension in the newborn (PPHN)**

## INTRODUCTION

Newborns admitted to the neonatal intensive care unit have an increased risk of hemodynamic failure for several reasons. The immaturity of the cardiovascular system in preterm infants, combined with impaired compensatory mechanisms, makes them prone to injury related to hypoperfusion and hypoxemia. Many conditions may affect the heart and vasculature, for example perinatal hypoxia-ischemia, sepsis, necrotizing enterocolitis, air leakage syndromes, and congenital anomalies, such as structural heart defects and congenital diaphragmatic hernia. Moreover, well-intended therapeutic interventions could potentially have adverse hemodynamic effects, for instance invasive ventilation with high mean airway pressure impeding venous return, iatrogenic tachycardia secondary to chronotropic properties of cardiovascular drugs limiting the filling phase (preload) of the heart, and drug-induced systemic vasodilation in case of sedation, analgesia, and muscle relaxation. It is therefor imperative that cardiovascular disturbances are detected as soon as possible or preferably prevented. The fact is, however, that this is easier said than done. It has been shown that severe systemic hypoperfusion can remain clinically undetected, irrespective of the level of experience of the health care professional (1, 2). This is probably related to the fact that hemodynamic monitoring and management is generally based on the interpretation of indirect markers of systemic perfusion, such as heart rate, blood pressure, urine output, blood gas analysis, capillary refill time, "peripheral perfusion" et cetera (3). For decades hemodynamic management could be classified as a "pressure-based approach," in which the presence of hypoperfusion was falsely predominantly evaluated by monitoring of blood pressure. This has resulted in a "one-size-fits-all" protocol with hypotension as main criterium to intervene, that has been used by many (4).

Nowadays, we are transitioning from an empirical approach via stratified medicine toward an individualized hemodynamic management (**Figure 1**). Not all newborn infants with hemodynamic instability are treated in a similar ("protocolized") manner (**Figure 1A**), since we stratify patients based upon presumed underlying pathophysiological mechanisms (diagnosis) and adapt our therapeutic interventions accordingly (**Figure 1B**). For example, systemic hypotension is treated different in an extreme preterm infant in transition, than in a full-term newborn with perinatal hypoxic-ischemic encephalopathy or congenital diaphragmatic hernia, or in a patient after surgery for severe necrotizing enterocolitis. However, not all patients within the same diagnostic

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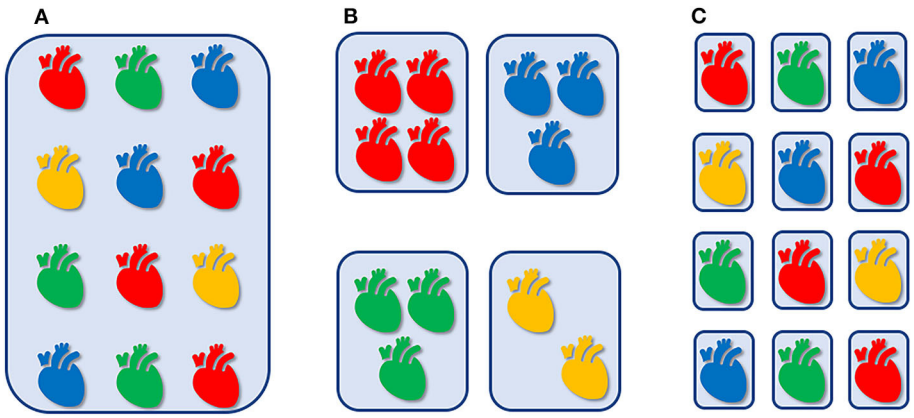


**FIGURE 1 |** Empirical, "one-size-fits-all" approach, irrespective of cardiovascular physiology and clinical context **(A)**. Hemodynamic management that is stratified by presumed underlying pathophysiology **(B)**. Individualized hemodynamic management that is tailored to the cardiovascular (patho-)physiology and the specific clinical characteristics of each individual patient **(C)**.

category necessarily share similar etiologic factors related to alike hemodynamic disturbances. As an example, systemic hypotension in a septic patient could be caused by either myocardial impairment or systemic vasodilation secondary to cardiodepressive or vasoplegic action of cytokines, respectively, requiring a totally different cardiovascular intervention. In other words, an individualized hemodynamic management is indicated, that is tailored to the cardiovascular (patho-)physiology and the specific clinical characteristics of each individual patient (**Figure 1C**).

Several aspects of personalized hemodynamic management will be discussed for patients with highly prevalent hemodynamic conditions, namely persistent patent ductus arteriosus (PDA), shock/hypotension, and persistent pulmonary hypertension of the newborn (PPHN).

## PATENT DUCTUS ARTERIOSUS

One of the most controversial issues in neonatology is whether or not a persistent PDA should be treated. Although a PDA is associated with serious adverse outcomes, such as mortality, pulmonary hemorrhage, bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis, firm evidence is currently still lacking for a causal relationship, despite many clinical trials. The question remains whether a PDA should be considered a binary entity in (extreme) preterm infants, that should either be treated and actively closed or can be managed conservatively awaiting spontaneous closure (5). Given the high spontaneous closure rate in preterm infants born after more than 28 weeks' gestation, there is generally no routine screening of the PDA performed in this subpopulation. From a pathophysiological perspective it would be highly informative to objectively quantify the transductal left-to-right shunt volume, since this would enable triage of preterm infants and identify those that could benefit from active medical closure of the ductus arteriosus. Higher transductal shunt volumes are associated with increased pulmonary and decreased systemic perfusion, that possibly explains associated morbidities as pulmonary hemorrhage, high need for respiratory support, bronchopulmonary dysplasia on the one hand and necrotizing enterocolitis, cerebral injury on the other hand. Neonatologist Performed Echocardiography (NPE) is used to estimate the degree of transductal shunt volume and determine the hemodynamic significance of the PDA (6, 7). Several scoring systems, incorporating clinical, and/or echocardiographic characteristics, have been published to stage the severity (hemodynamic significance) of the PDA (8–10). These scoring systems are used to estimate the risk of mortality or chronic lung disease in preterm infants with a PDA with a positive and negative predictive value of 92 and 82%, respectively (9).

When it is decided to treat a PDA in a preterm infant, NPE can be used to monitor the effect of the therapeutic intervention. It has been shown that the intensity of treatment with cyclooxygenase-inhibitors can safely be reduced by evaluating the vasoconstrictive action on the ductus arteriosus. Su et al. performed a non-blinded randomized controlled trial of conventional vs. echocardiography-guided treatment of a PDA with indomethacin in 93 preterm infants $<$1,500 g (11). In the echocardiography-guided study arm, an additional dose of indomethacin was only given if the transductal flow pattern persisted to be classified as "pulsatile" or "growing," suggestive of high transductal shunt volume. This resulted in a significant reduction in number of doses indomethacin (3.2 $\pm$ 1.4 vs. 1.6 $\pm$ 0.9 doses; $p < 0.01$) and less side-effects without causing a difference in ductal closure rate or obvious effects on mortality or incidence of chronic lung disease. In two similar studies, a subsequent dose of cyclooxygenase-inhibitor was only prescribed when ductal constriction was considered insufficient by evaluating the change in transductal diameter (12, 13). In the first, a next dose of indomethacin was only administered if the transductal diameter was more than 1.6 mm. This approach reduced the total number of doses indomethacin

from a median of three to only one without any increase in the incidence of ductal closure failure, ductal reopening, surgical ligation, pulmonary hemorrhage, chronic lung disease, necrotizing enterocolitis, or renal failure (12). Bravo et al. showed in a randomized trial that echocardiography-guided treatment with ibuprofen, i.e., only administration of a next dose of ibuprofen if the transductal diameter measured more than 1.5 mm, reduced the number of doses from a median of three [IQR 3–4] to two [IQR 1–5.7] (13). No differences in ductal closure failure, mortality of major neonatal morbidity was observed.

In addition to classification of the severity of transductal shunt volume and optimization of dosing schemes of indomethacin or ibuprofen, NPE is helpful in anticipating and identifying of complications of surgical ligation of the ductus arteriosus, such as post ligation cardiac syndrome (PLCS). PLCS is characterized by systemic hypotension secondary to myocardial impairment, oxygenation failure, and increased need of ventilatory support. Jain et al. observed that a left ventricular output <200 mL/kg/min in patients 1 h after ductal ligation was predictive of low cardiac output 8 h post-surgery (sensitivity (Se) 1.0; specificity (Sp) 0.89), systemic hypotension (Se 0.83; Sp 0.72), and need for inotropes (Se 1.0; Sp 0.65) (14). In a subsequent epoch study echocardiography was used to anticipate on PLCS and if a LVO <200 mL/kg/min was measured 1 h after ligation, milrinone was started in an attempt to prevent a low cardiac output state (14). This echocardiography-guided management resulted in a lower incidence of ventilatory failure (15 vs. 48%; $p < 0.05$), less inotropic support (19 vs. 56%; $p < 0.05$), and a trend toward less oxygenation failure (26 vs. 52%; $p = 0.08$). Despite the prophylactic use of milrinone, it was observed that about 50% of patients still developed PLCS, reason why other echocardiographic parameters are studied as potential preemptive indicators of myocardial impairment. For example, a prolonged isovolumic relaxation time (IVRT) in the preoperative phase, indicating myocardial diastolic dysfunction, is associated with an increased risk of PLCS (15).

As illustrated, hemodynamic management of preterm infants with a PDA can be adapted according the specific cardiovascular physiology and clinical context of the individual patient. In this way it is possible (1) to predict the hemodynamic significance of the PDA, that is associated with an increased risk of chronic lung disease and mortality, (2) to potentially reduce the number of doses of cyclooxygenase-inhibitors, and (3) to anticipate and prevent complications after surgical ligation of the ductus arteriosus.

## SHOCK/HYPOTENSION

The terms shock and hypotension are obviously not interchangeable. Shock is defined as a condition characterized by an imbalance between oxygen demand and delivery on a cellular level. Oxygen delivery is mainly determined by hemoglobin concentration, arterial oxygen saturation, and cardiac output. When the amount of oxygen delivered to the peripheral tissues doesn't fulfill the demands, compensatory mechanisms will aim to redistribute blood to the vital organs in an attempt to prevent these from hypoxemic-ischemic injury. Three stages of shock are distinguished, namely (1) compensated shock, (2) uncompensated shock, and (3) irreversible shock. In the first stage, neuroendocrine compensatory mechanisms are responsible for preservation of blood (and oxygen) supply to the brain, the heart and the adrenal glands. This phase is called the compensated shock and is characterized by selective constriction of the vasculature perfusing the non-vital organs, such as kidneys, intestines, muscles, liver and skin. By the counteracting effects of constriction of non-vital organ vasculature and dilation of vessels supplying the vital organs, blood pressure can remain unaffected. There is no linear relation between cardiac output and blood pressure, as can be understood from Hagen-Poiseuille's Law. According this law, cardiac output is determined by the pressure gradient (arterial blood pressure minus right atrial pressure) divided by the systemic vascular resistance. This means that blood pressure can be in the normal (or even high) range during a state of low cardiac output provided that vascular resistance is increased. Systemic hypotension will occur when compensatory vasoregulatory mechanisms fail, known as the uncompensated phase of shock. Relying solely on the level of blood pressure, a "pressure-based" approach, might lead to misinterpretation of the hemodynamic status of newborns and the initial, compensated phase of shock might be missed. Neglecting the possibility of the presence of a compensated shock might have detrimental effects. There are indications that in fetuses and very preterm infants the blood vessels perfusing the cortex are not acting as high-priority vasculature and therefore not dilating in response to decreased blood flow, as would be expected for a vital organ as the brain (16–18). One might speculate that this observation could be (partially) responsible for the fact that survival in (extreme) preterm infants is increasing without an equivalent reduction in neurodevelopmental impairment (19–23).

Hence, it is imperative to detect low cardiac output already in the compensated stage of shock and not wait for intervention after systemic hypotension occurs, suggestive for the uncompensated stage of shock. This requires comprehensive hemodynamic monitoring with at least simultaneous measurement of arterial blood pressure and cardiac output. Under normal physiological circumstances both systemic blood flow and blood pressure are in the normal reference range. The combination of low cardiac output with a normal or high blood pressure suggest a compensated shock, whereas in a uncompensated shock both blood pressure and cardiac output are in the lower ranges (24). Measurement of cardiac output is feasible and applicable in (preterm) newborn infants, such as transthoracic echocardiography, electrical biosensing technologies (bioimpedance and bioreactance), and transpulmonary ultrasound dilution (25). Interpretation of both cardiac output and arterial blood pressure enables the selection of the optimal cardiovascular drug that is based on the presumed etiology of impaired perfusion and oxygenation. For this it is imperative that the clinician is aware of the pharmacokinetic and pharmacodynamic characteristics of the available drugs. Algorithms are available for this purpose (24). Comprehensive hemodynamic monitoring has been shown to potentially improve outcome. Integrating clinical assessment with NPE and regional (cerebral and intestinal) oxygenation monitoring using

near infrared spectroscopy resulted in a shorter time to clinical recovery in critically ill infants with cardiovascular compromise (26, 27).

## PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN

Increased pulmonary blood pressure can be the consequence of either an increase in pulmonary blood flow (e.g., congenital heart disease with large left-to-right shunting), pulmonary wedge pressure (left ventricular failure; pulmonary vein stenosis), or pulmonary vascular resistance. Increased pulmonary vascular resistance is caused by maladaptation (reactive vasoconstriction on various stimuli), maldevelopment (remodeling of blood vessels), or underdevelopment (hypoplasia) of the pulmonary vasculature. The typical clinical presentation of a patient with PPHN is hypoxemic respiratory failure with sometimes signs of transductal right-to-left shunting (difference in pre- and postductal oxygen saturation $\geq$5%) and systemic hypotension. PPHN is associated with both left and right ventricular failure (28–32). Increased right ventricular (RV) afterload potentially leads to impaired systolic and diastolic myocardial impairment. Left ventricular (LV) preload is impeded by dilation of the right ventricle, deceased right ventricular output and right-to-left transductal shunting, resulting in decreased left ventricular output. Hypoxia and acidosis may further deteriorate myocardial performance. It is important to be informed about (imminent) ventricular failure in patients with PPHN, since treating systemic hypotension by increasing systemic vascular resistance might further impair myocardial performance, resulting in low cardiac output. Sehgal et al. found biventricular failure (ventricular output <150 mL/kg/min) in 67% of patients with PPHN (RV and LV failure in 64 and 76%, respectively) (29). In 70% of these patients signs of RV diastolic dysfunction was observed. NPE can be used to assess the severity of PPHN and estimate the risk of mortality or need for support with extracorporeal membrane oxygenation (ECMO) in order to timely transfer the patient to an ECMO center. Aggarwal studied the predictive value of the S/D-ratio, an indicator of systolic and diastolic global RV function reflecting ventricular loading and contractility (30). This ratio is calculated from the Doppler signal of tricuspid regurgitation, dividing the systolic duration by the diastolic duration within one heart cycle. An RV S/D>1.3 is associated with mortality or need for ECMO in patients with PPHN with a positive and negative predictive value of 68 and 81%, respectively. Advanced echocardiographic modalities can also stratify PPHN patients and predict adverse outcomes. In a retrospective study in 86 patients born after $\geq$ 35 weeks' gestation, diminished tricuspid annular plane systolic excursion (TAPSE) <4 mm and global longitudinal peak strain (GLPS) $\geq$9% was associated with the need for ECMO of risk of death.

In a case report pulmonary vasodilation could successfully be evaluated in a patient with pulmonary hypertension with RV failure with the use of speckle tracking echocardiography (33). In a retrospective observational study in 20 preterm infants with prolonged preterm rupture of membranes (PPROM) before 24 weeks' gestation, Shah and Kluckow showed that NPE identified the presence of PPHN earlier and enabled targeted treatment with nitric oxide inhalation (iNO) (34). This approach resulted in improved survival in this severely affected population. It is known that not all patient with congenital diaphragmatic hernia (CDH) respond successfully to iNO and its use is discouraged, based on the results of the NINOS trial, in which an increased mortality and ECMO requirement was observed in the iNO-treated patients (35). However, a recent study showed that the non-response to iNO in patients with CDH might be related to the presence of LV failure (36). Hypoplasia or myocardial dysfunction of the LV is more often observed in newborn infants with CDH. Decreasing pulmonary vascular resistance with iNO will augment pulmonary venous return, that might further deteriorate LV function. It was concluded that iNO treatment improved oxygenation and reduced the need for support with ECMO in CDH patients with preserved LV systolic function (36).

Being informed about myocardial performance is important in patients with PPHN, since hemodynamic management will be quite different in the presence of ventricular failure.

## INDIVIDUALIZED HEMODYNAMIC MANAGEMENT

The clinical scenarios discussed above illustrate the importance of a comprehensive hemodynamic assessment in order to timely diagnose cardiovascular failure, elucidate underlying pathophysiology, guide hemodynamic therapy, monitor the effects of the interventions, and adjust treatment as appropriate. This enables an individualized hemodynamic management that is optimized for the patient's specific pathophysiology and clinical situation. However, this personalized hemodynamic approach is more complex than a strict protocolized management and requiring more complicated algorithms. Moreover, accurate and precise monitoring of neonatal hemodynamics, using for example neonatologist performed echocardiography (NPE), non-invasive cardiac output assessment and near infrared spectroscopy (NIRS), is challenging (25). The implementation of individualized hemodynamic management in neonatal intensive care is more difficult and associated with higher variability in care (37). Nevertheless, our aim is to provide the best treatment for our patients, that takes into account the specific characteristics and cardiovascular physiology of the individual patient with the use of personal, adaptive target values for different hemodynamic variables. High quality trials are warranted to provide evidence for superiority of individualized hemodynamic management regarding short- and long-term outcomes.

## AUTHOR CONTRIBUTIONS

This minireview is written by WB.

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REVIEW
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# Perspectives on Probiotics and Bronchopulmonary Dysplasia

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Bronchopulmonary dysplasia (BPD) is a chronic respiratory disease of preterm infants, associated with high morbidity and hospitalization expenses. With the revolutionary advances in microbiological analysis technology, increasing evidence indicates that children with BPD are affected by lung microbiota dysbiosis, which may be related to the illness occurrence and progression. However, dysbiosis treatment in BPD patients has not been fully investigated. Probiotics are living microorganisms known to improve human health for their anti-inflammatory and anti-tumor effects, and particularly by balancing gut microbiota composition, which promotes gut-lung axis recovery. The aim of the present review is to examine current evidence of lung microbiota dysbiosis and explore potential applications of probiotics in BPD, which may provide new insights into treatment strategies of this disease.

**Keywords: bronchopulmonary dysplasia, probiotics, gut-lung axis, microbiota dysbiosis, inflammation**

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## INTRODUCTION

For more than 50 years, the definition, epidemiology, pathophysiology, and pathogenesis of BPD have been continuously updated (1–4). BPD was initially proposed by Northway and colleagues (5) in 1967 as a lung injury in preterm neonates due to mechanical ventilation and oxygen poisoning. However, current pathogenesis is more complex, involving exposure of infants to one or multiple pre- and/or post-natal high-risk events associated with lung immaturity, perinatal infection, inflammation, and altered blood vessel development (1, 2, 4, 6) (**Figure 1**).

The term probiotics derived from a Greek meaning "for life" (7), was coined by Lilly and Stillwell (8) in 1965, and described microorganisms with potential to release growth-promoting factors. In 2002, the FAO/WHO defined probiotics as "*live microorganisms which when administered in adequate amounts confer a health benefit on the host,*" but in 2013, The International Scientific Association for Probiotics and Prebiotics reached a consensus on modifying the concept to "*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host,*" including microbial species that have been shown to confer health benefits in controlled studies, and new commensal and consortium strains from human samples, with evidence of safety and efficacy (9). At present, commonly used probiotics derive from the *Bifidobacterium* and *Lactobacillus* genus.

In recent years, a large body of evidence has related BPD to microbiota dysbiosis, however, therapy approaches have rarely been discussed or investigated. Therefore, this review explores new frontiers of probiotics to ameliorate BPD based on microbiota recovery.

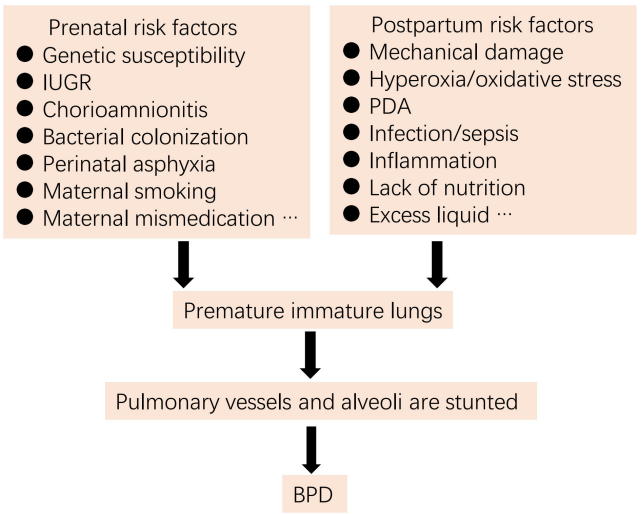


**FIGURE 1 |** Risk factors for BPD (IUGR, intrauterine growth restriction; PDA, patent ductus arteriosus).

## LUNG MICROBIOTA AND BPD

Microorganisms living in a given environment constitute a microbiota, whereas a microbiome relates to microbial genome, metabolism, and growth surroundings (10). It then becomes relevant to understand the impact of microbiota on human health and disease.

### Airway Microbiota Dysbiosis in BPD

Contrary to conventional wisdom, the human respiratory tract immediately acquires microbiota, detecting a low bacterial DNA load before or shortly after birth (11–13), which gradually builds up by the 1st month of life, developing colonization of relatively-stable bacteria at the phylum level (14). In this regard, different individuals and even distinct anatomical parts have their unique bacterial colonization patterns, and microbiota in each zone has its preponderant operational taxonomic units (OTUs) (14). Elucidating lung microbiota composition is a challenge, since it requires highly sensitive detection methods and reagents, and collected samples are susceptible to contamination by pathogenic microorganisms in the upper respiratory tract, such as oropharynx and nasopharynx (15). Furthermore, pre- and post-natal lung microbiota is compromised because of the use of antibiotics, maternal chorioamnionitis, mechanical ventilation, infection/inflammation, nutritional deficiencies, and abnormal colonization of the intestine (16). For example, antibiotics enhance the invasive potential of pathogenic bacteria by increasing their nutrient requirements such as organic acids, carbon, and nitrogen, thereby temporarily or permanently reducing the diversity and richness of the microbiota (17). As a result, some advances in research have generated inconsistent results in reporting lung microbiota at birth. However, in general terms, in a period after birth, the pulmonary microbiota composition at the phylum level is predominantly *Proteobacteria* and *Firmicutes* (12, 14).

The incipient composition of the lung microbiota cannot be neglected because it is closely related to the onset of mucosal immunity (18), the development of immune tolerance in the lungs (19), and healthy breathing (15). Several reports have shown alterations in the stability and diversity of the respiratory tract microbiota in BPD. Lohmann et al. (11) tested tracheal aspirates of 25 premature infants at different periods and showed that bacterial multiplicity of 10 patients with BPD significantly decreased, according to the observed species count and the Shannon index. As the disease progresses, *Firmicutes* and *Proteobacteria* populations increase and decrease respectively; at the genus level, the relative abundance of *Acinetobacter sp*. significantly decreases, whereas that of *Staphylococcus* and *Klebsiella* increases. However, microbiota in the non-BPD group invariably maintains high diversity and stability, indicating that lower diversity of airway microbiota may be associated with the disease.

A study by Lal et al. (12) showed that at the phylum level, the *Proteobacteria* amount was higher than that of *Firmicutes* and *Fusobacteria* in babies with BPD, whereas at the genus-level, *Lactobacillus* content was significantly low, which persisted during the disease. The reduction of *Lactobacillus* leading to an inflammatory response and consequently interfering with lung development is an important reason why the microbiota directly affects BPD, as many studies have shown that *Lactobacillus* possesses very strong anti-inflammatory properties. Furthermore, increased endotoxin was observed in the airways of patients with BPD, which was attributed to microbiota dysbiosis.

Lohmann et al. (11) and Lal et al. (12) put forward that conflicting results related to changes in the populations at the phylum level may indicate that lung microbiota is affected by multiple factors, including demographic characteristics, geographic position, living environment, methods and detection reagents, and sequencing platforms. In fact, both studies differed in criteria for the inclusion of children and timing of the sample collection. However, the ecological imbalance of the airway microbiota in children with BPD is an important characteristic in such investigations.

Imamura et al. (20) examined 169 infants with or without severe BPD and noted that all patients with the disease exhibited maladaptive changes in the lung microbiota. The detection rate of *Corynebacterium* species in the lower respiratory tract of severe BPD was higher than that of non-severe disease, and sepsis was commonly observed seven days after birth, speculating that airway microbiota dysbiosis is associated with infections, which may be a significant cause of BPD exacerbation.

Another longitudinal investigation of two research centers found that preterm infants with severe BPD contained abundant *Ureaplasma* after birth, and diversity in lung microbiota was more prominent with age (13). *Ureaplasma* colonization is considered an independent risk factor for BPD (21). On the one hand, it causes chronic infection of the uterine cavity to promote preterm delivery (22), whereas preterm birth is one of the most important causes of BPD. Furthermore, *Ureaplasma* damages the respiratory mucosa and interferes with lung development

by producing virulence factors and stimulating the release of pro-inflammatory mediators. Importantly, colonization of the respiratory tract by this species is negatively correlated with gestational age (22), which may partly explain the higher risk of BPD in preterm infants with low gestational age.

In addition, a recent systematic review of microbiota and BPD reported that microbiome disorders are present in patient airways and the frequency of microbiota transformation was associated with BPD impairment (23).

Taken together, lung microbiota composition establishes early in life, but the stabilization and diversity of microorganisms are altered by a number of factors, which are particularly relevant in children with BPD. Therefore, it becomes important to the relationship between microbiome dysregulation and this disease.

## BPD and Lung Microbiota Dysbiosis Association

Although the causal link between lung microbiota and BPD has not been fully demonstrated, it can be assumed that the lung maladaptive microbiome is an effective driver for other potentially harmful factors in this illness (24). Dysregulation of the lung microbiota elicits local or systemic infection/inflammation, activates the immune response (25–27), and is related to oxidative stress and metabolic disorders in the host (24), which impairs dysbiosis, thus creating a vicious circle involved in the disease onset and exacerbation.

### Gut-lung Axis in BPD

Lung microbiota dysbiosis in BPD may disrupt gut microbiota, which probably plays an important role in worsening this disease. Ryan et al. (28) analyzed stool samples of 50 preterm BPD infants and observed that among transvaginal babies, *Escherichia* and *Shigella* were significantly increased, whereas *Klebsiella* and *Salmonella* were in lower amounts, demonstrating that the disease is involved in gut microbiota dysbiosis. Furthermore, a case-control study of eight subjects with BPD and 10 subjects without the disease showed that gut microbiota diversity in the BPD group was significantly reduced (OTU, relative abundance, and Shannon index), and severe BPD may make gut microbiota more susceptible to destruction early in life (29).

In turn, gut microbiota dysbiosis also affects BPD. Cantey et al. (30) reported that for infants with a very low birth weight receiving antibiotics for 2 weeks significantly increased the risk of death or BPD, which still exists after controlling the severity of the disease. Furthermore, this risk increases with the duration of antibiotic exposure [i.e., every additional day of antibiotics increases the risk of BPD by ∼13% (30)]. A possible explanation is that antibiotics destroy the gut microbiota, and the dysbiosis exacerbates the disease. In a perinatal antibiotics-exposed BPD mouse model, it was shown that gut maladaptive microbiota increased pulmonary fibrosis and worsened the condition, which may be related to the reduced expression of lung IL-22 caused by gut microbiota dysbiosis (31). Moreover, gut microbiota, which controls trimethylamine N-oxide (TMAO) production (32), regulates BPD susceptibility by changing TMAO levels (33).

A growing body of research indicates an apparent bidirectional influence of intestinal and lung microbiota. The gut-lung axis hypothesis, which involves a complex cross-talk between lung/gut disease and gut/lung microbiota dysbiosis, has been extensively tested (34–37). Newborn mice that have been depleted of gut microbiota with antibiotics become more susceptible to *Streptococcus pneumoniae* infection, however, restoring intestinal microbiota increases neonatal and germ-free (GF) mice resistance (38) and potentiates phagocytosis of alveolar macrophages (39), thus protecting lungs from bacterial infection. In addition, two separate studies on severe pneumonia and childhood community-acquired pneumonia have shown that maladaptive gut microbiota may be relevant in the onset and development of pneumonia (40, 41). Moreover, the administration of lipopolysaccharide (LPS) in mouse lungs significantly alters gut microbiota (42).

Influenza virus-infected mice develop gut microbiota dysbiosis earlier, which reduces the secretion of metabolites by intestinal microorganisms, thus increasing the susceptibility of mice to *S. pneumoniae* infection (43), suggesting a close link between gut microbiota and the lung immune response. Dickson et al. (44) demonstrated live intestinal bacteria in lungs in a murine model of sepsis and in bronchoalveolar lavage fluid (BALF) of 68 patients with acute respiratory distress syndrome, which indicates that local or systemic inflammation mediates gut microbiota displacement, disrupting the lung microecology homeostasis, and in turn, dysregulated lung microbiota exacerbates the inflammatory response. These data suggest an important interrelationship between gut and lung microbiota, and BPD onset and development. Microbiota dysbiosis in these organs may trigger the inflammatory process, leading to immune disorders and exacerbating the disease outcome. Therefore, preserving the intestines and lungs microecological balance significantly improves BPD.

### Infection/Inflammation

Infection/inflammation may play a central role in BPD pathogenesis. Stressmann et al. (45) observed a number of pathogens (particularly *S. aureus*, *Enterobacter* sp., *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, and *Streptococcus* sp.) in the tracheal secretions of eight preterm infants at risk of developing BPD. Another investigation of 192 newborns found that lung infections in early newborns (particularly in the first 3 days of life) were related to the evolution of chronic lung disease (46). Under a combined effect of other BPD risk factors such as hyperoxia and mechanical ventilation, infection triggers a series of pro-inflammatory substances, such as IL-1β, IL-6, IL-8, NLRP3, TNF-α, and collagen I, which are further regulated by infiltrating neutrophils and macrophages (47–49). These inflammatory mediators in immature lungs of premature infants restrict the activity of surfactant proteins and the vascular endothelial growth factor (49), contributing to the development of alveolar and vascular alterations and other characteristic pathologies in BPD. Furthermore, TLR binding-induced reactive oxygen species (ROS) activate the NLRP3/caspase-1 pathway, which promotes IL-1b production (47), amplifying the inflammation process.

Microbiota dysbiosis may stimulate a robust inflammatory response and have a significant impact on BPD (**Figure 2**).

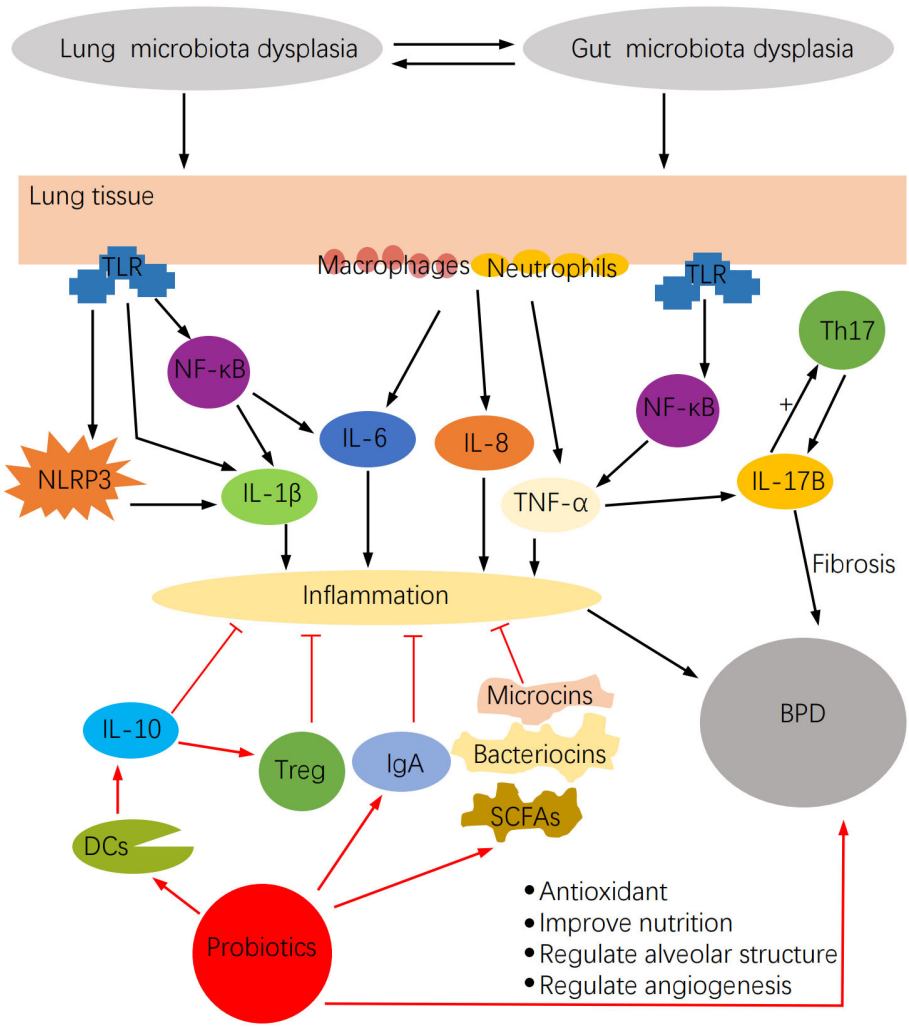


**FIGURE 2 |** The impact of microbiota dysbiosis on BPD and the potential function of probiotics in BPD.

Lung microbiota dysbiosis triggers the release of the pro-inflammatory cytokines IL-1β, IL-6, MIP-1α, IL-12p70, and CXCL8, which are associated with pulmonary fibrosis (50). Among them, IL-6 increases with the rise of *Firmicutes* richness, but IL-12P70 augments with the reduction of *Proteobacteria* richness. Similarly, a series of cytokines such as IFN-α2, IL-13, IL-4, IL-15, TNF-α, TNF-β, and MCP1 were detected in the BALF of hematopoietic cell transplantation recipients, and changes in their concentration were mostly associated with lung microbiota dysbiosis (51). In this regard, IFN-α2, IL-13, TNF-β, and TNF-α negatively correlated with the relative abundance of *Firmicutes*, whereas IL-4 and IL-13 positively correlated with *Bacteroidetes*. Another study demonstrated that the increased inflammation observed in the LPS-induced mouse lung injury model was associated with lung microbiota dysbiosis, which enhanced the IL-6 pro-inflammatory effect probably mediated by abnormally dominant OTUs (52). Moreover, in the bleomycin-induced lung fibrosis model, Th1 cells in GF mice were reduced, whereas $Foxp3^+$ T regulatory cells were higher compared with non-GF mice, thus demonstrating the regulatory effect of lung microbiota on cellular immunity (50).

The inflammatory reaction attributable to the lung maladaptive microbiota may also be closely related to Th17 activation (27). In this regard, the lung microbiota has been shown to modulate local mucosal barrier function by enhancing or reducing the release of IL-17 family cytokines, which are mainly secreted by Th17 cells (26). However, microbiota dysbiosis may activate pulmonary fibrosis by stimulating IL-17B production, which in turn acts on Th17 and neutrophil recruitment genes under TNF-α coordination in a feedback event (53). Furthermore, lung microbiota may intervene in inflammation by altering alveolar macrophages, DCs, invariant natural killer T cells, Treg cells, and lung-resident Tgd cells function (26). These cells induce neutrophil migration. They are involved in the intestine-lung axis by controlling pathogenic microorganisms, maintaining pulmonary homeostasis, and

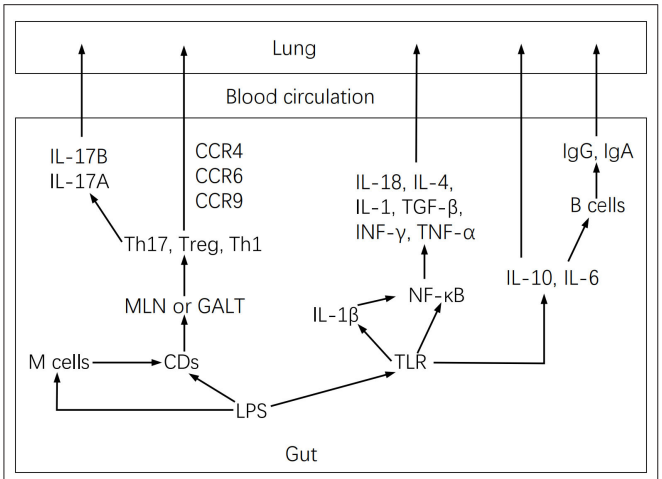


**FIGURE 3 |** Gut-lung axis diagram.

affecting chronic inflammatory activities of lung diseases (26), but microbiota dysbiosis may modify these effects. Dysbiosis activates the lung immune response through the gut-lung axis (**Figure 3**), by stimulating lymphocyte infiltration in the gut and lung mucosa (54). DCs first recognize pathogens in the intestine and present antigens to T lymphocytes of mesenteric lymph nodes or gut-associated lymphoid tissue, where T cell subsets are activated and migrate to the respiratory mucosa, attracted by chemotactic molecules, and stimulate a local inflammatory response (37). Furthermore, microbiota dysbiosis increases LPS, which stimulates TLR and NF-κB to produce IL-18, IL-4, IL-1, IL-6, TGF-b, IFN-g, and TNF-α involved in pulmonary immune responses (37, 55).

Taken together, these data suggest that gut and lung microbiota may have a significant impact on BPD, and that the disease outcome may be improved by protecting the microbiome.

## ROLE OF PROBIOTICS IN BPD

In recent years, the potential of probiotics to exert several biological activities is remarkable. *Lactobacillus reesei* has been shown to be useful in delaying tissue damage and relieving upper respiratory tract infections in patients with cystic fibrosis with mild to moderate lung disease (56), whereas *Bifidobacterium mixture* reduces the clinical manifestations of allergic rhinitis and improves the quality of life of children with the occasional paroxysm of asthma (57). In addition, prophylactic use of probiotics decreases lung infections in mechanically ventilated children (58) and lessens the likelihood of late-onset sepsis in preterm infants (59, 60).

It is not difficult to infer that probiotics have great potential in the treatment of respiratory diseases. More importantly, some studies have shown interest in probiotics and BPD. Consequently, we attempted to explore the feasible influence of probiotics on BPD.

### Anti-infection and Anti-inflammatory Roles of Probiotics

Probiotics, as living microorganisms, become useful in relieving microbiome dysregulations. It has been shown in animal studies that oral probiotics enhance the richness and diversity of airway microbial communities (61), which provide a starting point for understanding the interaction of probiotics and BPD. Furthermore, probiotics have unexpected anti-infection and anti-inflammatory elements and the potential to recover nutrition and antioxidant properties (**Figure 2**), which are essential for children with BPD.

Oral administration of *Lactobacillus plantarum* was observed to significantly reduce pulmonary inflammation in *Klebsiella pneumoniae*-infected mice, as shown by the decreased number of macrophages and neutrophils, and pro-inflammatory cytokines (KC, IL-6, and TNF-α), and the blocking of NF-κB activation by an interaction with TLR (62). *L. plantarum* also down-regulates T-bet and IL-2 levels, stimulates $Foxp3^+$ and z/70mRNA expression in lung tissue, and expands the number of $CD4^+CD25^+Foxp3^+$ cells in mediastinal lymph nodes (62). Although *L. plantarum* failed to increase the amount of DCs, it attracts them to produce IL-10, promoting Treg cell immunoregulatory action (62).

In a mouse model of *Neisseria meningitides* secondary to influenza A virus (IAV) infection, Belkacem et al. (63) observed that *L. paracasei* increased the amount of DCs, neutrophils, and monocytes in the lungs. Although some highly expressed inflammatory cytokines, such as IL-6, MCP1, KC, and IL-12p70, were detected, only IL-6 and MCP1 were statistically significant. They consider that *L. paracasei* simultaneously augments the health status of IAV and influenza-meningococcal infection in mice, by attracting interstitial monocytes and DCs (63). In addition, intratracheal administration of *Lactobacillus* probably suppresses the PAO1 virulence factor and reduces IL-6 and TNF-α activities, affecting lung infection outcome by *Pseudomonas aeruginosa* in mice (64).

Similarly, *Bifidobacterium bifidum* was shown to produce a significant anti-inflammatory response with a high production of IFN-g, IL-12, and IL-4 (cellular immunity) and IgG1 and IgG2α levels (humoral immunity) in mice infected with influenza virus, but reduced IL-6 production in lung tissue (65). In a mice model of severe asthma, *Bifidobacterium breve* significantly decreased the levels of the pro-inflammatory cytokines IL-1α and IL-1b and the chemokine CXCL-2, which stimulate neutrophil migration to pulmonary tissue, as compared with control, where an increase of IL-1α was observed (66). Furthermore, *B. breve* down-regulated activated $CD11b^+$ cells, up-regulated $CD4^+CD44^+$ cells and $CD4^+FoxP3^+$ cells, and increased the number of macrophages.

*In vitro* experiments showed antibacterial activity of *Bifidobacterium* and *Lactobacillus* against *Clostridium* (67), which was probably mediated by bacteriocins, microcins, and short-chain fatty acids (SCFAs) (68). Bacteriocins change the permeability of the inner membrane of pathogenic bacteria, affecting cell wall synthesis (69), whereas microcins directly impair the activity of enzymes required by pathogens in the process of gene replication and transcription (70).

SCFAs generated by probiotics interfere with pathogenicity by reducing intestinal pH, destroying pathogen cell membrane structure, accelerating oxidative phosphorylation, enhancing the antibacterial potential of other molecules (68), regulating the expression of histone deacetylase, and coupling with G protein receptors (71). In addition, SCFAs blocks secretion of IL-8 and macrophage inflammatory protein 2 by intestinal IL-1b in naive mice, evidencing the characteristic anti-inflammatory effect of SCFAs (72). Moreover, probiotics improve immunity by stimulating IgA production through the following mechanisms: (a) triggering the activation of TLR9 and TLR2, (b) promoting DCs maturation, (c) regulating B lymphocytes, (d) being involved in mucosa cytokines production, and (e) inducing the release of TGF-β, IL-10, and IL-6 (68, 71).

## The Antioxidant and Nutritional Effects of Probiotics

It is well-known that oxidative stress plays a central function in BPD pathogenesis. Hyperoxia exposure activates an excessive production of ROS, inhibits the development of alveolar and pulmonary blood vessels, and participates in the inflammatory process (73). In addition, ROS causes endothelial dysfunction and increases vascular permeability, leading to pulmonary tissue edema (74). Microbiota dysbiosis may initiate oxidative stress because lung microbiota stimulates the aryl hydrocarbon receptor, which regulates the activity of antioxidant enzymes, by changing the levels of tryptophan catabolites (74). *Lactobacillus* and *Bifidobacterium* are known to possess antioxidant properties (75). *In vitro* experiments showed that *Lactobacillus plantarum* MA2 inhibits hydrogen peroxide and lipid peroxidation, and possesses the great potential to chelate $Fe^{2+}$ and scavenge free radicals such as 1,1-diphenyl-2-picrylhydrazine (DPPH), hydroxyl radicals, superoxide anion radicals, and has exceptional reducing activity (76). Mechanistically, antioxidant genes (e.g., glutathione peroxidase, catalase, NADH oxidase, NADH peroxidase, and glutathione reductase genes) encode superoxide dismutase and glutathione peroxidase to exert antioxidant effects (76). Furthermore, exopolysaccharides extracted from *Lactobacillus plantarum* possess antioxidant activities. At a concentration of 10 mg/mL, exopolysaccharides reached the maximum 80.4, 65.5, and 60.5% scavenging rates for hydroxyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate), and DPPH radicals, respectively (77). Similarly, *Bifidobacterium* has a high potential to scavenge free radicals and an acceptable reducing activity (78). However, it should be recognized that the antioxidant properties of probiotics are unique. The free radical scavenging activity of probiotics greatly varies from strain to strain (79). In short, probiotics prevent oxidative stress in different ways, ① chelating metal ions, ② activating antioxidant enzymes and metabolites, ③ boosting antioxidant signaling pathways such as Nrf2-Keap1-ARE, mitogen-activated protein kinases, and protein kinase C pathway, ④ reducing ROS production, ⑤ protecting gut microbiota, and ⑥ increasing the host's antioxidant capacity (80).

The nutritional factor is also important in BPD onset. It has been observed that providing enrichment proteins and energy complements to children with protracted mechanical ventilation, reduces the disease incidence (81). Probiotics may increase the nutritional status of preterm infants. In this regard, it was observed that intestinal digestive enzymes (for example, α-amylase, lipase, and trypsin) of mice fed with *Bacillus subtilis* and *Bacillus velezensis* were significantly increased, as compared with control (82). In addition, it was shown that from weeks 2 to 3, the average daily weight gain of mice in the probiotic group was significantly higher and the feed conversion rate was lower, indicating that probiotics promote mice growth. Similarly, probiotics increase the appetite and weight of older dogs, improve immunity, and enrich gut microbiota (83). Moreover, a meta-analysis demonstrated that supplementing with probiotics reduces the time to full enteral feeding for premature infants to achieve better weight gain and growth development (84). Probiotics decrease nutrient wastage by protecting the integrity of the intestinal mucosa, promoting digestion and absorption, and blocking undesirable metabolic pathways (85).

*Lactobacillus sp.* may also alter alveolar structure and regulate alveolar growth (86). Furthermore, probiotics were shown in animal studies to be involved in angiogenesis (87).

In general, given the outstanding advantages of probiotics, we may need to broaden our horizons to fully comprehend the activities and mechanisms of probiotics, particularly associated with their potential to improve BPD outcome.

# CHALLENGE

As we envision the benefits of probiotics for BPD patients, we must also be very aware that there are still numerous problems that must be overcome. A meta-analysis indirectly evaluated the impact of probiotics on BPD (88). Unfortunately, the result was denied by the authors. However, all studies by the authors exclusively use BPD as a secondary result of the research, which may be one of their limitations. In addition, several studies have not given the importance the disease and probiotics deserve, possibly impacting judgment. In fact, the designated species and strains are likely to largely dominate the efficacy of probiotics, which has been certified in tests by Monteiro et al. (67) Different doses and preparations of probiotics may produce divergent conclusions as well. It is also probable that each host system possesses its own "*proprietary probiotics*."

Detection of high-quality probiotics that may relate to BPD requires a number of well-established, safe, reliable, and laborious scientific methods. However, in any case, this meta-analysis is suggested, as it opens a new direction in the discussion of probiotics and BPD.

For immensely frail infants with BPD, they can only face the following burdensome issues: How to determine the optimal therapy time window and duration of probiotics? Are combination medications used? How to choose the route of administration? Oral administration should anticipate whether probiotics will add to the burden of the gastrointestinal tract and whether a safe dose of probiotics achieves the therapeutic concentration. Theoretically, compared with oral administration, probiotics possibly have a more apparent curative effect by

aerosolized inhalation and intratracheal administration, since they directly act on the respiratory mucosa, but this assumption needs experimental verification.

Even though probiotics are beneficial bacteria for humans, they also can produce adverse effects, including involvement in systemic disease, harmful metabolic events, and excessive stimulation of the immune system (89). Thus, it is necessary to find more effective and precise probiotic action pathways to optimize their use in human health. They produce differential effects on the population. Particularly in infancy, premature babies may become a high-risk group to use probiotics, because of their immature immunity. Previous investigations reported that probiotic supplementation early after birth increases the occurrence of mucosal (oral, respiratory, gastrointestinal) infection diseases (90). Infants taking probiotics have complained of unpleasant taste, dry skin, bloating, vomiting, rash, and other adverse events (91). Some premature babies may be affected by bacteremia when receiving probiotics treatment, and the infected strain derives from the probiotics themselves (92, 93). In this regard, it is necessary to conduct a thorough long-term evaluation of the safety of probiotics in children (especially newborns) in consecutive trials, before using them in therapy.

In conclusion, probiotics have promising applications, particularly to improve BPD prognosis. However, it is essential to carefully select the probiotic strains, medication dosage, frequency, and routes. Furthermore, more valid scientific information and follow-up studies are required to support the adequate use of probiotics in human health.

## AUTHOR CONTRIBUTIONS

KY wrote the manuscript. WD audited the manuscript. All authors contributed to the article and approved the submitted version.

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# Renal Function Profiles in Preterm Neonates With Birth Asphyxia Within the First 24 H of Life

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The characteristics of early renal function in preterm neonates of different gestational ages (GAs) with birth asphyxia (BA) remain unclear. Kidneys are sensitive to oxygen deprivation, and renal insufficiency may occur within 24 h of BA. We aimed to elucidate the renal function profiles within the first 24 h after the development of BA among vulnerable preterm neonates of different GAs. The medical records of 128 preterm neonates born to mothers with normal renal function were retrospectively analyzed. Data regarding the serum creatinine (SCr) and urea nitrogen (BUN) levels in venous blood, estimated creatinine clearance (eCCl) within the first hours after birth, and urinary output (UOP) in the first 24 h after birth were compared between the preterm with BA population and GA-matched population without BA ($n = 64$ and $n = 64$, respectively). Significantly higher SCr levels and lower eCCl were observed in mid-late preterm neonates with BA than in preterm neonates without BA (84.05 versus [vs.] 64.20 μmol/L, $z = 4.41$, $p < 0.001$; 15.02 vs. 21.30 mL/min/1.73 $m^2$, $z = 3.57$, $p < 0.001$, respectively). Very preterm neonates showed a higher UOP (2.01 vs. 1.66 mL/kg/h, $z = 2.01$, $p = 0.045$) after the development of BA than before. In preterm neonates with BA, the incidence of SCr > 133 μmol/L, CCl < 16 mL/min/1.73 $m^2$ and UOP < 1.0 ml/kg/h, was 10.94%, 62.50%, and 20.31%, respectively. Within 24 h after birth, BA was associated with eCCl < 16 mL/min/1.73 $m^2$ ($p = 0.016$, odds ratio = 2.83, 95% confidence interval: 1.210–6.613) in preterm neonates. Different renal function profiles were observed in preterm neonates of different GAs within the first 24 h of life after the development of BA. Candidate therapies based on different renal function statuses will bring these vulnerable patient populations of different GAs closer to receiving precision medicine.

**Keywords: preterm, renal function, profiles, first 24 h of life, asphyxia, gestational age**

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## INTRODUCTION

Renal function in neonate changes markedly daily, and different degrees of renal immaturity exist in preterm neonates of different gestational ages (GAs) (1); Thus, evaluating the renal function of the preterm population is challenging. The estimated global preterm birth rate for 2018 ranged between 5 and 18% across 184 countries worldwide (2). Preterm neonates have multiple organ immaturity and high mortality, which increases with decreasing GA (3). Birth asphyxia (BA), a critical condition of transient anoxia experienced by neonates at or around the time of delivery, occurs at 1–10% per 1,000 live births and is more likely to occur in preterm neonates (4).

BA leads to dysfunction of the central nervous system and multiple organs, including the heart, lungs, kidneys, and bowel (5). Kidneys are sensitive to oxygen deprivation, and renal insufficiency may occur within 24 h of BA, which if prolonged may even lead to irreversible cortical necrosis (6). Changes, such as elevation of serum creatinine (SCr) levels in early kidney function have been associated with short- and long-term consequences, including fluid overload, increased length of hospitalization, and death (7–9). Most studies focused on early renal function status of term infants, and the earliest observation time was 3 days after birth (6, 10, 11). However, in vulnerable preterm neonates, the first 24 h after birth is a critical period, and detailed renal function is beneficial for reasonable management and timely interventions, such as antibiotic dosage choice and parenteral nutrition. Studies on the early renal function status of preterm neonates of different GAs within 24 h after the development of BA are rare. Therefore, this study aimed to clarify the early profiles of the conventional indicators of renal and micturition functions in preterm neonates with BA within the first 24 h after birth by comparing the levels of SCr, blood urea nitrogen (BUN), estimated creatinine clearance (eCCI), and urinary output (UOP) between preterm neonates of different GAs with and without BA, with the goal of providing advisable information for tailor clinical management. In addition, candidate therapies based on their GAs will bring these vulnerable patient populations closer to receiving precision medicine, including fluid and electrolyte management, drug choice, and for those drugs, dose selection.

# MATERIALS AND METHODS

## Study Design and Population

After obtaining institutional ethical clearance (2019-KY-023-01), this retrospective, matched case-control study was conducted in a neonatal intensive care unit (NICU) of our institution between March 2014 and March 2018.

The preterm neonates diagnosed with asphyxia by an experienced neonatologist team were included as preterm neonates with observed BA. Premature neonates (no history of fetal distress and Apgar score $< 7$ at 5 min) were continuously included as controls until the patients in each group were evenly distributed. The exclusion criteria were as follows: (a) congenital malformation in the newborn; (b) inherited metabolic diseases in the neonate; (c) congenital renal dysplasia detected on prenatal screening; (d) family history of kidney disease; (e) maternal use of opioids (fentanyl, remifentanil, or sufentanil), captopril, indomethacin, or other drugs that affect renal function during pregnancy; (f) maternal renal dysfunction during the perinatal period; (g) severe maternal anemia during pregnancy; (h) inadequate liquid intake within 24 h after birth; and (i) use of mannitol, ibuprofen, or diuretics within 24 h after birth.

**Abbreviations:** AKI, acute kidney injury; BA, birth asphyxia; BUN, blood urea nitrogen; eCCI, estimated creatinine clearance; GAs, gestational ages; GFR, glomerular filtration rate; NICU, neonatal intensive care unit; SCr, serum creatinine; UOP, urinary output.

Asphyxia was diagnosed based on a delivery history of fetal distress and/or 5 min Apgar score of <7, which was defined by the World Health Organization as failure to initiate and sustain normal breathing at birth (12–14). Fetal distress was recorded in the presence of bradycardia (fetal heart rate [FHR] <100 beats/min for >3 min) or persistent severe variable or late decelerations (persistent recording of a reduction in FHR by >15 beats/min from baseline for more than 15 s of recording with associated decreased beat-to-beat variability or reduction of FHR to <100 beats/min for >1 min) during fetal heart rate monitoring (15). GA was determined based on the date of the last menstrual period reported by the mother and on the modified Ballard maturational scoring system. Maternal complications were recorded, including eclampsia; hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, diabetes mellitus; hypothyroidism; placental abruption; intrapartum hemorrhage; and neonatal complications including shock, sepsis, respiratory distress syndrome, and multiple organ dysfunction, were recorded.

All observed premature neonates with BA and controls were admitted to the NICU within 30 min after birth, managed by the same experienced neonatology team, given timely treatment, and guaranteed standard input, but they did not received diuretics during the observation.

## Data Collection

Emergency care and resuscitation procedures took precedence over any other procedure. All neonates were uniformly managed as per standard NICU guidelines. All patient details were treated with the strictest confidence. The neonate's gender, GA, birth length, weight, and head circumference, 1 and 5 min Apgar scores, fluid intake, and UOP within 24 h after birth were recorded. Serum samples were isolated from the peripheral vein blood for routine admission examination within the first hours after birth. Then, serum concentrations of creatinine and BUN were determined by enzymatic assay using an automatic biochemical analyzer (Beckman Coulter, Miami, FL, USA). The eCCI rate was calculated using the Schwartz formula: eCCI (mL/min/1.73 $m^2$) = [0.34 × length (cm)]/SCr (mg/dL) (1 μmol/L = 0.0113 mg/dL) (16). UOP was systematically measured based on diaper weight every 3 h. The volumes of UOP and fluid intake within 24 h were obtained from the nursing records. We also analyzed the incidences of SCr > 133 μmol/L (6, 17), eCCI < 16 mL/min/1.73 $m^2$ (11), and oliguria (UOP < 1.0 mL/kg/h for 24 h) (18). The material's information, including primiparity, delivery mode, gestational complication, and perinatal SCr level was recorded.

## Sample Size Calculation

The sample size was calculated and showed that examining 128 neonates would have a power of 80% in detecting a significant difference in the SCr level using a two-sided F-test with a confidence level of 95% (type 1 error, 0.05).

## Statistical Analysis

Quantitative variables are expressed as median (interquartile range), whereas categorical variables are expressed as absolute

**TABLE 1 |** Demographic and clinical characteristics of preterm neonates and their mothers.

| Items | | | Preterm neonates with BA ($n$ = 64) | | Control ($n$ =64) | | *P*-value |
|---|---|---|---|---|---|---|---|
| | | | 28–31 week ($n$ = 32) | 32–36 week ($N$ = 32) | 28–31 week ($n$ = 32) | 32–36 week ($n$ = 32) | |
| Mother baseline | Primiparity | Yes | 37 | | 39 | | $p = 0.72$ |
| | | | 18 | 19 | 18 | 21 | |
| | Delivery mode | Nature labor | 10* | | 21 | | $p = 0.04$ |
| | | | 8 | 2 | 12 | 9 | |
| | Gestational complication | Yes | 41 | | 46 | | $p = 0.26$ |
| | | | 22 | 19 | 22 | 24 | |
| | Serum creatinine (μmol /L) | | 49.50 | | 47.10 | | $p = 0.416$ |
| | | | 49.50 | 49.75 | 47.25 | 47.1 | |
| Neonatal baseline | Gender | Male | 34 | | 37 | | $p = 0.86$ |
| | | | 18 | 16 | 19 | 18 | |
| | GA (weeks) | | 32.00 ± 2.79 | | 32.00 ± 2.52 | | $p = 1.00$ |
| | | | 30.00 ± 1.50 | 34.00 ± 1.70 | 29.87 ± 1.16 | 34.13 ± 1.52 | |
| | Birth weight (g) | | 1723.00 ± 673.74 | | 1703.90 ± 528.24 | | $p = 0.86$ |
| | | | 1232.40 ± 292.58 | 2207.00 ± 591.80 | 1335.31 ± 275.92 | 2072.00 ± 458.78 | |
| | Birth length (cm) | | 41.42 ± 4.58 | | 41.75 ± 4.14 | | $p = 0.67$ |
| | | | 38.31 ± 2.87 | 44.53 ± 3.79 | 38.875 ± 2.88 | 44.63 ± 3.09 | |
| | 24 h fluid intake (ml) | | 106.19 ± 37.55 | | 100.00 ± 30.00 | | $p = 0.31$ |
| | | | 118.93 ± 43.65 | 93.45 ± 25.08 | 107.28 ± 29.18 | 92.71 ± 31.21 | |
| | Perinatal complication | Yes | 43 | | 33 | | $p = 0.07$ |
| | | | 26 | 17 | 28 | 5 | |

*BA, birth asphyxia; GA, gestational age.*
**Statistically significant difference ($p < 0.05$) between gestational age-matched preterm neonates with asphyxia and control preterm neonates.*

frequency (percentage). The neonate characteristics were compared between the preterm BA and preterm control groups. Continuous variables with normally distributed values (i.e., eCCI) were compared using the independent Student' $t$-test, whereas those with non-normally distributed values (i.e., SCr, BUN, and UOP) were compared using the Mann-Whitney U-test. The chi-square or Fischer's exact test (as appropriate) was used to compare dichotomous variables between the groups. Univariate logistic regression analysis was performed to evaluate the potential associations of demographic and clinical variables with deteriorating renal function risk. A $p < 0.05$ was considered to indicate statistical significance. Statistical analysis was conducted using SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

# RESULTS

## Study Population

Between March 2014 and March 2018, 2,977 hospitalized preterm neonates were treated in the NICU. Seventy-one preterm neonates had a history of fetal distress and an Apgar score <7 at 5 min. After screening by the exclusion criteria, 64 preterm neonates with BA (BA group), including 32 very preterm neonates (GA: 28–31 weeks) and 32 mid-late preterm neonates (GA: 32–36 weeks), were enrolled in the observation group. Sixty-four GA-matched preterm neonates without BA were included as controls until patients in each group were evenly distributed. All 128 patients had NICU admission charts available for review, and their mothers had normal SCr levels.

Demographic and clinical characteristics of the neonates and their mothers are summarized in **Table 1**. No significant differences in gender, gestational age, birth weight, birth length, 24 h fluid intake, and perinatal complications were observed between the BA and control groups. No significant differences in primiparity, gestational complications, and SCr level of mothers were found between the matched groups. No abnormal SCr levels was observed in all 128 mothers throughout their pregnancy. Gestational complications, including hypertension/preeclampsia/HELLP syndrome, diabetes, abnormal thyroidal function, placental abruption, and postpartum hemorrhage occurred in 41/64 (64.06%) mothers of preterm neonates with BA and 46/64 (71.88%) mothers of control neonates ($p = 0.260$). The vaginal delivery rate was higher in the BA group than in the control group ($p = 0.040$).

## Differences in Renal Function Between Preterm Neonates With and Without Asphyxia Within the First Day After Birth

Preterm neonates with BA had a significantly higher SCr levels (85.00 versus [vs.] 69.70 μmol/L; z = 3.61, $p < 0.001$), but a significantly lower eCCI rate (14.47 vs. 18.00 mL/min/1.73 $m^2$; $t = 3.51$, $p < 0.001$), especially in the mid-late preterm neonates (SCr: 84.05 vs. 64.20 μmol/L; z = 4.41, $p < 0.001$; eCCI: 15.02 vs.

**TABLE 2 |** Comparison of renal function of preterm neonates with or without asphyxia within 24 h after birth.

| | Very preterm neonates ($n = 64$) | | Mid-late preterm neonates ($n = 64$) | | All preterm neonates ($n = 128$) | |
|---|---|---|---|---|---|---|
| | Control ($n = 32$) | BA ($n = 32$) | Control ($n = 32$) | BA($n = 32$) | Control ($n = 64$) | BA ($n = 64$) |
| SCr (μmol/L) | 73.60 (64.10–93.28) | 85.00 (67.38–106.60) | 64.20 (58.15–73.80) | 84.05 (72.00–114.25)* | 69.70 (60.80–83.30) | 85.00 (70–109.34)* |
| BUN (mmol/L) | 5.72 (3.86–7.49) | 5.60 (3.93–6.90) | 4.82 (3.66–5.80) | 4.88 (4.03–7.66) | 5.26 (3.75–6.84) | 5.00 (4.00–6.50) |
| eCCl (mL/min/1.73 $m^2$) | 14.88 (12.42–18.65) | 14.16 (10.08–16.90) | 21.30 (17.61–24.16) | 15.02 (11.76–19.23)* | 18.00 (13.92–22.95) | 14.47 (11.27–22.17)* |
| UOP (mL/kg/h) | 1.66 (1.17–2.40) | 2.01 (1.56–3.04) * | 1.45 (1.11–2.19) | 1.18 (0.83–2.17) | 1.58 (1.18–2.31) | 1.71 (1.07–2.44) |

*BA, birth asphyxia; BUN, blood urea nitrogen; eCCl, estimated creatinine clearance; SCr, serum creatinine; UOP, urinary output.*
**Statistically significant difference ($p < 0.05$) between gestational age-matched preterm neonates with asphyxia and control preterm neonates.*

**TABLE 3 |** Incidences of changed parameters related to renal function in asphyxiated preterm neonates within 24 h after birth.

| Abnormal renal function indicators | Very preterm neonates | | Mid-late preterm neonates | | Preterm neonates | |
|---|---|---|---|---|---|---|
| | Control ($n = 32$) | BA ($n = 32$) | Control ($n = 32$) | BA ($n = 32$) | Control ($n = 64$) | BA ($n = 64$) |
| SCr ≥ 133 μmol/L | 1 (3.13%) | 2 (6.25%) | 0 (0%) | 5 (15.63%) | 1 (1.56%) | 7 (10.94%) |
| eCCl ≤ 16 mL/min/1.73 $m^2$ | 19 (59.38%) | 22 (68.75%) | 5 (15.63%) | 18 (56.25%)* | 24 (37.50%) | 40 (62.50%)* |
| UOP < 1.0 mL/kg/h | 3 (9.38%) | 1 (3.13%) | 7 (21.88%) | 12 (37.50%) | 10 (15.63%) | 13 (20.31%) |

*BA, birth asphyxia; eCCl, estimated creatinine clearance; SCr, serum creatinine; UOP, urinary output.*
**Statistically significant difference ($p < 0.05$) between gestational age-matched preterm neonates with asphyxia and control preterm neonates.*

21.30 mL/min/1.73 $m^2$; z = 3.57, $p < 0.001$) than GA-matched controls. However, no significant difference was found in the SCr levels and eCCI rate between the very preterm neonates with and without BA. Although the very preterm neonates with BA had a significantly higher UOP than the GA-matched controls (2.01 vs. 1.66 mL/kg/h; z = 2.01, $p = 0.045$), there was no significant difference in the UOP between the mid-late preterm neonates with and without BA and between all preterm neonates with BA and without BA. Additionally, BA did not associated with the BUN level in all preterm neonates, including the mid-late and the very preterm neonates (**Table 2**).

## Incidences of Changed Parameters Related to Renal Function in Preterm Neonates With Asphyxia Within 24 h After Birth

Within 24 h after birth, in all 64 preterm neonates with BA, the incidences of SCr > 133 μmol/L, CCI < 16 L/min/1.73 $m^2$ and UOP < 1.0 ml/kg/h were 10.94% (7/64), 62.50% (40/64), and 20.31% (13/64), respectively. There was no significant difference in the incidences of SCr > 133 μmol/L (10.94% [7/64] vs. 1.56% [1/64] and oliguria (UOP < 1.0 mL/kg/h; 20.31% [13/64] vs. 15.63% [10/64]) between the 64 preterm controls and 64 preterm neonates with BA. However, the incidence of eCCI < 16 mL/min/1.73 $m^2$ was significantly higher in the 64 preterm neonates with BA than in the preterm controls (62.50 % vs. 37.50%; $\chi^2 = 8.00$, $p = 0.005$), especially in the 32 mid-late preterm neonates (56.25% [18/32] vs. 15.63% [5/32]; $\chi^2 = 11.47$, $p = 0.001$). The incidence of eCCI < 16 mL/min/1.73 $m^2$ was similar in the very preterm neonates with and without BA (68.75% [22/32] vs. 59.38% [19/32], respectively; $\chi^2 = 0.61$, $p = 0.430$ (**Table 3**).

## Predisposing Factors of Deteriorating Renal Function in Preterm Neonates Within 24 h After Birth

In the univariate logistic regression analysis, asphyxia ($p = 0.016$; odds ratio [OR]= 2.83; 95% confidence interval [CI]: 1.21–6.61), perinatal complications of neonates ($p = 0.003$; OR = 3.08; 95% CI: 1.48–6.43), Preterm birth with very small GA (GA < 32 weeks) ($p = 0.001$; OR = 3.41; 95% CI: 1.65–7.06), and natural birth ($p = 0.002$; OR = 4.91; 95% CI: 0.17–9.84) were significantly associated with an increased risk of eCCI < 16 mL/min/1.73 $m^2$ in all preterm neonates within the first hours after birth. Preterm birth with very small GA (<32 weeks) ($p = 0.002$; OR = 0.16; 95% CI: 0.05–0.50) and gestational complications ($p = 0.0260$; OR = 0.35; 95% CI: 0.14–0.88) were inversely associated with UOP < 1.0 ml/kg/h in preterm neonates within 24 h after birth. Primiparity and gender were not significantly associated with the change of renal function in preterm neonates within 24 h after birth (**Table 4**).

# DISCUSSION

Our research showed that the renal function of preterm neonates was altered after BA in the first hours after birth, and different renal function profiles were observed in neonates of different GAs after BA in the first day of life. In mid-late preterm neonates after BA, a higher initial SCr value and lower initial eCCI were observed. In the very preterm neonates after BA, higher UOP was seen within the first day after birth. The incidences of SCr > 133 μmol/L, eCCI < 16 mL/min/1.73 $m^2$ within the first hours after birth, and UOP < 1.0 ml/kg/h on the first day after birth were 10.94, 62.50, and 20.31%, respectively, in preterm neonates after BA. The influencing factors of early renal function

**TABLE 4 |** Maternal and neonatal risk factors for early deteriorating renal function in preterm neonates.

| Parameter | | *n* | SCr ≥ 133 μmol/L | Univariate logistic regression | | UOP < 1.0 ml/kg/h | Univariate logistic regression | | eCCI < 16 mL/min/1.73 $m^2$ | Univariate logistic regression | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Yes *N* = 8 | OR (95% CI) | *P*-value | Yes *N* = 23 | OR (95% CI) | *P*-value | Yes *N* = 29 | OR (95% CI) | *P*-value |
| Primiparity | Yes | 76 | 4 | 0.70 (0.52–2.80) | 0.579 | 17 | 2.21 (0.81–6.05) | 0.120 | 38 | 0.68 (0.52–1.53) | 0.683 |
| Delivery mode | Nature | 31 | 1 | 0.43 (0.05–3.63) | 0.437 | 5 | 0.84 (0.29–2.50) | 0.759 | 7 | 4.91 (0.17–9.84) | 0.002* |
| Gestational complication | Yes | 87 | 7 | 3.5 (0.42–29.43) | 0.246 | 11 | 0.35 (0.14–0.88) | 0.026* | 45 | 1.62 (0.78–3.37) | 0.196 |
| Gender | Male | 71 | 6 | 2.54 (0.49–13.09) | 0.260 | 12 | 0.85 (0.34–2.10) | 0.726 | 34 | 1.21 (0.60–2.43) | 0.594 |
| GA | <32 week | 64 | 3 | 0.58 (0.13–2.54) | 0.470 | 4 | 0.16 (0.05–0.50) | 0.002* | 53 | 3.41 (1.65–7.06) | 0.001* |
| Birth asphyxia | Yes | 64 | 8 | 7.74 (0.92–64.83) | 0.059 | 13 | 1.38 (0.56–3.42) | 0.491 | 40 | 2.83 (1.21–6.61) | 0.016* |
| Perinatal complication | Yes | 76 | 8 | 5.17 (0.62–43.38) | 0.130 | 12 | 0.69 (0.28–1.73) | 0.439 | 46 | 3.08 (1.48–6.43) | 0.003* |

*CI, confidence interval; eCCI, estimated creatinine clearance; OR, odds ratio; SCr, serum creatinine; UOP, urinary output.*
**The p < 0.05.*

in preterm neonates included BA, GA, maternal complication, and postnatal complications of neonates. Preterm birth with very small GA (<32 weeks) was inversely associated with UOP < 1.0 ml/kg/h. BA was associated with an increased incidence of eCCI < 16 mL/min/1.73 $m^2$, at an OR of 2.83. Therapies based on the different renal function statuses may bring these vulnerable patient populations of different GAs closer to receiving precision medicine after BA.

In preterm neonates hospitalized in the NICU, the incidence of acute kidney injury (AKI), functional renal failure, and intrinsic renal failure were reported to be 12, 48.14, and 51.85% respectively (19). despite the great advances in neonatal resuscitation, many preterm infants still suffer from asphyxia, which is an important risk factor for impaired renal function in preterm neonates (20). Severe asphyxia leads to diffuse tubular dysfunction, which results in impaired water and sodium reabsorption and decreased GFR (21). The damage caused by impaired renal function-related complications is significantly more severe in most immature neonates with impaired glomerulogenesis (2–4, 22). Therefore, it is critical to early identify the deterioration in renal function associated with asphyxia in these vulnerable premature neonates of different GAs within the first day of life, which would bring these vulnerable patient populations closer to receiving timely precision treatment and achieving a better outcome. These treatments included fluid and electrolyte management, drug choice (avoid nephrotoxic drugs and potential nephroprotective interventions), and for those drugs, dose selection (16).

Evaluating kidney injury in preterm neonates is challenging, but it is relevant for postnatal care and crucial for the future of a patient. Although the definitions of kidney impairment and AKI staging in adults and children have become widely adopted, no single-accepted definition exists for AKI in neonates, and no single accepted definition exists for AKI in neonates and no definition has been proposed for preterm neonates (4). Additionally, the measurement of preterm neonatal renal function is challenging (16, 20). GFR represents the most recognized measure of kidney function. Although the clearance rate of inulin is the gold standard of GFR, it was difficult to measure it because intravenous pre-infusion, followed by continuous infusion of insulin, was needed. The GFR of neonates is generally based on the eCCI (23). Accurate, easy-to-use markers to estimate the actual GFR of neonates are lacking. In clinical practice, the commonly used endogenous glomerular filtration markers are SCr and BUN, especially in developing countries such as China. However, SCr and BUN are affected by extrarenal factors (e.g., muscle mass, intake of nitrogen, and protein) and associated with age, sex, and body weight (20). The reference intervals for SCr, BUN, and eCCI in preterm neonates of each gestational age have been rarely reported until now.

In the context of the above challenges, studies on the early renal function profiles of asphyxiated preterm neonates with different GAs within the first day after birth have been rare. In this study, widely used renal function indicators, namely, SCr, BUN, eCCI, and UOP, were studied and compared between preterm neonates with BA and their GA-matched controls. Considering GAs related to renal function development, the renal function profiles of the very and mid-late preterm neonates with BA were separately observed. Our results suggest that the renal function of preterm neonates with BA change just a few hours after birth and that their early renal function profiles within the first day after birth were related to GAs. Changes in SCr levels and eCCI value in mid-late preterm and UOP in very preterm neonates should be carefully monitored. Within the first few hours after birth, higher SCr levels but lower eCCI values have already been observed in mid-late preterm neonates with BA. The very preterm neonates with BA only showed higher UOP within the first 24 h after birth. Both the very and mid-late preterm neonates showed no asphyxia-related change in the initial BUN level in this study. Pan et al. also reported on the renal function status of asphyxiated preterm neonates (GA ≤ 34 weeks) at 24 h after birth, and similar SCr, BUN, and UOP values but a reduced eCCI value was observed (20). The differences in changes of renal function parameters between the above two studies may be related to the

different time points of serum collection and the composition of GAs. Precision medicine is urgently needed in early renal function evaluation for the vulnerable preterm neonates of different stages of development. SCr values reflected a pattern with an initial increase and subsequent decrease during postnatal life, as observed in a cohort of 1,140 neonates (GAs 23–42 weeks) in the first 42 days of postnatal life (16, 24). In preterm neonates with gestational age ≥32 weeks, the SCr level may increase for the first several days following birth and the degree and duration of the increase is proportional to the degree of prematurity. This increase in creatinine is thought to be secondary to tubular resorption of creatinine by the immature kidney, compounded by the total body fluid loss and intravascular volume contraction typically encountered in preterm neonates (25, 26). Absolute SCr ≥133 μmol/L on the second or third day of life were frequently proposed to indicate AKI in most neonatal AKI studies (19, 27). All mothers in this study had normal SCr levels during pregnancy, labor, and delivery. The renal function of preterm neonates whose SCr levels have already been ≥133 μmol/L within the first day after birth should be paid more attention to. Therefore, we evaluated the incidence of SCr level ≥133 μmol/L according to routine examination results on admission and found that 10.94% of preterm neonates with BA had already shown an SCr level ≥ 133 μmol/L within the first hours after birth. However, the incidence of SCr ≥ 133 μmol/L in these neonates was not significantly different from that in their GA-matched controls. More sensitive cut-off values of creatinine in preterm neonates of different GAs still need to be explored. Thus far, no unifying accepted cut-off values of eCCI or GFR exist for preterm neonates of different GAs. Kastl (24) reported that GFR measured by renal inulin clearance may be as low as 10–20 mL/min/1.73 $m^2$ at birth, and a rapid increase is then seen within the first 14 days of life and GFR is typically between 35 and 45 mL/min/1.73 $m^2$. VIEUX et al. (28) reported that median GFR reference values in infants aged 27 to 31 weeks' gestation ranged from 7.9 to 30.3 mL/min per 1.73 $m^2$ on day 7. In the study conducted by Nkidiaka et al. (11), AKI was diagnosed within 3 days of life when the eCCI was ≤16 mL/min/1.73 $m^2$ and occurred in 42.85% of the 70 full-term neonates with perinatal asphyxia. Considering that very few studies have explored the cut-off values of eCCI or GFR in preterm neonates within the first day, a tentative comparison of the incidence of eCCI ≤ 16 mL/min/1.73 $m^2$ was conducted between the preterm neonates with and without BA in our present study. We found that the eCCI ≤ 16 mL/min/1.73 $m^2$ occurred in 62.50% of the 64 preterm neonates with BA within the first hours after birth, when compared to GA-matched preterm neonates without BA, and eCCI ≤ 16 mL/min/1.73 $m^2$ was significantly seen more often in the preterm neonates with BA, especially the mid-late preterm neonates with BA. Compared to the occurrence of SCr ≥ 133 μmol/L, the occurrence of eCCI < 16 mL/min/1.73 $m^2$ may have more potential as an index for the early evaluation of renal function of preterm neonates after the development of BA, considering that the eCCI contains much information related to GAs and SCr reabsorption in the proximal tubules varies according to the prematurity level (22). Further studies of more preterm neonates should be conducted to detect more precise interval range values of eCCI. Oliguria is common after perinatal asphyxia, which is a manifestation of renal dysfunction. This is due to the reduction of cardiac output or AKI secondary to renal tubular necrosis (21).

The Acute Kidney Injury Network (AKIN) criteria; the modified pediatric risk, injury, failure, loss, and end-stage (RIFLE) kidney disease criteria, and the Kidney Diseases: Improving Global Outcomes (KDIGO) criteria were used in numerous researches for the diagnosis of neonatal AKI based on the increase in SCr levels and/or decrease in UOP, and UOP < 1.0 ml/kg/h was the most often used cut-off value for oliguria (17, 29). Almost all neonates urinate within 24 h after birth (19). However, the prognosis is poor, and the mortality rate is high if oliguria develops (4). Therefore, in our present study, a tentative comparison of the incidence of UOP < 1.0 ml/kg/h was conducted between the preterm neonates with and without BA within the first 24 h after birth. The incidences of oliguria were similar in the two groups. The very preterm neonates with oliguria should be more carefully monitored within the first 24 h after the development of BA.

BA has been often reported as one of the most common early and late causes of renal function decline in neonates (30). In this study, we also found that asphyxia was significantly associated with an increased risk of impaired renal function in preterm neonates within the first day after birth. BA was associated with a significantly higher incidence of eCCI < 16 mL/min/1.73 $m^2$ in the preterm neonates. The potential risk factors of a higher incidence of eCCI < 16 mL/min/1.73 $m^2$, including very small GA (<32 weeks), perinatal complications of neonates, and natural birth, were also observed in this study. The precise cut-off value of eCCI for neonates of different GAs needs to be further studied with a large sample in the future. Additionally, we found that preterm birth with very small GA (<32 weeks) and mothers' gestational complications were negatively associated with UOP < 1.0 ml/kg/h in preterm neonates within 24 h after birth. Oliguria was not likely to occur in very preterm neonates, which may be related to the high proportion of water content in their body and the immature function of renal tubular concentration and dilution (27). Our results suggest that the influencing factors of early renal function in preterm neonates include BA, GA, maternal complication, and postnatal complications of neonates. Preterm neonates' renal function may be protected by active treatment of the basic maternal diseases during pregnancy, timely and effective asphyxia neonatorum resuscitation, and then reasonable treatment of neonatal complications.

There are several strengths and limitations of this study. Early renal function evaluation in preterm neonates of different GAs after BA within the first 24 h of life were beneficial to the vulnerable population in decreasing the risk of developing AKI within 2–3 days after birth, and better clinical outcomes were achieved after the preterm neonates received reasonable fluid and electrolyte management, drug and dose choice, and timely interventions by a neonatologist. Several measures have been taken to ensure the objectivity of this study, as all neonates were given guaranteed standard input and timely treatment; mothers' SCr levels were all normal during pregnancy, labor, and delivery; and serum was all isolated from peripheral venous blood. This study was a cross-sectional survey on the renal

function profiles of preterm neonates after BA. It was difficult to obtain multiple blood samples for continuous evaluation of renal function because premature neonates have a small blood volume and very preterm neonates, especially, have a low body weight. Further research should extend the observation period to > 48 h after birth to fully clarify the early renal function profiles according to the GAs of neonates. Additionally, our sample size in this study was limited since the number of premature neonates with BA has decreased substantially owing to improvements in perinatal screening and management. Large multicenter studies are needed in the future. Finally, studies using novel and more sensitive blood or urinary markers must be conducted in the future to detect the decline in neonatal renal function as early as possible. Recently, biomarkers of renal GFR or tubular damage as predictors of early renal damage prior to SCr elevation have been explored in term and preterm neonates (16). Compared with the classic endogenous glomerular filtration markers (e.g., SCr and BUN), neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a promising indicator of kidney injury. NGAL was found 34 h earlier than SCr to detect AKI (20). Protein cystatin C is not filtered by the placenta, and elevated cystatin C levels directly reflect fetal and neonate GFRs. Urinary cystatin C shows approximately 90% sensitivity and >80% specificity in predicting AKI in preterm neonates, meaning that these measures have excellent reliability for positively identifying disease and ruling out the possibility for not having the disease (31). Moreover, cystatin C had good distinguishability between asphyxiated and non-asphyxiated preterm neonates, irrespective of GA (<28, 28–32, or ≥32 week subgroups), and further discriminated between mild, moderate, and severe asphyxia (16).

Kidneys are sensitive to oxygen deprivation, and renal insufficiency may occur within 24 h of BA. Accurate evaluation of renal function status remains difficult in the newborn period. Thus far, in clinical practice, commonly used endogenous glomerular filtration markers have still been SCr, BUN, and UOP. Our results based on these classic biomarkers are helpful for understanding the asphyxia related to the early renal function profile of preterm neonates with different developmental maturity statuses, especially in developing countries. When the early renal function assessment is conducted in preterm neonates with BA within the first 24 h after birth, the changes in SCr levels and eCCI should be monitored more intensively in mid-late preterm neonates with BA, whereas oliguria should be carefully monitored in very preterm neonates. Early identification of deterioration of renal function associated with asphyxia and tailored clinical care and pharmacotherapy of the vulnerable preterm neonates of different GAs are beneficial to improve preterm neonate outcomes.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

This study was reviewed and approved by the Ethical Review Commitment (2019-KY-023-01) of Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China. The clinical and research activities being reported are consistent with the principles of the Declaration of Helsinki. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

YZ and H-HZ contributed to the study conception and design. Data collection was performed and the first draft of the manuscript was written by YZ. Data analysis was performed by H-HZ. All authors commented on previous versions of the manuscript, read, and approved the final manuscript.

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REVIEW
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# Precision Medicine in Neonates: Future Perspectives for the Lung

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Bronchopulmonary dysplasia (BPD) is the most common complication of pre-term birth with long lasting sequelae. Since its first description more than 50 years ago, many large randomized controlled trials have been conducted, aiming to improve evidence-based knowledge on the optimal strategies to prevent and treat BPD. However, most of these intervention studies have been performed on a population level without regard for the variation in clinical and biological diversity (e.g., gestational age, ethnicity, gender, or disease progression) between patients that is driven by the complex interaction of genetic pre-disposition and environmental exposures. Nevertheless, clinicians provide daily care such as lung protective interventions on an individual basis every day despite the fact that research supporting individualized or precision medicine for monitoring or treating pre-term lungs is immature. This narrative review summarizes four potential developments in pulmonary research that might facilitate the process of individualizing lung protective interventions to prevent development of BPD. Electrical impedance tomography and electromyography of the diaphragm are bedside monitoring tools to assess regional changes in lung volume and ventilation and spontaneous breathing effort, respectively. These non-invasive tools allow a more individualized optimization of invasive and non-invasive respiratory support. Investigation of the genomic variation in caffeine metabolism in pre-term infants can be used to optimize and individualize caffeine dosing regimens. Finally, volatile organic compound analysis in exhaled breath might accurately predict BPD at an early stage of the disease, enabling clinicians to initiate preventive strategies for BPD on an individual basis. Before these suggested diagnostic or monitoring tools can be implemented in daily practice and improve individualized patient care, future research should address and overcome their technical difficulties, perform extensive external validation and show their additional value in preventing BPD.

**Keywords: individualized medicine, targeted treatment, personalized medicine, newborn, neonatal intensive care**

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## INTRODUCTION

Improvements in neonatal care have led to an increased survival of very low birth weight (VLBW) infants over the past decades (1, 2). Evidence based intervention aiming to improve the pulmonary condition, such as exogenous surfactant treatment, antenatal corticosteroids, more gentle modes and restrictive use of invasive ventilation have greatly contributed to this improved survival (3–5). However, many of the VLBW infants will be at high risk of developing bronchopulmonary

dysplasia (BPD), which is considered the most common complication after pre-term birth (6). BPD is histologically characterized by an arrest in normal lung development, resulting in a prolonged need for respiratory support and (re)hospitalization (7). Although studies have consistently shown the independent association between BPD and increased risk for repeated respiratory infections (bacterial and viral), asthma, and a compromised lung function lasting into adolescence (7, 8), not every infants with the diagnosis BPD will suffer from these pulmonary sequelae. However, there is also a general concern that pre-term infants with BPD have more risk of developing chronic obstructive pulmonary disease in later life (9). In addition to pulmonary sequelae, BPD is also associated with an increased risk of cerebral palsy and developmental delay (10). BPD is considered a multifactorial disease with genetic susceptibility, intrauterine growth restriction, nutritional deficits, oxygen toxicity, pulmonary inflammation, and direct mechanical injury caused by mechanical ventilation as the most important risk factors (11–13).

Over the last decades, several interventions aiming to reduce the incidence of BPD have been studied in large high quality randomized controlled trails, but the results have so far been disappointing. In contrast to other morbidities related to pre-term birth, the incidence of BPD as not declined over time (2). It is important to acknowledge that most intervention studies, although restricting eligible participants to infants below 30 weeks of gestational age, used a population based approach, targeting infants based on a single, often indirect, risk factor for BPD, such as having a gestational age below 30 weeks gestational age or treatment with invasive mechanical ventilation. This approach does not account for the complexity of developing BPD, and the individual diversity that is often present in infants randomized in these studies. At an individual level, a pre-term infant might have to be differentiated into different respiratory disease phenotypes using for instance biomarkers, metabolomics, and genomics (14). However, in order to prevent pre-mature phenotyping based on clinical intuition, the hypothesis of multiple BPD phenotypes needs to be investigated extensively using large subgroups of datasets after external validation before it can be used in neonatal precision medicine of the lung (15). Using a similar line of reasoning, the intervention applied should probably be tailored to the individual patient and lung characteristics instead of using the "one size fits all" approach routinely investigated in large randomized controlled trials (16). This individualized approach using both prognostic enrichment or predictive enrichment in randomized controlled trials requires individual monitoring of the BPD risk profile, the underlying respiratory phenotype, and the correct application of the intervention (17, 18).

Precision medicine, also referred to as personalized or individualized medicine has become an increasingly used approach in adult and pediatric research (19). Prognostic enrichment in precision medicine focusses on patient stratification based on a combination of clinical (e.g., gestational age, ethnicity, gender) information, genetic pre-disposition, and individual biomarkers, enabling more precise differentiation of different phenotypes within this group of pre-term infants (20), whereas predictive enrichment identifies subgroups of patients with a higher chance of responding to a specific therapy based on a specific clinical or biological phenotype (18). However, a literature search in MEDLINE combining the words "precision" or "individualized" and "pulmonary" and "pre-term" results in zero citations. This suggests that individualized risk profiling and tailored intervention for BPD, are not part of daily practice and/or research. This is largely caused by lack of appropriate monitoring tools to assess the individual risk of developing BPD and the successful and correct application of intervention to reduce BPD. This narrative review highlights some promising developments in neonatal pulmonary research enabling the use of precision medicine in the search for more effective interventions to prevent BPD.

## ELECTRICAL IMPEDANCE TOMOGRAPHY

As previously mentioned, invasive mechanical ventilation is one of the risk factors for developing BDP. Pre-clinical studies have indicated that overdistension (volutrauma) and collapse (atelectrauma) of alveoli play a major role in ventilator induced lung injury and subsequent development of BPD. Pre-term infants are prone to loss of lung volume due to their immature lung physiology and underlying lung disease. This leads to impaired lung function and respiratory failure and the need for non-invasive or invasive respiratory support to restore gas exchange. Ideally, respiratory support should reverse atelectasis and avoid alveolar overdistention, resulting in homogeneous aeration and ventilation of the lungs. Reaching this goal requires an individual and dynamic approach, as lung condition differs between patients and lung disease is often heterogeneous in nature. This heterogeneity results in co-existence of overdistended and collapsed lung regions in the same lung (21, 22). It is clear that monitoring of regional aeration and ventilation is essential to individualize respiratory support in pre-term infants with respiratory failure and at risk for developing BPD.

Monitoring regional ventilation distribution and end expiratory lung volume in pre-term infants is challenging, especially at the bedside. Currently available monitoring tools such as chest X-ray, tracer gas wash-in/out methods, or respiratory inductive plethysmography have serious limitations, including lack of regional and online information, cumbersome to perform at the bedside, and use of radiation. Electrical Impedance Tomography (EIT) is a technique developed in the early 1980's that uses differences in tissue conductance in response to an electrical currents to visualize changes in lung aeration (23). EIT is non-invasive, radiation free, and generates continuous bedside information on relative changes in regional ventilation distribution and end expiratory lung volume, which shows a high correlation to actual intra-thoracic changes in air-content (24). Measurement requires placement of a belt containing non-sticky electrodes placed around the chest at the level of the nipple.

In pre-term infants, EIT research has mainly focused on the pathophysiology of lung disease and the impact of interventions

on (regional) lung aeration. Studies have shown that EIT is able to detect and monitor changes in (regional) aeration caused by pneumothoraces (25–27), postural changes (28, 29), atelectasis (30), incorrect endotracheal tube placement (31), endotracheal suctioning (32, 33), (minimal) invasive surfactant administration (34, 35), changes in nasal continuous positive pressure levels (36, 37), and lung recruitment procedures during conventional and high frequency ventilation in pre-term infants (**Figure 1**) (38, 39).

These observational studies show the potential of EIT to individualize respiratory care in pre-term infants at high risk of developing BPD. EIT can assist the clinician in the challenge to optimize ventilator support at an individual level, and thereby achieving the goal of homogeneous non-injurious ventilation. Although EIT has important potential, there are still some technical and practical issues that need to be resolved before it can be implemented in clinical practice. First, results from most studies are based on off-line EIT data analysis. Software allowing continuous online analysis of EIT data is currently being developed. Second, well-designed easily applicable equipment for neonates is still lacking. A user-friendly textile electrode belt, a well-tolerated skin conductance substance, and wireless recording are needed for successful implementation in clinical practice. These improvements will also allow testing of a longer recording time than what was used in most observational studies (1–2 h). Third, developmental and production costs of the hardware need to be reduced in order to make implementation feasible from an economic perspective. Finally, the effect of EIT on important clinical outcomes needs to be investigated in future studies.

### Key Messages

- EIT can visualize regional lung volume and ventilation changes at the bedside and individualize pulmonary treatment.
- Future development should focus on improvement in EIT hardware and software so the final step to clinical implementation can made.

## ELECTROMYOGRAPHY OF THE DIAPHRAGM

Respiratory failure is a common complication in pre-term infants primarily caused by an impaired control of breathing and a compromised lung function (40). Impaired control of breathing leads to apnea of pre-maturity, which can lead to hypoxemia and bradycardia, thereby increasing the risk of adverse neurodevelopmental outcome (41). In an attempt to stabilize the respiratory system and reduce the work of breathing, pre-term infants often receive respiratory support. Non-invasive support is the preferred modality, which can be applied via continuous nasal positive airway pressure (nCPAP), heated humidified high flow nasal cannula, and nasal intermittent positive pressure ventilation (nIPPV) (42). If this is insufficient to restore gas exchange and work of breathing, endotracheal intubation and invasive mechanical ventilation may be necessary. Despite the frequent use of respiratory support, objective criteria to select the optimal mode and setting are not well-established (43, 44). Conventional parameters such as oxygen need and blood gas analysis are not very specific and lack information on work of breathing. As a result, respiratory support modes are often selected and set according to general protocols and timely individual titration is often lacking. This might have important consequences, because both too little and too much support may injure the lungs. In case non-invasive support is adjusted, most clinicians use a "trial and error" strategy (43). For example, the nCPAP pressure is lowered and if the patients' respiratory condition deteriorates, the pressure is increased to the previous setting. Ideally, selection and weaning off the mode and level of respiratory support should be based on bedside, continuous and quantitative individual information on breathing activity or the work of breathing. As the diaphragm is the main respiratory muscle in pre-term infants, retrieving information on its activity might provide objective information on breathing activity. Electrical activity of the diaphragm can be with electromyography (dEMG) and there are currently two methods to detect the electrical signal of the diaphragm; the transcutaneous method in which sensors are placed on the skin and the invasive transesophageal method in which sensors are mounted on a catheter positioned in the esophagus. Recent studies have shown that both techniques are feasible in pre-term infants (45, 46), and are able to detect changes in diaphragmatic activity (47, 48) (**Figure 2**). However, transesophageal dEMG is relatively invasive, expensive and only available on one specific ventilator. The transcutaneous method is less invasive, cheap, and uses stand-alone equipment allowing its use during all modes of respiratory support, independent of the ventilator. Observational studies in pre-term infants have shown that transcutaneous dEMG is able to detect changes in diaphragmatic activity in response to weaning the mode of respiratory support from nCPAP to low flow nasal cannula. Furthermore, the diaphragmatic activity was significantly higher in those infants that failed this transition compared to those in who weaning was successful (49).

In addition to unloading the additional work of breathing, the delivered respiratory support should be, if applicable, synchronized to the individual breathing efforts of the infants. Historically, changes in airway pressure or flow are used for synchronization of invasive mechanical ventilation (50). However, these parameters are not always accurate in the presence of leak, either around the endotracheal tube or via the upper airways during nasal positive pressure ventilation (51). Being independent of airway flow or pressure, dEMG might therefore also be an ideal candidate for triggering respiratory support. Indeed, measuring the electrical activity of the diaphragm by the transesophageal method can be used for synchronizing the individual breaths with the ventilator (52). A Cochrane review performed in 2017 identified one small underpowered randomized control trial comparing conventional ventilation to ventilation triggered by diaphragmatic activity with no significant effects on the primary outcomes. However, the conclusion of that review was that lower level evidence studies, such as case series and non-randomized cross over studies are suggesting a physiological benefit associated with diaphragma

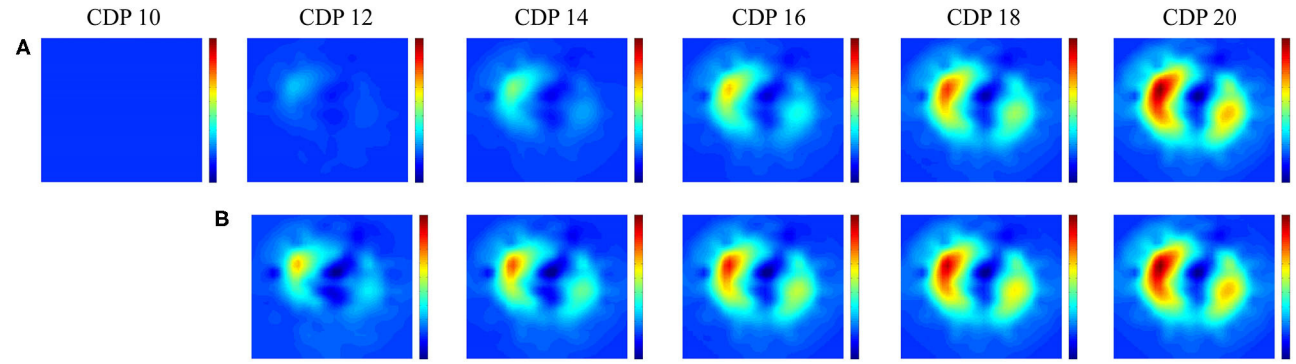


**FIGURE 1 |** Lung recruitment visualized by Electric Impedance Tomography. Change in functional electrical impedance tomography in end expiratory lung impedance during an oxygenation guided lung recruitment procedure in a high frequency ventilated pre-term infant (945 grams). The impedance changes are referenced to the starting pressure of 8 cm $H_2O$. Row **(A)** shows the inflation and row **(B)** the deflation limb of the recruitment procedure (39). All images have the same scale where red indicates a large change and blue a small change in impedance.

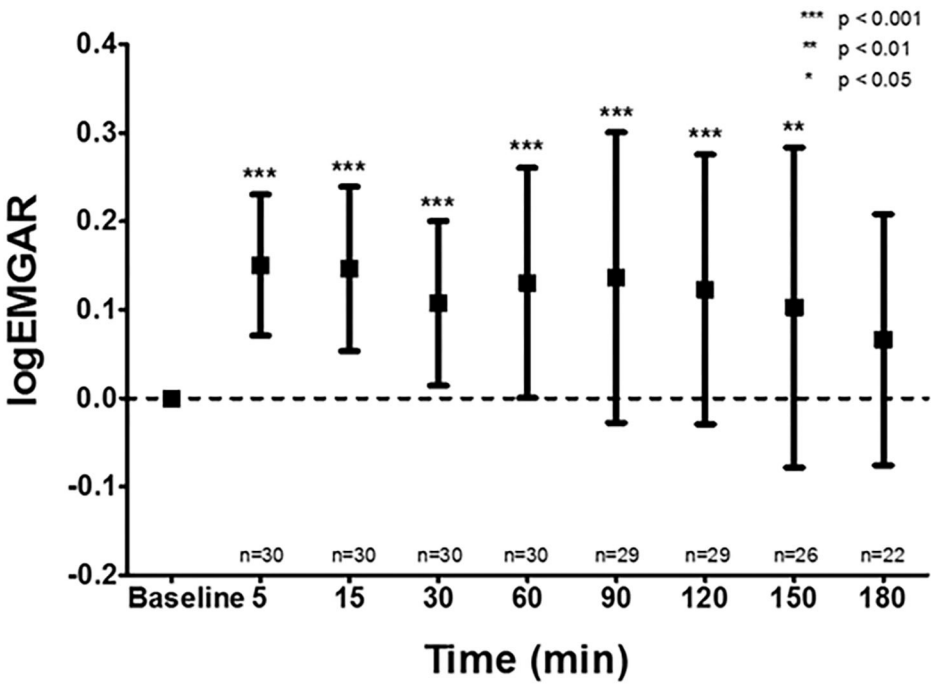


**FIGURE 2 |** Effect of caffeine on the amplitude of the diaphragm measured by electromyography. The transcutaneous electromyographic (EMG) analysis of the diaphragm showed a significant increase in logarithm of the EMG Activity Ratio (logEMGAR) after a loading dose caffeine, compared to baseline. The logEMGAR described the relative changes in EMG activity, either increasing or decreasing symmetrical around unity.

triggered ventilators, and that new high quality randomized controlled studies are urgently needed (53).

Studies have also shown that (spontaneous) breath detection is feasible and appropriate via transcutaneous dEMG (54). Optimal triggering might also have clinical implications, as studies suggest that synchronized nasal positive pressure ventilation reduces the need for invasive mechanical ventilation (55).

It is clear that measuring diaphragmatic activity with dEMG has the potential to individualize the application of respiratory support in pre-term infants. However, more and larger studies are needed to determine which dEMG output parameters provide the best information on the individual patients' needs. Furthermore, transcutaneous dEMG triggering of non-invasive support still needs to be tested in pre-term infants.

## Key Messages

- Most clinicians use a "trial and error" strategy when selecting and setting the mode of respiratory support. Furthermore, synchronizing support to the individual spontaneous

breathing effort is often not optimal, especially during non-invasive support.
- The activity of the diaphragm can be measure with electromyography and provides objective information on the patients' breathing effort. Furthermore, breath detection is feasible and accurate with dEMG.
- dEMG can potentially be used to titrate and trigger the mode and level of respiratory support but future studies are needed to explore these potential indications in pre-term infants.

## PHARMACOGENETICS AND CAFFEINE

Administration of caffeine to pre-term infants is standard of care in all neonatal intensive care units. A large randomized controlled trial provided solid evidence that besides reducing apnea of pre-maturity (56), pre-term infants treated with caffeine have a reduced risk of BPD (57), and an improved neurodevelopmental outcome at 2 years corrected age (58, 59). Despite this clear evidence, there are uncertainties regarding the exact working mechanism of caffeine and the optimal caffeine dose (60). Comparable to the adult population, effective and safe dosing probably differs between pre-term infants (61). Standardized regimens, i.e., the same dose to all infants, lead to high variation of serum levels of caffeine and its metabolites (62). This observation is important because it has been shown that in some infants a high caffeine loading-dose is associated with negative effects on pre-term infant brain development (63). On the other hand, low caffeine concentrations may lead to insufficient apnea treatment, increased use of invasive mechanical ventilation posing a higher risk for BPD and neurodevelopmental impairment (64). Since most studies evaluated the effect of a standardized caffeine dosage regimen on important neonatal outcomes at population level, they were neither able to determine patient-specific risks and benefits nor incorporate these in the evaluation of an optimal dosing regimen. Heterogeneity due to genomic differences likely contributes to the lack of a strong correlation between serum caffeine levels and clinical effects. Precision medicine might help to optimize caffeine dosing and treatment-associated outcomes. Caffeine metabolism is limited in pre-term infants because cytochrome P4501A2 (CYP1A2) enzyme activity is markedly reduced relative to term neonates, leading to a prolonged half-life and increased urinary excretion of unmetabolized caffeine. Enzyme activity increases with increasing gestational age and advancing post-natal age (65). Although the exact molecular mechanisms underlying the benefits of caffeine administration have not been elucidated (57), it is likely that genomic and metabolomic heterogeneity influences optimal patient dose due to infant-specific caffeine metabolism and risk susceptibility (66–68). Several candidate genes have been suggested based on the known genetic associations with caffeine (e.g., cytochrome P450 enzymes, adenosine receptors) (66, 69–72).

It is highly likely that there is a dose-specific response to caffeine treatment in pre-term neonates. Individual genomic variants are a potential indicator for its effectiveness but also the risk to develop complications due to caffeine treatment. Genomic variance probably explains why the correlation between caffeine plasma concentrations and clinical outcome remains poor. An observational cohort study including infants with a gestational age ≤30-weeks and treated with caffeine should be conducted to investigate to which extent genomic variation contributes to this poor correlation, and to what extend adopting individualize treatment in this population can improve outcome. In the future, clinicians may be able to evaluate a genomic profile at birth which they can then utilize to determine a personalized caffeine dose.

### Key Messages

- Caffeine treatment is a well-established evidence-based standard of care treatment for the prevention of BPD.
- Similar to adults, pre-term infants might need individual dosing of caffeine based on their genomic profile.
- Future research should investigate the correlation between genomic variation in caffeine metabolism to optimize and individualize caffeine treatment in pre-term infants.

## VOLATILE ORGANIC COMPOUNDS

Many interventions to reduce the risk of BPD have been tested in randomized clinical trials, but to date few have shown to be effective and safe. It has been suggested that these disappointing results might be caused by failure of clinical variables or biomarkers to accurately predict the risk of BPD at an early stage in life. This is also called a lack of prognostic enrichment: the study population has a low a-priori risk for the outcome of interest and therefore many patients are exposed to the treatment without any chance of a positive effect, limiting efficacy, yet experiencing the side effects, increasing safety concerns (73). Consistent with this assumption, a systematic review summarizing all published clinical prediction models failed to show accuracy in discriminating and calibrating performance after external validation using a large individual patient database (74). Therefore, clinical prediction models are infrequently used in current clinical practice and research.

Besides prognostic enrichment, trials may further benefit from predictive enrichment: increasing the likelihood of the included patient for a beneficial treatment response. Although BPD is multifactorial in nature, inflammation and growth failure are considered important risk factors and mediators in its development. Most BPD associated inflammatory and growth factor biomarkers investigated, such as interleukin-6 or−8, monocyte chemoattractant protein-1, vascular endothelial growth factor, keratinocyte growth factor, angiopoietin 2 and interferon-γ failed as predictors of BPD development (75) emphasizing the complex pathophysiology. Irrespective of this underwhelming result for BPD prediction, a set of biomarkers that identifies a homogeneous group of patients with a shared pathogenesis might serve as a predictive tool for treatment response. However, it is important to emphasize that most evaluated biomarkers require sampling of urine, blood or saliva, and need complicated laboratory analysis techniques. This may hamper implementation in clinical practice as these techniques are usually not available in every hospital. This means that the search for better predictive indices with bedside availability,

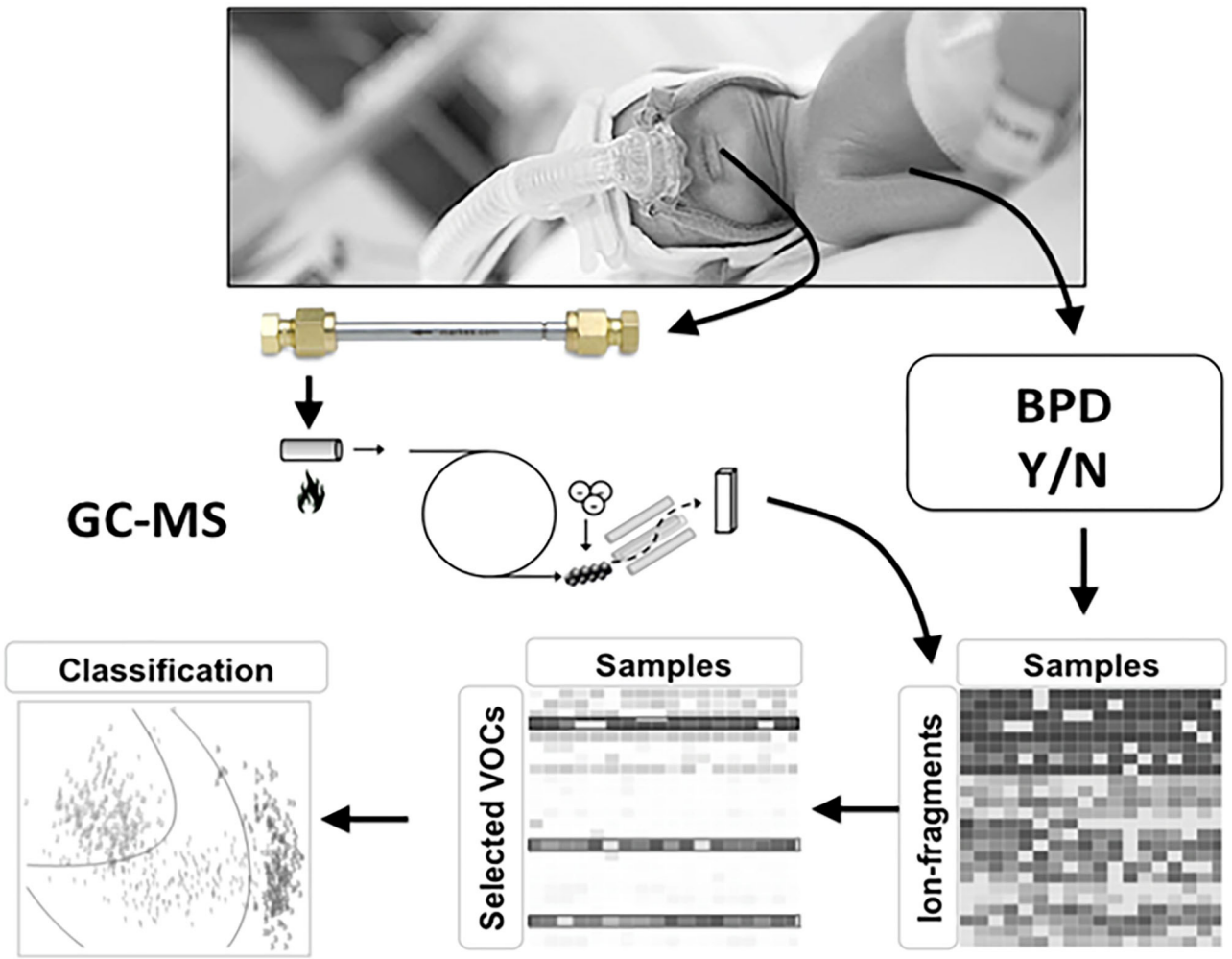


**FIGURE 3 |** Study design for developing BPD specific eNose. Volatile organic compounds (VOCs) are absorbed onto a stainless-steel tube containing Tenax GR 60/80 (Interscience, Breda, The Netherlands) for 5 min at a flow rate of 50 ml/min. The captured VOCs in the tubes are released by re-heating the tubes after which the fragment ions are detected using a quadrupole mass spectrometer (GCMS-GP2010; Shimadzu, Den Bosch, The Netherlands) with a scan range of 37–300 Da. Ion fragment peaks were used for statistical analysis. GC-MS analysis will be performed in all infants.

without the need for complicated laboratory techniques, and preferably without requiring blood needs to be continued.

Exhaled breath might be the medium that meets the requirements for such a prognostic and predictive test in preterm infants. Collection is fully non-invasive and analysis may be rapid when sensor technology is used. Hundreds to thousands of volatile organic compounds (VOCs) have been described in exhaled breath, which represent metabolic processes in the host, bacterial metabolism, and organ function (76). VOCs are reported to serve as potential biomarker in several adult respiratory diseases, such as pleural mesothelioma, pulmonary sarcoidosis, asthma, chronic obstructive pulmonary disease, ventilator associated pneumonia and acute respiratory distress syndrome (77–79).

There are several distinct technologies to analyze exhaled breath of which gas chromatography-mass spectrometry (GC-MS) and electronic nose analysis (eNose) are most frequently used. VOCs can be separated, quantified and identified by GC-MS, which remains the gold-standard for untargeted biomarker analysis in exhaled breath. GC-MS analysis is a time-consuming and off-line analysis, making it unpractical as a clinical prediction instrument. However, it does enable researchers to measure a broad range of compounds and identify unknown compounds semi-quantitatively. A diagnostic study that uses GC-MS for breath analysis will therefore result in a list of potential biomarkers. These potential markers can be used to develop a disease-specific measurement tool based on technologies that can provide rapid, bedside results. One technique that has gained substantial traction is the eNose, which relies of on sensor technology (79). The eNose enables real-time analyses of the patterns of selected VOCs in complex gas mixtures. It does not allow measurement of individual VOCs, but uses pattern recognition to capture composite VOC mixtures by cross-reactive sensors, called a breath-print (80). This device is

highly attractive in daily clinical practice since it can be used at the bedside and because it provides instant results, which is so highly needed in predicting BPD at an early age.

Given the multifactorial etiology of BPD, analysis of exhaled breath of pre-term infants with GC-MS might enable us to quantify the prognostic accuracy of individual and combinations of VOCs in exhaled breath. As BPD has several pathophysiological links to lung injury in adults, we expect that the VOCs might also be equipped to detect markers of BPD. One of the challenges will be the collection of breath in pre-term infants. The minute volume ventilation is quite low and there is a relatively high bias flow delivered by the devices used for respiratory support.

A recent study suggested that the eNose can discriminate pre-term infants developing BPD at an early age from those who do not. However, this study only included mechanically ventilated infants, using headspace analysis of tracheal aspirates rather than exhaled breath (81). Nowadays, more and more infants are initially managed without invasive ventilation and tracheal aspirates are therefore not available in these infants (4). We studied breaths of four pre-term infants on non-invasive respiratory support in a pilot study. Several breath collection techniques were investigated, but the introduction of a suction catheter positioned under the nasal mask was the only technique that provided positive results defined as a range of known human VOCs above the detection limit. We are currently collecting exhaled breaths at multiple time points in 100 pre-term infants born <28 weeks of gestation, treated with both invasive and non-invasive respiratory support (**Figure 3**). The GC-MS analyses will be performed, allowing us to determine which selected set of VOCs to focus on when developing the eNose for identifying infants at risk of BPD within the first week of life. Once the GC-MS analyses of exhaled breath in pre-term infants show which selected VOCs predict BPD, an eNose specific breath print for BPD can be developed following an efficient translation from promising biomarker to established bedside tool for predicting BPD as described in the BEST (Biomarkers, EndpointS, and other Tools) Resource (82). This has to be validated in a large population of pre-term infants in a multicenter setting. After showing a good external validation, the final step will be performing an impact analysis showing the additional value of this prediction model. Another potential application of exhaled breath analysis might be discriminating between different (main) pathophysiological causes of BPD. An appropriate grown pre-term infant born after severe chorioamnionitis might develop a different BPD phenotype than a pre-term infants born after severe intrauterine growth retardation. If the development of the eNose shows promising discrimination between infants with and without the development of BPD, it might also be possible to differentiate in infants with BPD what is the main pathophysiological pathway is and select the optimal treatment accordingly.

### Key Messages

- To date, there is no prediction model based on clinical characteristics or biomarkers with accurate discriminating ability to detect BPD at an early stage.
- Measuring VOCs is increasingly being used in adult respiratory medicine.
- Observational cohort studies should investigate which VOCs in exhaled breath accurately predict BPD at an early stage of the disease.

## LIMITATIONS

First, given the narrative nature of the article, this review has the limitation that we did not identify systematically all publications regarding precision medicine for pre-term lungs. We have selected these four items on precision medicine because the recent publication dates, and are deemed most promising to have an important clinical impact. Second, every of these promising technological, diagnostic or prognostic tools need further development to go from bench to bedside tools, need extensive internal and external validation before submitted to impact analyses in daily practice.

## CONCLUSION

These four presented potential developments in pulmonary research might improve precision medicine in order to prevent development of BPD. EIT and dEMG are bedside monitoring tools with the potential to individualize invasive and non-invasive respiratory support, whereas pharmacogenetic research of caffeine metabolism and VOC analysis of exhaled breath might optimize preventive drug therapy for BPD on an individual basis.

## AUTHOR CONTRIBUTIONS

MM and AVK had specific focus on the section on electrical impedance tomography. WO and AHM-vdZ had a specific focus on the section of pharmacogenetics and caffeine. WO, PB, and LB had a specific focus on the section of volatile organic compounds. JH and AVK had a specific focus on the section of electromyography. All authors provided crucial pulmonary expertise on these sections and the introduction. All authors approved the final version of the paper.

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# Clinical Study of Correlation for the Intestinal and Pharyngeal Microbiota in the Premature Neonates

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**Objective:** There are mutual influences between intestine and lung, that propose a concept of the gut-lung axis, but the mechanism is still unclear. Microbial colonization in early life plays an important role in regulating intestinal and lung function. In order to explore the characteristics of early microbiota on the gut-lung axis, we studied the correlation between intestinal and pharyngeal microbiota on day 1 and day 28 after birth in premature neonates.

**Methods:** Thirteen neonates born at 26–32 weeks gestational age (GA) hospitalized at the neonatal intensive care unit (NICU) of the West China Second Hospital of Sichuan University were enrolled in this study. Stool samples and pharyngeal swabs samples were collected from each neonate on the first day (T1) and the 28th day (T28) after birth. Total bacterial DNA was extracted and sequenced using the Illumina MiSeq Sequencing System based on the V3–V4 hyper-variable regions of the 16S rRNA gene. Based on the sequencing results, the composition of the intestinal and pharyngeal microbiota was compared and analyzed.

**Results:** At T1, the difference in microbial composition between intestine and pharynx was not statistically significant. The intestinal microbiota was mainly composed of *Unidentified Enterobacteriaceae*, *Ralstonia*, *Streptococcus*, *Fusobacterium*, *Ureaplasma*, etc. The pharyngeal microbiota was mainly composed of *Ureaplasma*, *Bacteroides*, *Fusobacterium*, etc. *Ureaplasma* and *Fusobacterium* were detected in both intestine and pharynx. At T28, there was a significant difference in microbial composition between intestine and pharynx ($p < 0.001$). The intestinal microbiota was mainly composed of *Unidentified Clostridiales*, *Klebsiella*, *Unidentified Enterobacteriaceae*, *Enterobacter*, *Streptococcus*, etc. Pharyngeal microbiota was mainly composed of *Streptococcus*, *Rothia*, etc. *Streptococcus* was detected in both intestine and pharynx.

**Conclusions:** The intestine and pharynx of premature neonates have a unique microbial composition, and share some common microbiota. Whether these microbiotas play a role in the mechanism of gut-lung crosstalk needs further study.

**Keywords: intestinal micriobiota, pharyngeal microbiota, 16S rRNA sequencing, preterm neonate, gut-lung axis**

## INTRODUCTION

Intestinal microbiota plays an important role in human health and disease (1). In recent years, numerous studies have shown that infant intestinal microbiota affects the growth and development of children (2, 3), and are associated with neonatal sepsis, neonatal necrotizing enterocolitis (4), childhood obesity (5), asthma, eczema (6), and diabetes (7), hypertension (8), and other diseases in adulthood. It is commonly believed that the uterus is sterile and the colonization of microbiota began after birth. But recent research results suggested that the intestinal microbiota could colonize in neonates before delivery. The concept of sterile uterus has been challenged, but the results are still controversial and worth further study (9, 10). The initial colonization of microbiota in the lung may affect the development of the respiratory system and even lead to disease (11, 12). Chronic respiratory diseases can affect the composition of the intestinal microbiota, and in turn, intestinal microbiota may affected the function of the respiratory system (13, 14). These results suggest that there is a crosstalk between the respiratory system and the intestine, which proposes a concept of the gut-lung axis (15). There are many studies on the gut-lung axis, but the mechanism is still unclear (16). Therefore, it is very crucial to study the microbial characteristics of the intestine and lung for further exploring the mechanism of the gut-lung axis.

The pharynx connects the oral cavity, nasal cavity, lower respiratory tract, and digestive tract. It communicates with the outside world and is exposed to a variety of exogenous and endogenous microbes. Thus, the pharynx is an ecological niche for potentially pathogenic microbe, which may cause local inflammation or lead to lung diseases (17). Previous studies have shown that there was a large amount of overlap between the pharyngeal microbiota and the respiratory microbiota (18), so the pharyngeal microbiota could, to some extent, reflect the characteristics of the respiratory microbiota. Besides, the pharyngeal swab is a fairly accurate method to define the composition of the respiratory microbiota. Therefore, studying the composition and characteristic of pharyngeal microbiota may open a window for exploring respiratory microbiota.

The concept of the gut-lung axis was born out of the observation that different lung diseases can be influenced by intestinal microenvironment changes and vice versa. The microbiota is an important factor responsible for interactions between these two sites in asthma (19). In addition, the intestinal microbiota of patients with severe bacterial pneumonia (20), cystic fibrosis (19), and influenza differs from that of healthy controls (21). Many studies show that early life is the most important period during which microbiota dysbiosis in the intestine may lead to the development of many respiratory diseases, as the intestine microbiota has a significant influence on immune cell maturation and resistance to pathogens (22).

In this study, we took premature neonates with GA <32 weeks as study subjects to analyze intestinal and pharyngeal microbial characteristics and influencing factors. Meanwhile, we studied the correlation between intestinal and pharyngeal microbiota, to provide some evidence for the role of early microbiota in the mechanism of the gut-lung axis.

## PATIENTS AND METHODS

### Study Participants

Premature neonates who were delivered and hospitalized in the NICU of West China Second Hospital of Sichuan University from December 2019 to May 2020 were enrolled. Selection criteria were as follows: GA < 32 weeks; single birth; the birth weight of the infant appropriate for gestational age (between the 10–90th percentile of the average weight of the infant of the same gestational age) (23, 24). Exclusion criteria were as follows: infants with congenital malformations; intrauterine growth retardation; neonatal hypoxic-ischemic encephalopathy; immunodeficiency or severe infectious diseases; mother's antibiotic therapy for more than 3 days within 2 weeks before delivery; the neonate using antibiotics after birth more than 3 days within 2 weeks before delivery; the mother or the neonate used probiotics or prebiotics during perinatal period; and the guardian does not agree to participate or withdraws from the study. The following information was obtained from the medical records: duration of ruptured membranes; prenatal antibiotic use; delivery mode; GA; birth weight; gender. The study protocol was approved by the medical ethics committee of the West China Second Hospital of Sichuan University, and written informed consents were obtained from the parents or guardians of the neonates.

### Sample Collection Method

Stool samples were collected from baby diapers with sterile test tubes at T1 and T28. Pharyngeal secretions samples were collected with sterile pharyngeal swabs within 30 min after birth and T28. Stool and pharyngeal swabs samples were quickly placed in a −20°C refrigerator after being collected, and stored in a −80°C refrigerator within 24 h until further processing.

### DNA Extraction and Amplification

Total genome DNA from samples was extracted using Hexadecyltrimethy Ammonium Bromide (CTAB) method. DNA concentration and purity were detected by a spectrophotometer. According to the concentration, DNA was diluted to 1 ng/μl using sterile water. The bacteria genomic DNA was amplified with the 341F and 806R primers specific for the V3–V4 hypervariable regions of the 16S rDNA gene. All DNAs were amplified by following a protocol described previously (25). Samples were sequenced on an Illumina MiSeq platform according to the manufacturer's recommendations. The sequencing service was provided by Beijing Novogene Genomics Technology Co. Ltd (China).

### Data Processing

Paired-end reads from the original DNA fragments are merged by using FLASH (26), which is designed to merge paired-end reads when there are overlaps between reads 1 and reads 2. Paired-end reads were assigned to each sample according to the unique barcodes. Sequences were analyzed using QIIME software package (Quantitative Insights Into Microbial Ecology), and in-house Perl scripts were used to analyze alpha (within samples) and beta (among samples) diversity. First, reads were filtered by QIIME quality filters. Then we use pick_de_novo_otus. py to pick

**TABLE 1 |** Clinical characteristics of neonates ($N = 13$).

| Subject | Antibiotics, yes/no* | GA, weeks | Delivery, cesarean/ vaginal | Gender, male/ female | Prolonged rupture of membranes, yes/no |
|---|---|---|---|---|---|
| 01 | Yes | 27.6 | Cesarean | Female | Yes |
| 02 | Yes | 27.6 | Cesarean | Female | Yes |
| 03 | No | 29.4 | Vaginal | Male | No |
| 04 | No | 28.1 | Vaginal | Female | No |
| 05 | Yes | 29.9 | Vaginal | Male | Yes |
| 06 | Yes | 29.0 | Cesarean | Male | No |
| 07 | Yes | 29.7 | Cesarean | Male | Yes |
| 08 | Yes | 28.7 | Cesarean | Female | No |
| 09 | Yes | 31.4 | Cesarean | Female | No |
| 10 | No | 31.9 | Vaginal | Male | No |
| 11 | Yes | 26.1 | Vaginal | Male | Yes |
| 12 | Yes | 27.9 | Cesarean | Female | Yes |
| 13 | Yes | 29.9 | Vaginal | Male | Yes |

**Mother's intrapartum antibiotic therapy (duration < 3days).*

**TABLE 2 |** The result of 16S rRNA was amplified from samples (O: microbiota was detected).

| Subject | Meconium (T1s) | Pharyngeal swabs (T1y) | Stool (T28s) | Pharyngeal swabs (T28y) |
|---|---|---|---|---|
| 01 | O | | O | O |
| 02 | | O | O | O |
| 03 | O | | O | O |
| 04 | O | O | O | O |
| 05 | | O | O | O |
| 06 | | | O | |
| 07 | O | | O | O |
| 08 | | | O | O |
| 09 | | | O | O |
| 10 | | | O | O |
| 11 | O | O | O | O |
| 12 | O | | O | |
| 13 | | | O | O |

operational taxonomic units (OTUs) by making an OTU table. Sequences with ≥97% similarity were assigned to the same OTUs. We pick representative sequences for each OTU and use the RDP classifier (27) to annotate taxonomic information for each representative sequence. In order to compute Alpha Diversity, we rarify the OTU table and calculate the Shannon index and Chao1 estimator. QIIME calculates weighted unifrac, which are phylogenetic measures of beta diversity. We used weighted uniFrac for Principal Coordinate Analysis (PCoA). LefSe tool was implemented to identify differentially abundant taxa.

## Statistical Analysis

Continuous variables were reported as means ± standard deviations, and categorical data were presented as ratios or percentages. Non-parametric Wilcoxon rank-sum test was used to study differences in continuous variables and Fisher's exact tests were used to analyze categorical variables. $p < 0.05$ was considered statistically significant.

**TABLE 3 |** Analysis of influencing factors of intestinal and pharyngeal microbial colonization.

| Factors | | Microbiota was detected in meconium | Microbiota was detected in pharyngeal swabs |
|---|---|---|---|
| Antibiotics | Yes (Total: 10) | 4 | 3 |
| | No (Total: 3) | 2 | 1 |
| | *P*-value | 0.559 | >0.999 |
| Delivery | Vaginal (Total: 6) | 3 | 3 |
| | Cesarean (Total: 7) | 3 | 1 |
| | *P*-value | >0.999 | 0.266 |
| GA | >28 w (Total: 9) | 3 | 2 |
| | <28 w (Total: 4) | 3 | 2 |
| | *P*-value | 0.266 | 0.530 |
| Gender | Male (Total: 7) | 3 | 2 |
| | Female (Total: 6) | 3 | 2 |
| | *P*-value | >0.999 | >0.999 |

# RESULTS

## Clinical Characteristics, and Influencing Factors of Microbial Colonization

In this study, 22 infants with GA <32 weeks in the NICU of West China Second Hospital of Sichuan University were included. Based on the exclusion criteria, 9 cases were excluded, and the remaining 13 cases were included in the final analysis (**Table 1**). At T1, 13 meconium samples were collected and included in the T1s group, and 13 pharyngeal swabs samples were collected and included in the T1y group. At T28, 13 stool samples were collected and included in the T28s group, and 13 pharyngeal swabs samples were collected and included in the T28y group. In total, 52 samples were collected from the enrolled neonates, 16S rRNA was amplified from 34 samples (6 samples in T1s group; 4 samples in T1y group; 13 samples in T28s group; 11 samples in T28y group; **Table 2**), and 2,944,688 reads were obtained. At T1, maternal antibiotics, GA, delivery mode, and gender had some effect on the microbial colonization of meconium and pharyngeal swabs, but these were not significant (**Table 3**).

## Comparisons Between Intestinal and Pharyngeal Microbiota

At T1, 1,340 OTUs were detected in the intestine, and 2,404 OTUs were detected in the pharynx, among which 179 OTUs were commonly shared. At T28, 536 OTUs were detected in the intestine, and 1,377 OTUs were detected in the pharynx, among which, 120 OTUs were commonly shared (**Figure 1A**). At T1, there was no significant difference in Shannon index and Chao1 index between the T1s group and the T1y group. At T28

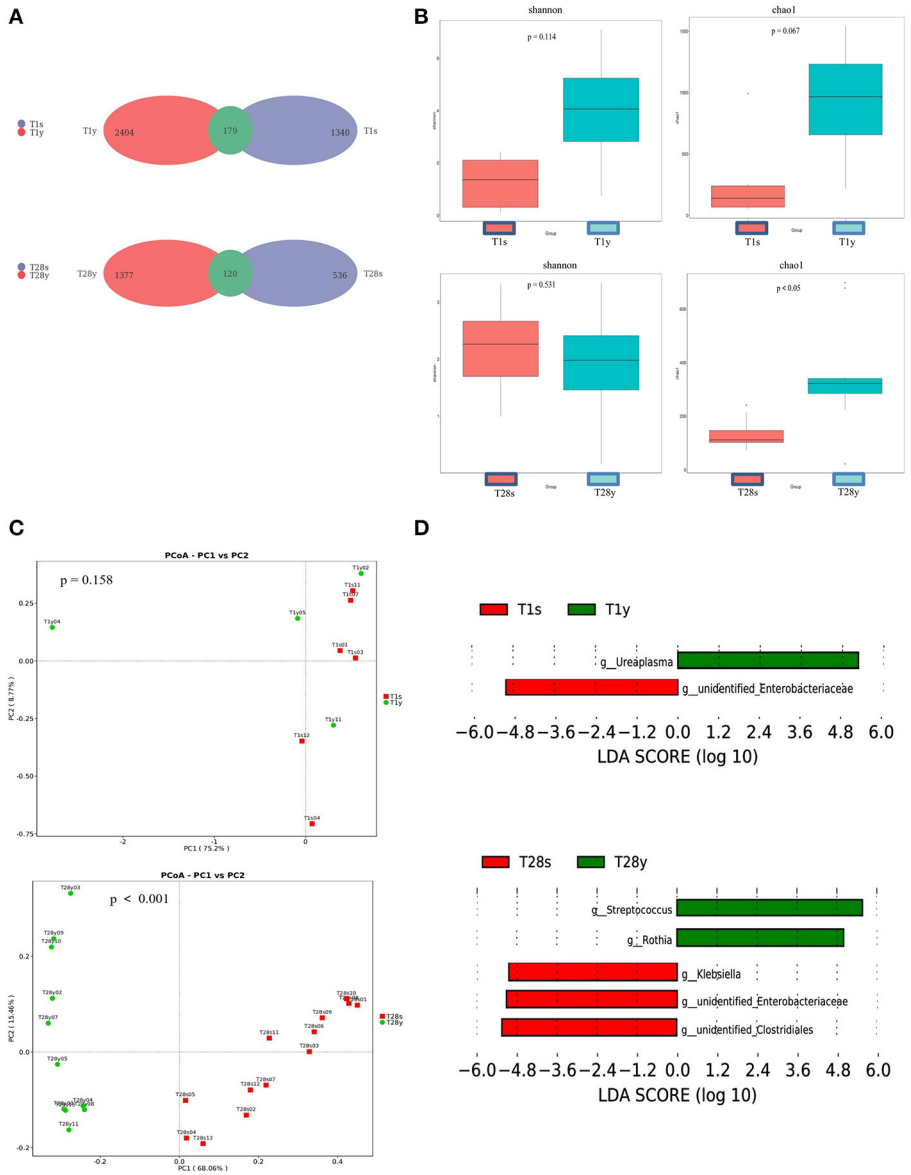


**FIGURE 1 |** Comparisons between intestinal and pharyngeal microbiota. **(A)** OTUs were shared by the intestine and pharynx. **(B)** Comparison of the microbial biodiversity between intestine and pharynx, the shannon index, and chao1 were shown as estimators. **(C)** PCoA plot based on OTU abundance. Each point represents the intestinal or pharyngeal microbiota of a subject. **(D)** Histogram of the LDA scored for differentially abundant genera between intestine and pharynx. LDA scores were calculated by LDA effect size using linear discriminant analysis.

there was no significant difference in Shannon index between the T28s group and the T28y group; the Chao1 index of the T28y group was higher than that of the T28s group, the difference was significant ($p < 0.05$; **Figure 1B**).

In order to compare the composition of the overall microbiota of the intestine and pharynx, PCoA was implemented based on the OTU level. At T1, the results of PCoA showed no difference in microbiota composition between the T1s group and the T1y group. At T28, the results of PCoA showed a significant difference in microbiota composition between the T28s group and the T28y group ($p < 0.001$; **Figure 1C**). Next, the LEfSe tool (28) was used to analyze microbiota in stool samples and pharyngeal swabs samples, and to detect potential significant differences in relative abundances between the intestinal and pharyngeal microbiota. There were significant differences in 2 genera between the T1s group and the T1y group. *Ureaplasma* was relatively more abundant in the T1y group, whereas *Unidentified Enterobacteriaceae* was relatively more abundant in the T1s group. There were significant differences in some genera between the T28s group and the T28y group.

*Streptococcus*, *Rothia* were relatively more abundant in the T28y group, whereas *Unidentified Clostridiales, Unidentified Enterobacteriaceae*, *Klebsiella* were relatively more abundant in the T28s group (**Figure 1D**).

## The Microbial Composition of the Intestine and Pharynx at the Genus Level

We analyzed the microbial composition of the intestine and pharynx at the genus level and found that all samples were dominated by a specific genus (**Figure 2A**). At T1, intestinal microbiota was mainly composed of *Unidentified Enterobacteriaceae* (18.8%), *Ralstonia* (16.7%), *Streptococcus* (16.6%), *Fusobacterium* (14.2%), *Ureaplasma* (11.9%), etc. Pharyngeal microbiota was mainly composed of *Ureaplasma* (38.1%), *Bacteroides* (14.9%), *Fusobacterium* (1.8%), etc. At T28, intestinal microbiota was mainly composed of *Unidentified Clostridiales* (30.0%), *Klebsiella* (19.6%), *Unidentified Enterobacteriaceae* (18.8%), *Enterobacter* (7.0%), *Streptococcus* (2.0%), etc. Pharyngeal microbiota was mainly composed of *Streptococcus* (70.7%), *Rothia* (17.6%), etc. At T1, the relative abundance of *Ureaplasma* between the T1s group and the T1y group was 11.9 vs. 38.1% ($p = 0.053$); The relative abundance of *Unidentified Enterobacteriaceae* between the T1s group and the T1y group was 18.4 vs. 0.08% ($p < 0.05$). At T28,

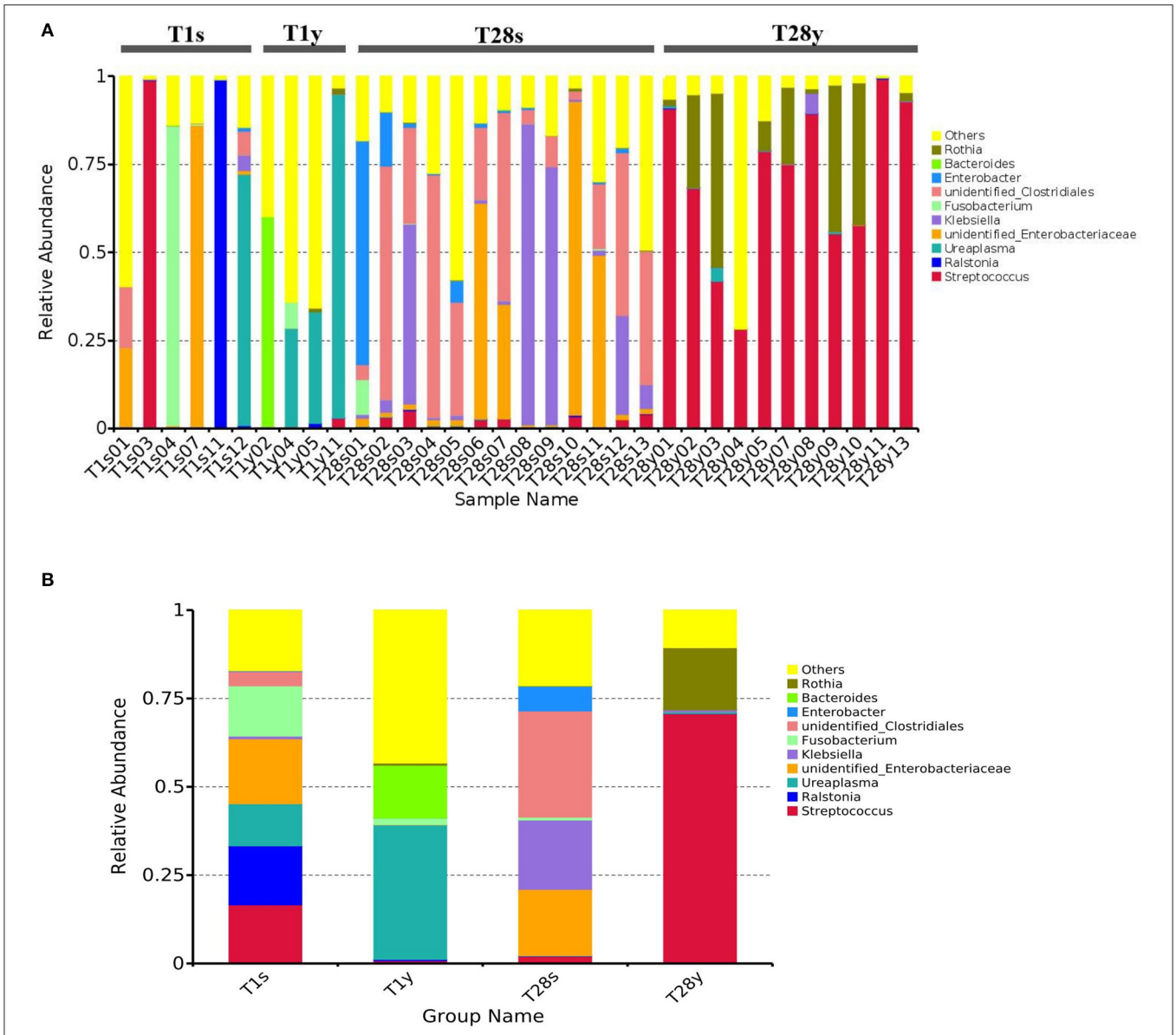


**FIGURE 2 |** The composition of intestinal and pharyngeal microbiota.at the genus level. **(A)** The microbial composition of each sample. **(B)** Average microbiota of T1s, T1y, T28s, and T28y (T1s: meconium samples at T1; T1y: pharyngeal swabs samples at T1; T28s: stool samples at T28; T28y: pharyngeal swabs samples at T28).

the relative abundance of *Streptococcus* between the T28s group and the T28y group was 2.0 vs. 70.7% ($p < 0.05$); the relative abundance of *Rothia* between the T28s group and the T28y group was 0.1 vs. 17.6% ($p < 0.05$); the relative abundance of *Unidentified Clostridiales* between the T28s group and the T28y group was 30.0 vs. 0.002% ($p < 0.05$); the relative abundance of *Unidentified Enterobacteriaceae* between the T28s group and the T28y group was 18.8 vs. 0.02% ($p < 0.05$); the relative abundance of *Klebsiella* between the T28s group and the T28y group was 19.6 vs. 0.5% ($p < 0.05$; **Figure 2B**).

## DISCUSSION

Microbial colonization is a complex and dynamic process. In this study, premature neonates with GA <32 weeks were selected as study subjects. Microbiota was detected in 6 meconium samples and 4 pharyngeal swabs samples at birth, which seems to be a challenge to the concept of a sterile uterus, but it needs to be determined by a larger sample size and the exclusion of more interfering factors. Maternal antibiotics, GA, delivery mode, and gender had no significant effect on the microbial detection rate of meconium and pharyngeal swabs, this is inconsistent with the results of some previous studies that the delivery mode affects the microbiota colonization of the neonates (29). The possible reason is that our sample collection was carried out immediately after birth, and the colonization of environmental microbiota has not been completed.

Through Venn diagram analysis, at T1, intestine and pharynx shared more OTUs than at T2. We may speculate that this pattern of colonization can be driven by the developing host immunity (30), or, alternatively, one pioneer group of bacteria (31) might metabolically prime the environment for replacement by a particular successor. Our observations support the hypothesis that temporal factors influence the microbial colonization of preterm infants, such as there was no significant difference in Shannon index between the T28s group and the T28y group. The Chao1 index of the T28y group was higher than that of the T28s group, and the difference was significant. The results show that the pharyngeal microbiota diversity was higher than that of the intestinal and that the low abundance and rare species are present in the community at T28y group. Through PCoA analysis, there was no significant difference in microbial composition between the T1s group and the T1y group. At T28, there was a significant difference of microbial composition between the T28s group and the T28y group. *Streptococcus*, *Rothia* were relatively more abundant in the T28y group, whereas *Unidentified Clostridiales*, *Klebsiella*, *Unidentified Enterobacteriaceae*, *Enterobacter* were relatively more abundant in the T28s group.

The analysis of microbial composition show that, at T1, the intestine was dominated by *Unidentified Enterobacteriaceae*, *Ralstonia*, *Streptococcus*, etc. among which *Unidentified Enterobacteriaceae* and *Streptococcus* are potential pathogenic microbes, and the rest are mostly pathogenic microbes. It suggests that premature neonates have very few beneficial microbes in the intestine, but a large number of potentially pathogenic microbes and pathogenic microbes, which is consistent with previous studies (32). At T1, pharyngeal microbiota was mainly composed of *Ureaplasma*, *Bacteroides*, *Fusobacterium*, etc, which are similar to the oropharyngeal microbiota of infants (33, 34). *Ureaplasma* was detected in both intestine and pharynx, the relative abundance has no significant difference between the T1s group and the T1y group. This may indicate that the origin of intestinal and pharyngeal microbiota may be the same at birth. The relative abundance and composition of the intestinal and pharyngeal microbiota was significantly different on the 28th day after birth. *Streptococcus* mainly existed in the pharynx, while *Unidentified Clostridiales* mainly existed in the intestine. We analyzed the characteristics of intestinal microbiota at different time, the results show that the intestinal microbiota colonization with beneficial bacteria is delayed in preterm infants, while the number of potentially pathogenic bacteria is high. *Ureaplasma* was relatively more abundant in the T1y group, *Streptococcus* was relatively more abundant in the T28y group. There was significant difference between early and late pharyngeal microbiota. These preliminary and explorative results suggested that the neonatal microbial colonization is a highly dynamic process, which is sensitive to environmental factors.

However, several potential limitations should be taken into consideration. Firstly the sample amount might not be adequate. Secondly, if the characteristics of neonatal microbiota and maternal microbiota were studied together, it is more meaningful to know the initial source of microbial colonization.

In conclusion, the study's results suggest that, in some neonates, the microbiota may exist in both the intestine and the pharynx at birth. There was no significant difference between intestinal and pharyngeal microbiota composition on day 1, but on day 28, site-specific microbial was already established. Despite the initial similarity of pharyngeal and gut microbiotas, the specific bacterial genera were determined in both sites. Further we hypothesize that these genera may contribute to the functioning of the gut-lung axis.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s. https://figshare.com/s/81253de749d49bcb0898.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of West China Second University Hospital, Sichuan University. Written informed consent to

participate in this study was provided by the participants' legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

SY: data collection, management and analysis, and manuscript drafting and revision. LQ and JS: data collection and manuscript revision. LX: site oversight and manuscript revision. YX and HL: study concept and design, interpretation of results, manuscript revision, and final approval. All authors contributed to the article and approved the submitted version.

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REVIEW
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# The Controversy Persists: Is There a Qualification Criterion to Utilize Inhaled Nitric Oxide in Pre-term Newborns?

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Inhaled nitric oxide (iNO) use in premature newborns remains controversial among clinicians. In 2014, the American Academy of Pediatrics, Committee on Fetus and Newborn released a statement that the available data do not support routine iNO use in pre-term newborns. Despite the absence of significant benefits, 2016 California data showed that clinicians continue to utilize iNO in pre-term infants. With studies as recent as January 2017, the Cochrane review confirmed no major advantages of iNO in pre-term newborns. Still, it recognized that a subset of pre-term infants with pulmonary hypertension (PHTN) had not been separately investigated. Furthermore, recent non-randomized controlled trials have suggested that iNO may benefit specific subgroups of pre-term newborns, especially those with PHTN, prolonged rupture of membranes, and antenatal steroid exposure. Those pre-term infants who showed a clinical response to iNO had increased survival without disability. These findings underscore the need for future studies in pre-term newborns with hypoxemic respiratory failure and PHTN. This review will discuss the rationale for using iNO, controversies regarding the diagnosis of PHTN, and additional novel approaches of iNO treatment in perinatal asphyxia and neonatal resuscitation in the pre-term population < 34 weeks gestation.

**Keywords: pre-term infants, pulmonary hypertension, cardiovascular, inhaled nitric oxide, inhaled nitric oxide (iNO), persistent pulmonary hypertension of the newborn**

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## IMPACT STATEMENT

- The report aims to explain why neonatologists prescribe inhaled nitric oxide to premature newborns, despite recommendations against it.
- The article reviews biological factors that may predispose premature newborns to benefit from inhaled nitric oxide.
- The manuscript takes a different approach from systematic reviews. It discusses non-randomized controlled trials to analyze the rare events of pulmonary hypertension in pre-term newborns that appropriately responded to inhaled nitric oxide.

## INTRODUCTION

Nitic oxide (NO) is a known free radical important as a chemical intermediate in multiple environmental reactions (1–4). In the 1990's, Nitric oxide's function was better elucidated as an endothelium-derived relaxing factor and signaling molecule (5, 6). Later, many investigations targeted NO in the cardiovascular, nervous, immune, and gastrointestinal systems, demonstrating its significant regulatory effects in physiological and pathophysiological processes (7–14).

Nitric oxide has been extensively investigated in the fetus and newborn due to its ability to influence basal pulmonary vascular tone (15–17). Term and late-pre-term newborns with poor postnatal vascular adaptation, leading to pulmonary hypertension of the newborn and hypoxemic respiratory failure (HRF), experienced significant benefits after inhaled nitric oxide (iNO) treatment. Systematic reviews confirmed a decreased need for extracorporeal membrane oxygenation (ECMO) and related complications after iNO (18). In 1999, the US Food and Drug Administration approved iNO therapy for HRF in term and late pre-term newborns (19). Subsequently, researchers investigated the benefits of iNO in pre-term infants. The investigations focused on iNO use in reducing chronic lung disease development in extremely premature infants. These randomized controlled trials showed mixed results, and no consensus has been achieved for recommending iNO use in pre-term infants <34 weeks gestation. This review discusses the findings and controversies regarding iNO administration in pre-term newborns. A focused PubMed® search with the term *nitric oxide* appearing with either *pre-term* or *premature* resulted in 272 articles through February 2020. Case report and case series were excluded. Retrospective case controls, comparative or larger population-based studies were the primary source of this analysis showing clinical improvement after iNO exposure.

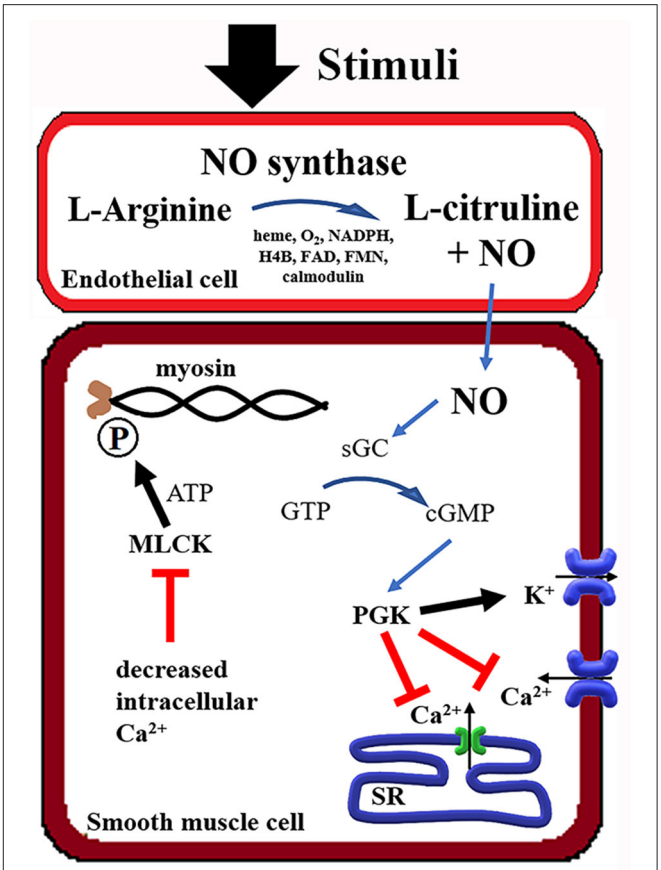


**FIGURE 1 |** Summary of the Nitric Oxide (NO) mechanism in smooth muscle relaxation. NADPH, Nicotine adenine dinucleotide phosphate; H4B, tetrahydrobiopterin; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate; sGC, soluble guanylate cyclase; PGK, phosphoglycerate kinase; SR, sarcoplasmic reticulum; MLCK, myosin light-chain kinase; ATP, adenosine triphosphate.

## PHYSIOLOGY AND PRE-TERM NEWBORN CONSIDERATIONS FOR INHALED NITRIC OXIDE TREATMENT

### Vascular Mechanism of Action

Nitric oxide released by endothelial cells, which covers the entire vascular system, acts on adjacent smooth muscle cells by paracrine signaling, causing vasodilation (20, 21). Nitric oxide is produced by nitric oxide synthase (NOS), which has three different gene isoforms. Neuronal NO synthase (nNOS), cytokine-inducible NOS (iNOS), and endothelial NOS (eNOS), as the name suggests, have a preferential expression, but all participate in vascular tone regulation (22). Different stimuli, including acetylcholine, bradykinin, histamine (calcium-dependent), and shear stress, 17β-estradiol (calcium-independent), induce NOS phosphorylation and production of nitric oxide. NO is generated when L-arginine is converted to L-citrulline, which requires multiple co-factors as heme group, oxygen ($O_2$), nicotine adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin (H4B), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and calmodulin (23–27). In the smooth muscle cells, nitric oxide combines with soluble guanylyl cyclase (sGC) and promotes the formation of cyclic guanosine monophosphate (cGMP) (28). Cyclic GMP mobilizes protein kinase G, decreasing intracellular calcium by stimulating calcium reuptake by the sarcoplasmic reticulum (SR) and opening calcium-activated potassium channels (29). The reduction of intracellular calcium decreases phosphorylation of myosin by myosin light chain kinase (MLCK), causing muscle relaxation (30, 31) (**Figure 1**). The arterial vasodilation effect of iNO in pulmonary circulation maximizes perfusion in well-aerated airspaces, improving oxygenation, and decreasing ventilation-perfusion mismatch (32).

Inhaled nitric oxide supposedly has a half-life of 2–6 s since it is degraded by various mechanisms (33–35). However, it directly affects the lung endothelium by diffusion, reaching the alveolar lining (36). As pre-term newborns have limited peripheral vascular tone control and may present with left ventricular

dysfunction, side effects, including systemic hypoperfusion, must be considered during iNO administration (37–40).

## Other Effects of Nitric Oxide

Nitric oxide has multiple effects on cellular function, which can be protective or detrimental in pre-term newborns during major organogenesis (41). These conflicting results demonstrate that iNO should be used judiciously in clinical practice. The most complicating factor is that most of the studies were conducted *in vitro* or in animal models that are not easily extrapolated to pre-term human newborns.

### Toxicity vs. Protective Effective

Nitric oxide is a free radical (NOx). In mammalian tissues, it can be converted to other toxic compounds, including peroxynitrites ($ONOO^-$), nitrogen dioxide ($NO_2$), nitrous acid ($HNO_2$), and S-nitrosothiols (SNOs). Such compounds cause DNA breakage, lipid peroxidation, protein oxidation, protein nitration, and mitochondrial respiratory enzyme inhibition, leading to apoptosis and necrosis (42–51). One important discussion in pre-term infants is the interaction between nitric oxide and surfactant. In animal experiments with iNO exposure $>48$ h at doses of $>$ 80 ppm, there was a decrease in surfactant adsorption. In contrast, the surfactant's lipid peroxidation was decreased in isolated surfactant complex exposed to nitric oxide during surface cycling due to reduced conversion of surfactant to small vesicles (52). It is worth noticing, in term newborns, no significant surfactant function change, cytokine profile, or lipid peroxidation has been identified in analyzed airway specimens after iNO treatment. Furthermore, one study detected cell damage markers related to nitric oxide 10 days after the end of iNO treatment, which could be related to endogenous nitric oxide other than iNO (53). Another toxic iNO effect is methemoglobin (metHb) (54). Term and pre-term infants are at higher risk for this condition due to easier fetal hemoglobin oxidation and metHb reductase system immaturity (55, 56). However, metHb above 5% (outside safety range) is rare in newborns of all gestational ages (GA), as long as the iNO dose is below 40 ppm (57–59).

On the other hand, in multiple *in vitro* and animal studies, nitric oxide had protective effects according to environmental factors. Endogenous NO produced in response to reactive oxygen species due to pathogens and pathophysiological conditions served as a free radical scavenger and decreased peroxide-mediated cell damage (51, 60–62). Furthermore, in mature rats and 1-month-old ventilated pigs, iNO decreased inflammatory response by inhibiting the NFκβ pathway (63, 64). In another rat model, iNO reduced leukocyte adhesion and vascular permeability dysfunction in the mesenteric venules (65). Also, in a swine model of cardiopulmonary bypass, iNO decreased interleukin−8 (a chemoattractant) and induced early programmed apoptosis, reducing long-term inflammation (66, 67). The inflammatory regulation of iNO may be beneficial in combating pathogens in the airway. In a rat model of pneumonia, iNO decreased signs of inflammation and improved bacterial clearance (68). In another study in patients with cystic fibrosis and antibiotic resistance microbes, clinical improvement was noticed after iNO exposure (69).

### Cellular Effects

Nitric oxide can regulate multiple mechanisms and cellular functions, as shown in *in vitro* animal model studies. For example, Cook et al. and Nakaki et al. showed that NO-producing vasodilators decrease DNA synthesis through cAMP and cGMP signaling (70, 71). Furthermore, in rat's smooth muscle cells, induction of endogenous NO played a role in the depression of growth cells (72, 73). In other *in vitro* studies, NO-releasing agents interfered with essential immunologic system pathways by inhibiting cytochrome P450 (74, 75). Even though these studies were not related to NO's inhaled administration, a similar mechanism occurs *in vivo* after iNO exposure in the lung endothelium and smooth muscle.

Another considerable iNO side effect is the modification of platelet function with increased bleeding time in newborns, as shown by George et al. (76). Such an effect can predispose pre-term patients to intracranial hemorrhage, pulmonary edema, and pulmonary hemorrhage. However, a metanalysis of pre-term infants exposed to iNO had no increased complications from bleeding (77).

### Alveolar and Lung Development

Animal trials of iNO showed a promising effect in improving lung development, which drove clinical trials of the inhaled treatment in pre-term newborns. Following are some of the findings that justified such human trials. Newborn rats exposed to hyperoxia have impaired alveolarization. The use of iNO restored growth factors known to participate in developing the airway, like fibroblast growth factor and vascular endothelial growth factor (78, 79). Curiously, Lopez et al. showed rats treated with iNO had similar mortality, lower weight gain, and capillary alveolar density than non-treated animals (78). On the other hand, Lin et al. showed enhanced lung growth and alveolarization in the recovery period if animals were treated with iNO at 10 ppm (79).

In animal models of pre-term ventilated lambs, Kinsella et al. exposed their animals to iNO for a short period of time after birth (3–4 h). Treated animals had a decrease in neutrophil migration, no significant changes in vascular permeability, and improved gas exchange (80). These findings were expanded by Bland et al. with a model of chronic lung disease. Lambs were ventilated and treated for 3 weeks. Animals in the iNO group had improved radial alveolar count and capillary surface density (81).

McCurnin et al. applied a similar strategy to a baboon model of neonatal chronic lung disease. Improvement was noticed in pulmonary-to-systemic blood flow ratio (Qp/Qs), ventilation index, lung compliance, and expiratory resistance, mostly in the first 8–10 days after iNO exposure. Yet, after the day of life 10, there was no statistical significance, indicating the initial benefit only for early and short use of iNO, but not chronic administration. However, lung weight and DNA content were improved after the study if animals were treated with iNO for a total of 14 days (82).

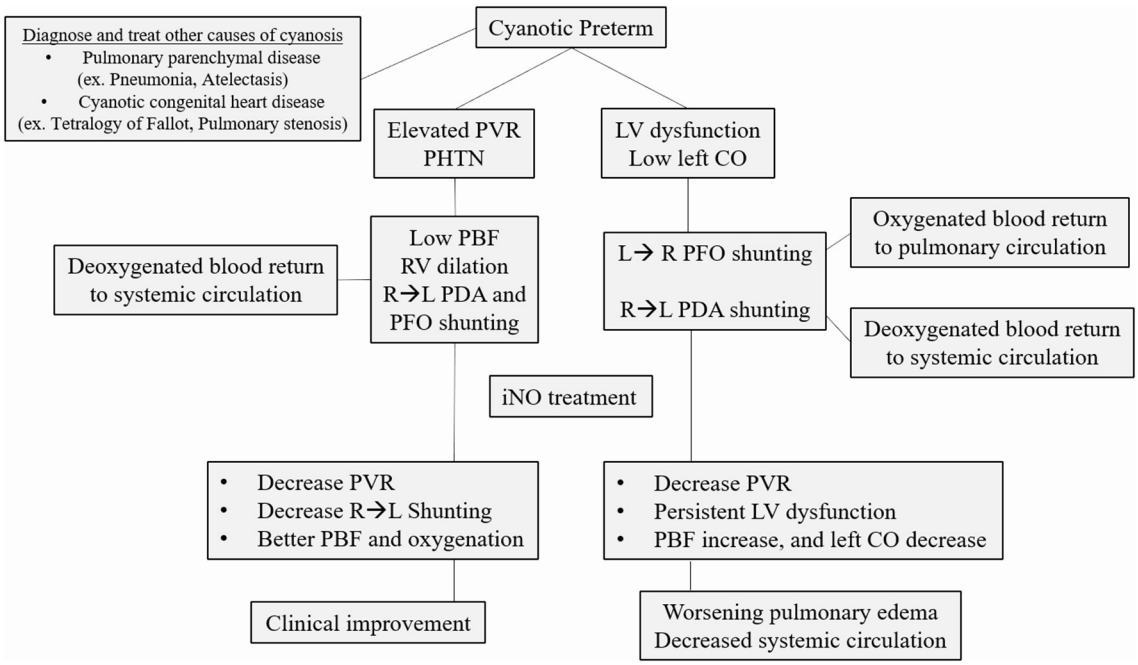


**FIGURE 2 |** Common causes of cyanosis in the pre-term infant. After ruling out pulmonary parenchymal disease and cyanotic congenital heart disease, the clinician should determine the primary cause of hypoxic respiratory failure. If elevated pulmonary vascular resistance (PVR) with a right to left shunt (RL) is the primary determinant of cyanosis, inhaled nitric oxide (iNO) is the recommended treatment. If there is significant left ventricle (LV) dysfunction, not related to RV dilation from PHTN, iNO can decrease left cardiac output (CO) even further, worsening the clinical status. PBF, Pulmonary blood flow; PHTN, pulmonary hypertension; PFO, patent foramen ovale; PDA, patent ductus arteriosus.

### Cardiovascular Effects

In adult studies, when used in pulmonary hypertension associated with right heart failure, inhaled nitric oxide has been shown to lower pulmonary vascular resistance (PVR) and improve left ventricle (LV) output (83). However, when used in patients with LV dysfunction, the resultant increase in pulmonary venous blood flow further compromised LV function and decreased cardiac output. One exception is LV dysfunction related to RV dilation due to PHTN and major bulging to the interventricular wall compressing the LV cavity (84). For this reason, neonatologists have to understand the hemodynamics and the cause of the hypoxic failure before using iNO. Patients with high PVR causing right-to-left shunt at the PDA level will benefit from pulmonary vasodilator as iNO. If the right to left shunt is related to LV dysfunction or low systemic vascular resistance (SVR), inotropic drugs and volume expansion can reverse this shunt by increasing arterial blood pressure, and iNO could be detrimental if used as a primary treatment agent (**Figure 2**) (85–87). Another consideration, PHTN rebound after iNO discontinuation, is not commonly reported but is a potential complication before full endothelium recovery (88–90).

#### *Summary*

The nitric oxide role of vascular relaxation is well-determined in the developed lung. Animal and human data still demonstrate the controversial role of iNO in inflammation and toxicity. Furthermore, the clinician should identify the cause of the hypoxic failure in the pre-term infant since iNO can be detrimental if used in patients with primary LV dysfunction.

## Physiologic Considerations of Pulmonary Hypertension in Pre-term Newborns

At any GA, the fetus PVR is elevated compared with systemic vascular resistance (SVR) since the lungs have constricted vessels and are fluid-filled *in utero*. After birth, PVR reduction occurs due to air entry, establishing functional residual capacity, higher $PaO_2$, decreased lung fluid, and multiple chemical pathways, including intrinsic nitric oxide (91). The reduction in PVR/SVR ratio diminishes right-left shunting and increases blood flow to the lungs, resulting in improved gas exchange. In situations where this transition is impaired, PHTN may develop, for which iNO is the current standard treatment in late pre-term and term infants (92, 93).

Multiple factors are involved in PVR regulation after birth in pre-term newborns. Nevertheless, the anatomical and physiological lung structure of the pre-term lung may influence iNO response (94–97). Primarily, the endothelium of lung vasculature is underdeveloped, and the lack of intra-acinar arterial muscular layer is the most significant barrier for proper iNO response (98, 99). For example, in fetal lamb studies, increased PVR and response to oxygen are likely related to better vascular smooth muscle development with the advancement of GA (100, 101).

### Summary

Inhaled NO can facilitate a decrease in PVR by smooth muscle relaxation. However, the pre-term lung vasculature is underdeveloped to respond to the vasodilatory effects of iNO appropriately.

# MAJOR CONTROVERSIES OF iNO USE IN PRE-TERM NEWBORNS

## Current Recommendations vs. Utilization of iNO in Pre-term Newborns

The existing literature on iNO in term and late-pre-term newborns in the presence of PHTN has raised a question about the possible benefits of the same drug in newborns ≤ 34 weeks GA. Early case reports proposed the benefits of iNO in pre-term infants with HRF (102–105). As discussed above, some animal models of lung injury have shown positive results, with enhanced lung growth and development, when exposed to iNO (78–81). These findings suggest that iNO could be used as a preventive agent for bronchopulmonary dysplasia (BPD). On the other hand, no pre-clinical trial has investigated the ideal GA to offer iNO since pre-term lungs may lack the structures necessary to respond to the treatment properly (101).

Prematurity-related complications are not decreasing because of increased survival of extremely pre-term infants (106), including infants with BPD (107, 108). According to the Institute of Medicine, prematurity's overall financial burden reached $26 billion in 2005. To combat this increased burden, many investigators focused on overcoming these complications in pre-term neonates by utilizing iNO to prevent BPD, as well as a treatment for acute HRF.

### Current Recommendations

The first randomized placebo-controlled iNO trials were published in 1999. The results demonstrated the efficacy of iNO in improving oxygenation in HRF, but it failed to improve survival or BPD in pre-term newborns (109, 110). Other randomized trials have been published with similar or contradictory results, leading to the first Cochrane review in 2010. This systematic review was divided into three categories based on initial iNO exposure and the following criteria; (1) initial 3 days of life based on hypoxia criteria, (2) initial 3 days due to pulmonary disease, and (3) later recruitment according to an elevated risk of BPD. No statistically significant positive results were found in any of these groups for decreases in BPD, infant mortality, or adverse events, such as an increase in intraventricular hemorrhage (IVH) or neurodevelopmental impairment (111). Besides, two other independent comprehensive data reviews, JHU EPC and MAPPiNO, found no significant benefits for the pre-term newborns exposed to iNO (112, 113).

It should be noted that these studies varied in iNO dosing, age at first dose, and treatment duration. Despite these irregularities, an NIH Consensus and the Committee on Fetus and Newborn published a policy statement that the current data do not support the routine use of iNO in pre-term infants (114, 115). The consensus made it clear that subpopulation analysis, including demographics (gestational age, ethnic groups) and medical factors (pulmonary hypertension and lung hypoplasia), were not sufficiently evaluated. Therefore, targeted clinical trials are needed with predefined subgroups (116). The latest Cochrane review concluded that no significant benefits are gained when pre-term infants are exposed to iNO. As with the earlier studies, these studies did not evaluate the use of iNO for common medical conditions, such as PHTN, in pre-term newborns (117).

### Increasing Use of iNO in Pre-term Newborns

Inconsistent with published recommendations, 2016 data from California showed that 2.61% of newborns ≤34 weeks GA had been treated with iNO. The highest percentage of utilization was in newborns with gestational ages from 22 to 24 6/7 weeks GA (10.6% in regional centers). Furthermore, newborns < 27 weeks GA had the highest increase in iNO exposure since 2007 (118).

According to Manja et al. a national survey showed that most neonatologists continue to prescribe iNO to pre-term newborns based on the imperative that physicians should "do something/do everything" to treat HRF on maximal ventilatory support (119). Taken together, these data make clear that clinicians remain uncertain about the possible benefits of iNO in the vulnerable population of extremely pre-term infants, mainly due to a lack of studies on its efficacy in treating specific medical conditions in this population. This underscores the importance of specifically targeted clinical trials that are needed to resolve this uncertainty.

#### *Summary*

Despite the lack of evidence for using iNO in pre-term newborns, the use of iNO continues to increase.

## Pulmonary Hypertension Diagnosis in Premature Newborns

The benefits of iNO in pulmonary hypertension in term infants are very well-described. However, diagnosis and characterization of pulmonary hypertension in premature neonates are incredibly challenging due to the presence of intra- (patent foramen ovale-PFO) or extra-cardiac shunts (patent ductus arteriosus–PDA) and continued change in hemodynamics according to gestational age. Multiple echocardiography parameters have been beneficial as modified shunt blood flow allows accurate hemodynamic assessment and can be used to follow response to the prescribed therapy.

PHTN is consistent with a right-left communication or a bidirectional PDA shunt >60% right-to-left (120). When shunts are not present or conclusive, other indirect measures, such as tricuspid regurgitation jet, leftward/flattened intraventricular septum, right ventricular dysfunction or dilation, and low left ventricular output, are used to indicate the presence of PHTN. Unfortunately, there is no normative data for post-conception age due to the multitude of factors implicated in shunt direction as systemic blood pressure, PFO size, right and left ventricular function, and pathology causing elevated PVR (85). In 2016, Levy et al. used echocardiogram-derived

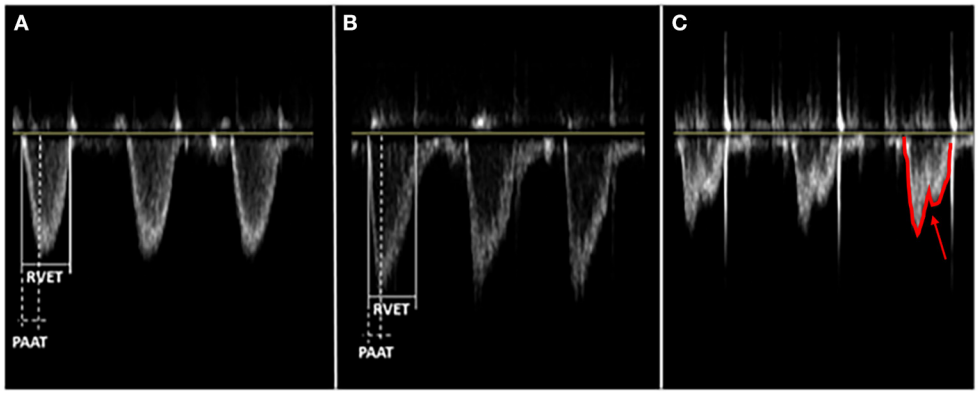


**FIGURE 3 |** Different main pulmonary artery (MPA) flow velocity profile patterns depend on the pulmonary vascular resistance (PVR). PVR is an index ratio of right ventricular ejection time (RVET) and pulmonary artery acceleration time (PAAT), both obtained from the right outflow tract's pulse-wave doppler. PAAT, as shown, is the interval from the onset of ejection through the pulmonic valve to the peak flow velocity. These are three distinct patterns of flow velocity envelope: **(A)** Parabolic or normal PVR - Isosceles triangle, RVET:PAAT <4; **(B)** Right-angle triangle or increased PVR - RVET:PAAT>4; **(C)** Notched pulmonary artery doppler or severe increase in PVR - two distinct peaks (arrow) from midsystolic flow deceleration.

pulmonary artery acceleration time (PAAT) with 95% feasibility, 97% sensitivity, and 95% specificity when compared with right heart catheterization in diagnosing children (121). In 2019, Patel et al. demonstrated that PAAT could reliably be used in pre-term newborns (122). Due to the recent validation of PAAT, no studies presented in this review used PAAT as criteria for diagnosing PHTN (**Figure 3**).

Considering that the echocardiography diagnosis of PHTN is challenging in pre-term infants, adding clinical and echocardiography parameters may maximize the accuracy of diagnosing PHTN (85). The classical findings of a differential oxygen saturation >10% between pre-and post-ductal with pre-ductal being greater is often used to diagnose the presence of right-to-left shunts (85). The absence of differential saturation does not exclude the presence of a right-to-left shunt if the shunting is predominantly at the atrial level.

In recent years, the combination of clinical findings and echocardiogram results triggered the development of targeted neonatal echocardiography (TnECHO) or functional echocardiogram by neonatologists. This patient's overall evaluation provided novel physiologic insights and permitted individualized care of these neonates. For example, Ahmed et al. developed a scoring system using echocardiogram findings associated with clinical findings to better diagnose PHTN in pre-term newborns in a cohort study, specifically using the relationship between right ventricular and systolic pressure (123). Such a scoring system requires bedside evaluation of the neonatologist from the TnEcho team. The real-time assessment of the hemodynamic changes, early pathological situations assessment, and response to treatment interventions improves the outcomes of pre-term neonates (124–127). Still, it is essential to mention the importance of the pediatric cardiologist's echocardiogram or review of the echocardiogram done by TnEcho to rule out anatomical congenital heart disease.

## Summary

Diagnosis of PHTN in pre-term newborns clinically or by echocardiogram continues to be controversial, but with the development of TnEcho better outcomes could be expected.

# A PERSONALIZED EVALUATION AND TREATMENT OF PHTN IN PRE-TERM NEWBORNS

In the era of personalized and precision medicine, prevention and treatment strategies are increasingly sensitive to individual differences (128). One recent application of specialized medicine in the pre-term newborn population is the Askie et al. meta-analysis showing that African-American pre-term newborns respond better to iNO therapy than do infants of other racial groups (129). This finding suggests that large studies with average data across a diverse population, regardless of their unique medical status, can mask the specificity of the benefits in subsets of patients (130). Therefore, we evaluated non-randomized controlled trials (RCT) studies showing the benefits of providing iNO to pre-term newborns. Though the quality of evidence in these non-RCT studies is limited, continued evaluation of available retrospective or prospective data may give new insight into the apparent conflicts between studies and in differing clinical practices.

Following the rationale of precision medicine, we identified and then narrowed the range of clinical indications for which iNO could be recommended to decrease side effects while reducing costs. The rationale that iNO inhalation might benefit a subset of pre-term newborns was based on existing case reports and series. Initially, the investigated pre-term infants presented with PHTN and HRF associated with pre-term premature rupture of membranes (PPROM), as this was proposed to cause PHTN in term and pre-term newborns, possibly by chest

compression or decreased distal airway pressure (103, 131–134). However, the observation that iNO and effective ventilation management dramatically improve oxygenation in newborns with a short period of PPROM is not always consistent with the theory of severe lung hypoplasia. In fact, retrospective studies found a correlation of PPROM >120 h as a poor predictor for iNO treatment failure (135, 136). Aikio et al. showed that PPROM patients decreased nitrites and nitrates, which improved after iNO administration. The vasodilation that proposedly improved oxygenation does not explain the increase in lung compliance and ventilation (137). Likely the same relaxation of smooth vascular cells could be applied to acinar smooth muscle cells but has not been evaluated in term and pre-term infants (138). Pre-term infants can still present with similar pathophysiology as term babies due to slow or complicated pulmonary transition, which can benefit from iNO (139).

The limiting factor to this research is that this patient subset (pre-term newborn with acute PHTN) is frequently underdiagnosed if early echocardiogram assessment is not pursued. With the advancement of TnECHO techniques, placebo-controlled clinical will be possible, as shown in recent PDA treatment trials (140). One example, Cheng et al. utilized TnECHO in newborns <34 weeks GA to determine early PHTN diagnosis (141). Their findings were consistent with other studies showing that early iNO initiation in pre-term newborns more than 1,000 g produce better outcomes than does in smaller newborns with late-onset HRF attributable to sepsis

Another critical factor is the safety of iNO in pre-term infants. Both Carey et al. and Ellsworth et al. indicated that pre-term newborns receiving iNO within 7 days of birth had no increase in mortality (142, 143). Furthermore, most of the studies presented here reinforced the safety of iNO, even when no benefit was found.

The findings of these non-randomized studies, however, remain equivocal. To provide maximal clarity, they will be grouped and reviewed by the following characteristics: GA and BW, chronological age, inclusion criteria, dose of iNO, clinical features that predict better iNO response, and outcomes. This will allow readers to apply these findings to clinical practice appropriately. **Table 1** summarizes the key elements of each reference.

## Gestational Age and Birth Weight

This discussion focuses on pre-term newborns <34 weeks GA and <2,500 g. However, some of the retrospective studies also addressed BW limits that involved late-pre-term newborns. In all studies, the most common finding was that newborns with birth weight above 750 g were more likely to benefit from iNO administration. *Post-hoc* analysis of two large RCTs, the PINO trial and the Large Preemie Pilot trial, found that newborns with BW >1,000 g had lower mortality than newborns with lower BW (146). Other retrospective studies suggested that newborns>1,000 g were more likely to benefit from iNO (136, 145). Conversely, three studies reported that newborns as small as 750 grams could still benefit from iNO, likely because of receiving surfactant before iNO initiation (123, 135, 147). Since most studies focused on birthweight, few studies mentioned GA as a limiting factor. The three studies' threshold was around 27–29 weeks, which could correlate with lower BW, as discussed above (136, 145, 150).

### Summary

Newborns with a birth weight of ≥750 g and older than 27 0/7 weeks GA may benefit from iNO.

## Time and Duration of iNO Administration

The goal of all non-RCTs presented in **Table 1** was to evaluate the effect of iNO on acute PHTN and HRF. Consequently, newborns receiving iNO for BPD prevention and BPD with PHTN were excluded. The patients analyzed were under 4 weeks of age. Pre-term newborns benefitting most from iNO administration were those treated between the first hour of life and 3 days of life (123, 135, 144, 147, 148). Treatment duration also correlated with hypoxia improvement after treatment initiation. The included studies varied in the criteria used to indicate a clinical response to iNO administration; however, a decrease in $FiO_2$ by 0.15–0.30 or an increase in $PaO_2$ by at least 20 mmHg were the most common outcome measures. OI was not reported in all studies. Kettle et al. indicated a median improvement in $FiO_2$, $PaO_2$, and OI, even when non-responders were included (149). Most studies reported clinical response latencies between 30 min and 6 h of treatment onset (135, 145, 147–150). Due to its short half-life and rapid clinical effect, $FiO_2$ and $PaO_2$ should be assessed no later than 6 h after iNO treatment initiation. iNO discontinuation and dose adjustments should be considered to decrease potential side effects and eliminate unnecessary costs in newborns with inadequate iNO treatment response.

### Summary

iNO should be started early in the disease course, preferentially before 72 h of life. Evaluation of treatment response should be done within the first 6 h of treatment.

## Inhaled Nitric Oxide Dosing

The most common iNO dosing in the studies analyzed was between 10 and 20 ppm due to the risk of methemoglobinemia with higher dosing (57, 58). iNO dosing was extensively evaluated in multiple RCTs; dosing outside this range should be closely monitored (117). Only one study, Uga et al. used a higher dose range, between 30 and 40 ppm. However, this group did not find an increased incidence of IVH, BPD, or metHb above 2% in the treatment group (144). Three of the studies analyzed iNO dosing as low as 5 ppm. These protocols adjusted doses up to 20 ppm. Except by Rallis et al. (protocol increased up to 20 ppm in all patients), it is unclear how many patients had iNO increased to the maximum dose or if any clinical benefits resulted from iNO adjustment (145, 146, 148).

### Summary

iNO dosing should begin between 5 and 10 ppm in pre-term infants. There is no evidence of clinical benefits with dosing above 20 ppm, and the risk for side effects increases at higher doses.

**TABLE 1 |** Characteristics of pre-term infants that responded to iNO treatment.

| References | Type of study | GA and/or weight | *N* | Inclusion criteria | PHTN on echo | Intervention | Significant findings | Major weakness |
|---|---|---|---|---|---|---|---|---|
| Uga et al. (144) | Retrospective comparative study | <1,500 g | 18 | PPROM >5 days; HRF; PEEP>8 | 7 out of 7 | iNO 30 ppm, up to 40 ppm; < 24 h of life | iNO increased PaO2 and survival at 28 days | Small N; no echocardiogram in controls. |
| Kumar et al. (145) | Retrospective, case-control analysis | <37 wks | 61 | Echocardiogram evidence of PHTN up to 4 weeks of life | 61 | iNO 5 ppm, up to 15 ppm | Newborns >1,000 g were more likely to respond to iNO; Non-responders have higher mortality | No echocardiogram in the control group. |
| Chock et al. (146) | Retrospective *post hoc* subset analysis from PiNO trial & larger preemie pilot | <34 wks, <1,500 g (PiNO) & <34 wks >1,500 g (large preemie) | 12 | Pulmonary hypoplasia; PPROM or oligohydramnios. iNO vs. $O^2$ placebo | 4 out of 5 | iNO 5 ppm, up to 10 ppm | iNO group: increased PaO2; decreased ventilation and oxygen days | Small N; echocardiogram not done routinely. |
| Chandrasekharan et al. (136) | Retrospective comparative Study | <34 wk | 93 | iNO use in first 28 days of life | Not clear | iNO 20 ppm; < 28 days of life | iNO responders, responded early with better survival. PPROM and antenatal steroids predicts survivors | Echocardiogram not done routinely. |
| Baczynski et al. (135) | Retrospective cohort study | <35 weeks | 89 | iNO for early PHTN at <3 days of life | Echocardiogram done, but not detailed | iNO 20 ppm; <3 days of age after acute PHTN was diagnosed by echocardiogram or clinical diagnosis | Responders: females, PPROM, received surfactant, 1st DOL, PaO2 improvement at 1 h; responders had higher survival and lower disability. | No control group, echocardiogram not done routinely. |
| Dani et al. (147) | Retrospective cohort Study | <30 wks, <1,250 g | 42 | Severe RDS despite surfactant | 28 out of 42 | iNO 20 ppm, up to 40 ppm, | PHTN group had faster and better improvement after iNO; responders had a higher birth weight (>750 g), FiO2 ≥ 0.65, PPROM did not have a better response | Small N to evaluate responder vs. non-responders with or without PHTN. |
| Rallis et al. (148) | Retrospective cohort Study | <34 wks | 55 | HFR with evidence of PHTN | 52 out of 52 | iNO 5 ppm, up to 20 ppm. PHTN by echocardiogram or clinical diagnosis | PHTN and oligohydramnios had better response; early PHTN (<72 h) had higher survival. PPROM alone did not have a better response to iNO | No control group |
| Kettle et al. (149) | Retrospective observational study | < 34 wks | 72 | Pulmonary hypoplasia treated with iNO | 30 out of 44 | iNO 20 ppm | Non-responders had higher mortality. PHTN by echo did not predict response to iNO | Voluntary data submission to a registry. No control group. No clear definition of pulmonary hypoplasia. |
| Rhine et al. (150) | Retrospective registry analysis | < 34 wks, mean of 27.1 wks GA | 431 | Received iNO ≤7 days of life | Echocardiogram done, but not detailed | iNO 20 ppm | 60% improvement, mostly in the 1st h. 99.5% presented with HRF and PHTN. | No control group. Echocardiographic findings not described. |
| Ahmed et al. (123) | Retrospective Cohort study | <36 weeks | 213 | FiO2 ≥ 0.6, OI ≥ 10, Echocardiogram within 24 h of iNO initiation | 53 out of 73 | Targeted neonatal echocardiogram (TnECHO) followed by iNO and vasopressors/inotropes | Presence of PHTN on echo and treatment before 72 h of life had better response. IVH III/IV higher in the iNO group. | TnECHO not done in all patients. Head US not standardized. |

*N, number.*

## Clinical Characteristics Predicting Improvement After iNO Treatment

Identifying the best iNO response predictor in pre-term newborns varies among studies. Differential inclusion criteria and research methodologies (cohort vs. case-control) prevent the identification of reliable recommendations. For example, Uga et al. and Chock et al. used PPROM as a typical inclusion criterion. While most patients received an ECHO to document PHTN, ECHOs were not always done on controls. In both studies, iNO was shown to improve $PaO_2$ with increased survival and decreased ventilator support days (144, 146). Chandrasekharan et al. and Baczynski et al. followed pre-term newborns receiving iNO from birth and reported outcomes. Both studies identified PRROM >18 h as a significant predictor for improvement after iNO. Interestingly, patients with PPROM exceeding 120 h had lower iNO treatment response (135, 136). Kumar et al. proposed that patients exposed to PPROM with oligohydramnios and low Apgar scores were more likely to develop PHTN. Newborns with PHTN responded better to iNO treatment, with higher survival rates than non-responders in all conditions except sepsis-induced PHTN (145). Conversely, studies focusing on HRF and PHTN as inclusion criteria did not consistently identify PPROM as a common characteristic of iNO responders. For example, Dani et al. and Rallis et al. evaluated patients with HRF or severe RDS despite surfactant administration. Patients diagnosed with PHTN had a better iNO response than non-PHTN patients. However, PPROM did not predict iNO response. Alternatively, oligohydramnios was a positive predictor of iNO treatment (147, 148). It should be noted that not all patients with PPROM develop pulmonary hypoplasia unless they have prolonged oligohydramnios.

One obvious but often overlooked finding is that pre-term newborns with PHTN were more likely to respond to iNO treatment than were newborns without ECHO confirmed PHTN (123, 147). Also, patients exposed to prenatal steroids and receiving surfactant postnatally had better iNO responses, possibly related to improved ventilation from surfactant, ultimately maximizing ventilation-perfusion (V/Q) ratio (135, 136). Clinicians dealing with pre-term newborns progressing toward HRF should also know that $FiO_2$ of 1.0 is not required before considering iNO. Dani et al. showed significant improvement in pre-term newborns receiving $FiO_2$ between 0.6 and 1.0 with a baseline of 0.77 (147). This provides evidence for starting iNO therapy before PHTN advances to an irreversible status.

### Summary

Pre-term newborns with PPROM, oligohydramnios, and echocardiography or clinical PHTN diagnosis are more likely to benefit from iNO if they progress to HRF. The surfactant should be given before iNO if clinically indicated. iNO should be started before $FiO_2$ reaches 1.0.

## Positive iNO Response Predicts Better Outcomes

The current literature shows a positive iNO response (PiR) from 43 to 78% in pre-term newborns with HRF and even better response rates for newborns with PHTN diagnosis who receive early iNO treatment (123, 135, 136, 147, 148, 150). However, not all studies included long-term outcomes other than clinical improvements, such as $FiO_2$ decrease or $PaO_2$ increase. Studies analyzing long-term disabilities and survival showed that PiR experienced benefits in addition to oxygenation improvement.

Survival rates for PiR exceeded those of iNO non-responders (NiR) in four different studies. Survival rates were 74 vs. 33% (148), 88 vs. 70% (136), 80 vs. 41% (149), and 66 vs. 29%, respectively, for PiR and NiR (135). The latter study further compared mortality by comparing early (<7 days) and late (>7 days) iNO exposure, finding significantly higher survival rates for those receiving iNO in the 1st week. Furthermore, PiR had lower IVH grades and PVL (2.8 vs. 28.5%) and higher disability free-survival at 18 months (51 vs. 15%) (135, 136). Other comorbidities, such as cerebral palsy and chronic lung disease, were similar in PiR and NiR (135, 149).

### Summary

iNO responders are more likely to survive than were non-responders. Responders may experience lower neurodevelopmental disability rates but with similar chronic lung disease rates.

# NOVEL iNO USE IN PREMATURE NEWBORN RESUSCITATION

This review found that many controversies remain in pre-term newborn care, including delivery room resuscitation. Presently, optimal $FiO_2$ in premature newborns during resuscitation is unknown (151). Current resuscitation guidelines recommend maintaining target oxygen saturation for both term and pre-term infants (152, 153). Despite this, pre-term infants may be exposed to high oxygen concentration during resuscitation to maintain optimal oxygen saturation targets (154). The morbidities associated with increased oxygen concentration exposure in premature infants are well-known (155). The primary emphasis of current NRP guidelines is on ventilation to facilitate pulmonary vasodilation. However, there is no alternative to such measures for non-responders. Hence, iNO may be an adjunct therapy to decrease PVR and reduce excessive oxygen exposure. In the neonatal lamb model of asphyxia and studies of premature newborns, lower $FiO_2$ use during resuscitation after birth decreased oxidative stress (156, 157). Therefore, the administration of iNO may facilitate less oxygen exposure to premature newborns during neonatal resuscitation by improving V/Q mismatch. This novel hypothesis was studied in a randomized controlled pilot trial in premature newborn infants between 25 0/7 and 31 6/7 weeks GA. Premature infants were randomized to oxygen + nitrogen as a placebo or oxygen + iNO starting at 20 ppm and titrated down during the first 17 min of resuscitation. The results showed a trend toward lower oxygen exposure in the oxygen + iNO group, with significantly less cumulative oxygen exposure than the oxygen-only group. Besides, the placebo group had significantly higher

exposure to hyperoxia ($FiO_2$ > 60%) than did the treatment group. There were no differences in the secondary outcomes measured, including IVH. This first pilot human trial suggested that the administration of iNO during resuscitation is feasible and decreased exposure to supplemental oxygen in premature infants (158). It should be noted that an acute reduction in PVR during the transition with iNO may increase the pulmonary venous return and increase organ perfusion, particularly to the brain, increasing the risk of IVH. Therefore, caution should be exercised when iNO is administered during early adaptation. A larger randomized trial is needed to validate this novel approach further.

## CONCLUSION

Non-randomized controlled trials suggest that inhaled nitric oxide can be a treatment option for pre-term newborns with hypoxic-respiratory failure associated with pulmonary hypertension. To achieve better outcomes, we advocate for early assessment with echocardiogram or TnECHO for the certainty of diagnosis and prompt treatment with iNO. However, the debate on the risks and benefits of iNO in pre-term newborns is far from over. Investigations of pathophysiology, pharmacology, and pharmacogenetics are needed to validate such treatment in pre-term newborns with pulmonary hypertension.

## AUTHOR CONTRIBUTIONS

FV, MM, and KS contributed to the concept, design, literature review, and writing of the article. All authors contributed to the article and approved the submitted version.

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REVIEW
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# Stratified Management for Bacterial Infections in Late Preterm and Term Neonates: Current Strategies and Future Opportunities Toward Precision Medicine

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Bacterial infections remain a major cause of morbidity and mortality in the neonatal period. Therefore, many neonates, including late preterm and term neonates, are exposed to antibiotics in the first weeks of life. Data on the importance of inter-individual differences and disease signatures are accumulating. Differences that may potentially influence treatment requirement and success rate. However, currently, many neonates are treated following a "one size fits all" approach, based on general protocols and standard antibiotic treatment regimens. Precision medicine has emerged in the last years and is perceived as a new, holistic, way of stratifying patients based on large-scale data including patient characteristics and disease specific features. Specific to sepsis, differences in disease susceptibility, disease severity, immune response and pharmacokinetics and -dynamics can be used for the development of treatment algorithms helping clinicians decide when and how to treat a specific patient or a specific subpopulation. In this review, we highlight the current and future developments that could allow transition to a more precise manner of antibiotic treatment in late preterm and term neonates, and propose a research agenda toward precision medicine for neonatal bacterial infections.

**Keywords: neonatal bacterial infection, diagnostics, antibiotic stewardship, precision medicine, late preterm and term neonates**

## BACKGROUND

### Neonatal Bacterial Sepsis and Infections

Bacterial infection can lead to sepsis, a state in which dysregulation of the hosts' response to the infection leads to potentially fatal organ dysfunction (1). Consensus on specific criteria to define this state in neonates is still lacking, and hitherto the most common proxy definition of neonatal sepsis is the presence of a positive blood culture (indicating bacteremia), or a positive cerebrospinal fluid culture (indicating meningitis). This paper will acknowledge the limitations of this proxy definition by incorporating the uncertainties it carries when making clinical decisions,

demonstrating how precision medicine can help with those decisions and highlighting how a future consensus definition can further advance precision medicine in treating neonatal sepsis (2, 3).

Neonatal bacterial infections (bacterial infections presenting in the first 28 days of life) affect an estimated 3.0 million neonates yearly, resulting in significant morbidity and mortality (4–6). Early-onset sepsis (EOS), defined as bacteremia within the first 72 h after birth, affects an average of 10 per 1,000 live births among neonates born below 33 weeks of gestation. Lower incidences of 0.73 and 0.56 per 1,000 live births are seen among late preterm neonates [gestational age (GA): 34–36 weeks] and term neonates (GA $\geq$ 37 weeks), respectively (7). Although the incidence and mortality remain much higher among extreme preterm neonates, the absolute number of cases of EOS is higher among late preterm- and term neonates, since prematurity (GA $<$ 37 weeks) affects about 11% of total live births of which 85% occurs in the late preterm period (GA 32–37 weeks) (8). Pathogens associated with EOS include both Gram-positive and -negative pathogens with significantly higher rates of Gram-negatives, especially *Escherichia coli* (*E. coli*) infections among preterm neonates compared to term neonates. In contrast, Group B *Streptococcus* (GBS) infections seem to affect term neonates more frequently (7). Late-onset sepsis (LOS) involves infections occurring >72 h after birth. However, the onset of late-onset GBS infection is frequently defined in literature as an infection that occurs >7 days after birth (9). LOS develops due to contact of the host with environmental organisms and includes both hospital-acquired infections (nosocomial infections) and community-acquired infections. Causative pathogens include skin commensals such as coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus*, and gut-associated microbiota such as *E. coli*. The latter is thought to reach the bloodstream through translocation across the immature intestine (10). CoNS can be pathogenic, especially for preterm neonates, and these infections are therefore often seen in hospitalized preterm neonates undergoing invasive procedures or with intravenous catheters (5, 11).

## Host Susceptibility

The neonatal immune system is a complex network, constantly adapting and undergoing an age-dependent maturation during gestation and after birth. It is shaped by intra- and extra uterine exposures such as antigens, medication, and environmental factors, necessitating both immunotolerance (to prevent immunoreactivity between mother and fetus) and pro-inflammation (infection protection) (12). Reviewing the neonatal immune system and the development of neonatal sepsis is beyond the scope of this review. Several excellent reviews have been published elaborating on this topic (13–15). In summary, in the presence of a pathogen, the host' immune system dysregulates; alternating phases of hyper inflammation ("cytokine storm"), potentially causing multi-organ failure, and immunosuppression (window for opportunistic infections). As pathogen exposure *in utero* is limited, and thus memory function is lacking, neonates primary rely on innate immunity and maternal transplacental immunoglobulin G (IgG) in early life (13).

Consequently, neonates are vulnerable for infections. The differences between preterm and term neonatal immune development do partly explain the differences observed in infection and sepsis incidence and severity between both groups. However, this does not explain interpatient variability in infection susceptibility seen within each group. Many late preterm and term neonates, fortunately, develop only mild symptoms when exposed to a pathogen. But a small group of late preterm and term infants, without any apparent co-morbidities, does develop severe infection (16).

## Balancing Under- and Over-Treatment

In case of a clinical suspicion of neonatal bacterial infection, empirical therapy using intravenous administration of broad-spectrum antibiotics is generally started without further delay. Although lifesaving in case of a true infection, unnecessary and inadequate antibiotic use has many downsides for both patient and health care system including gut microbiome alterations, multi-drug resistance and costs (17, 18). The balance between timely and proper diagnosis and overtreatment of neonatal infections remains a daily clinical challenge. This is illustrated by the fact that, in most cases, antibiotics can be discontinued after 36–48 h when clinical and laboratory signs are reassuring, or are continued in the presence of clinical and laboratory signs of infection, despite culture negativity (culture-negative infection) (19, 20). As a result, a substantial number of late preterm- and term neonates are exposed to intravenous antibiotics in their first weeks of life and antibiotics are among the most prescribed drugs on the neonatal medium and intensive care units (NICU) (11). Wide variation in neonatal antibiotic exposure between countries and hospitals, unexplained by infection rates, demonstrates the difficulty in ascertaining neonatal infection This, together with the differences in susceptibility, highlights the need for precision medicine in neonatal sepsis (21, 22).

## Precision Medicine

The term precision medicine has emerged in the last years. Yet, no consensus definition exists and many other terms such as "personalized" or "stratified" medicine are used interchangeably. However, experts view precision medicine as a novel, improved concept that goes beyond the personal doctor-patient relation (23). Precision medicine is viewed as a way to identify, stratify, and treat patients using large-scale data that relate to the underlying causes of their disease (24). It implicates deep phenotyping of patients in which information is gathered at

**Abbreviations:** EOS, early-onset sepsis; GA, gestational age; *E. coli*, *Escherichia* coli; GBS, group B *Streptococcus*; LOS, late-onset sepsis; CoNS, coagulase-negative staphylococci; NICU, neonatal intensive care unit; CRP, C-reactive protein; HRV, heart rate variability; HRC, heart rate characteristics; MIC, minimal inhibitory concentration; MIPD, model-informed precision dosing; PK, pharmacokinetics; PD, pharmacodynamics; Cmax, maximum concentration; AUC, area under the curve; %*f*T, percentage of time fraction of the free, unbound concentration; TDM, therapeutic drug monitoring; IgG, immunoglobulin G; IVIG, iv immunoglobulins; PTX, pentoxifylline; PCT, procalcitonin; RNA, ribonucleid acid; MMP8, metalloproteinase-8; TNF-$\alpha$, tumor necrosis factor-alpha; PROM, premature rupture of membranes; ETEC, enterotoxigenic *e.coli*; SMS, Sepsis MetaScore.

different levels ("big data") and involves the use of clinical- and life style data, omics and biomarkers. Collected data are used for the development of algorithms and models for disease or therapy risk assessment, screening, diagnosis, treatment selection, prognosis, prevention, and surveillance or monitoring. Those tools allow a more tailored and targeted therapy (24). Precision medicine is not yet widely practiced in the field of neonatal sepsis. However, recent studies evaluating different tools for neonatal sepsis have been performed and results could be a step toward better understanding of the disease-specific pathophysiology (25).

This could be achieved through the use of newer techniques, such as "*omics*," in addition to conventional methods. The suffix *-omics* generally refers to the biotechnology that characterizes and quantifies biological molecules and structures at different levels of an organism. It compromises *genomics*, *transcriptomics*, *proteomics*, and *metabolomics* and allows detection of a "unique barcode" that could predict the underlying response to infection for an individual patient (26). We will discuss some of the current "omics" findings applicable to late preterm and term neonates.

## Genomics

Variation in the host genetics could partly explain the variability in disease susceptibility. Genetic signatures or polymorphisms have been discovered for several infectious diseases. One of the well-known examples is malaria, where patients suffering from haemoglobinopathies are protected against malaria because of an altered erythrocyte structure (27). Another example, applicable to the pediatric population, is the PAI-1 polymorphism in meningococcal sepsis. PAI-1 is an acute phase protein and elevated concentrations correlate with disease severity and mortality. The 4G/4G PAI-1 polymorphism is associated with higher concentrations of PAI-1, and thus worse prognosis, compared to other genotypes (28). Sex has also been found to influence sepsis susceptibility and outcome as reflected in the increased vulnerability to infections and higher sepsis mortality in male neonates compared to females. The X-chromosome encodes multiple genes related to the immune system. Moreover, it is involved in the generation of the sex hormones, of which estrogen has showed to influence several pathways of innate immunity, possibly explaining a better sepsis outcome in females compared to males (29–31). Finally, pharmacogenomics allows us to study genetic polymorphisms associated with pharmacokinetics or –dynamics (drug response).

## Transcriptomics

Transcriptomics refers to the study of the ribonucleic acid (RNA) transcripts allowing to study changes in gene expression over time or under certain circumstances, such as sepsis. Studies using next-generation sequencing and RNA-sequencing have showed that differences are present in gene expression between septic and non-septic neonates with overexpression of genes related to innate immunity and inflammation [CD177; Matrix metalloproteinase-8 (MMP8); tumor necrosis factor-alpha (TNF-α)] (25, 32). Moreover, Cernada et al. reported that genome wide expression profiles differ between Gram-negative and Gram-positive sepsis (32). A recent published study by Ng et al. reported whole blood transcriptomic profiles of very preterm infants ($n = 18$) with proven, possible, and no sepsis. Significant differences were seen in gene expression between proven and no sepsis cases. Altered genes were associated with cytokine signaling, pattern recognition and metabolism (33). With regard to gestational age, Cernada et al. (32) reported no differences in gene expression between very preterm- and late-preterm and term neonates. However, the proportion of late preterm and term neonates included in the study is low, and those are, in most cases, suspected sepsis episodes, not culture proven sepsis. Wynn et al. (34) reported significant differences between the transcriptome of septic neonates and that of septic infants and older children, illustrating an association with developmental age (35).

## Proteomics

Protein-coding genes eventually lead to the expression of specific proteins, and the structure, function and interaction of those proteins can be studied revealing potentially useful biomarkers for neonatal sepsis. One of the advantages of proteomics is that these new biomarkers can be discovered through a hypothesis-free approach as more than a thousand proteins and modifications can be screened using mass spectrometry (25).

## Metabolomics

The metabolome includes all low molecular weight molecules produced by the human body and is considered to be a reflection of a patient's phenotype and real-time physiological condition (36). Metabolomic perturbations due to a higher energy demand and oxidative stress during sepsis can therefore be used as possible predictors or biomarkers for neonatal sepsis (37, 38). Only a few studies have used metabolomics in neonatal sepsis revealing different metabolic pathways involved in neonatal sepsis. Levels of metabolites of energy and glucose metabolism (glucose, glutamine, and lactate) were significant altered in septic neonates (39, 40).

## Microbiome

The microbial community of the gut ("gut microbiome") is shown to be an important influencer of health and disease. It protects from potential pathogens through both the development of barrier function and by shaping the immunological and metabolic pathways. Alterations in early life have been associated with several diseases, such as asthma and obesity, at a later age (41, 42). Significant differences are seen between the gut microbiome of preterm neonates compared to that of term neonates, independent of other environmental factors ("exposome") that influence bacterial colonization such as the mode of delivery, type of feeding or the administration of antibiotics (43). Decreased bacterial diversity in preterm neonates is associated with LOS, although the microbiome was reported to be highly variable in time (44, 45). Moreover, the causative pathogen retrieved in blood culture is often the most dominant species present in the gut microbiota. A recent prospective study by El Manouni et al. (46) showed that the causative pathogen could already be detected 3 days prior to LOS

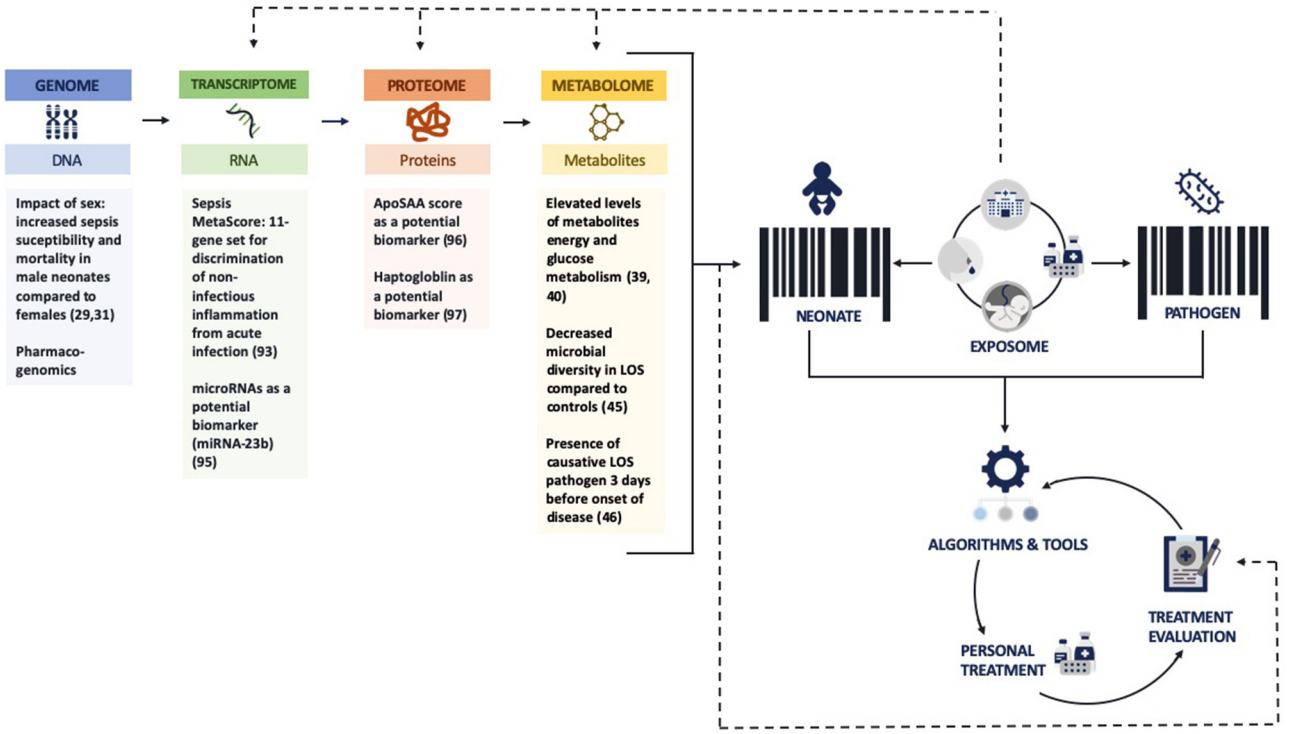


**FIGURE 1 |** Concept of precision medicine and -omics techniques and its potential for neonatal bacterial infections. ApoSAA score, Serum Amyloid A (SAA) and Apolipoprotein (Apo)C2 score; LOS, late-onset sepsis. Figure (with all the icons) is created using https://www.flaticon.com/.

onset in fecal samples. These finding support the hypothesis of bacterial translocation, gut dysbiosis, and the occurrence of LOS.

In the next paragraphs, we will elaborate on the current and future options to move toward a more stratified approach in the antibiotic management of proven and probable bacterial infection, focusing on late preterm and term neonates. These options include a wide variation of antibiotic stewardship programs and guidelines, clinical decision tools, pharmacological advances, biomarkers, and prevention strategies. We separate these options for each stage of decision-making that can be personalized: prevention, treatment initiation, treatment modality and optimization, and treatment duration. **Figure 1** shows the concept of precision medicine and the use of -omics techniques with some recent findings relevant for neonatal bacterial infections. **Figure 2** illustrates opportunities for precision medicine in neonatal bacterial infections during different phases of disease management. Finally, **Table 1** presents a research agenda toward more precise medicine for neonatal bacterial infections.

# PREVENTION

## Current Strategies

Mother-to-child transmission is considered to be the main route of transmission for EOS. In the presence of risk factors such as premature rupture of membranes (PROM) and signs of sepsis before birth, broad spectrum antibiotics are usually administered to the mother. To date, intrapartum prophylactic antibiotic therapy is only used in mothers with risk factors for GBS and has showed to be an effective way to decrease the transmission and incidence of GBS by 50–80% (47). However, several components and effects of this strategy are under debate, especially in the context of late preterm and term pregnancies.

First, considerable variation exists in maternal GBS colonization testing strategies (21). For instance, the American Academy of Pediatrics recommends universal antenatal GBS colonization testing in all pregnant women, and the use of intrapartum antibiotic prophylaxis in case of GBS colonization (48). In contrast, countries like the United Kingdom, Switzerland, and the Netherlands, opt for a risk-based management and only screen and treat high risk pregnancies (49). A disadvantage of the risk-based approach can be that cultures may be obtained too late to allow timely prophylaxis, whereas universal screening in the third trimester may not reflect the actual colonization status at birth. Second, the evidence with regard to optimal dose and timing of intrapartum antibiotics is evolving; it appears that the duration prior to birth is less important than previously thought (50). Third, there are concerns and uncertainties about potential adverse effects of intrapartum antibiotics on the neonate, such as perturbations of the developing microbiome (51, 52). A promising development for precision medicine in preventing

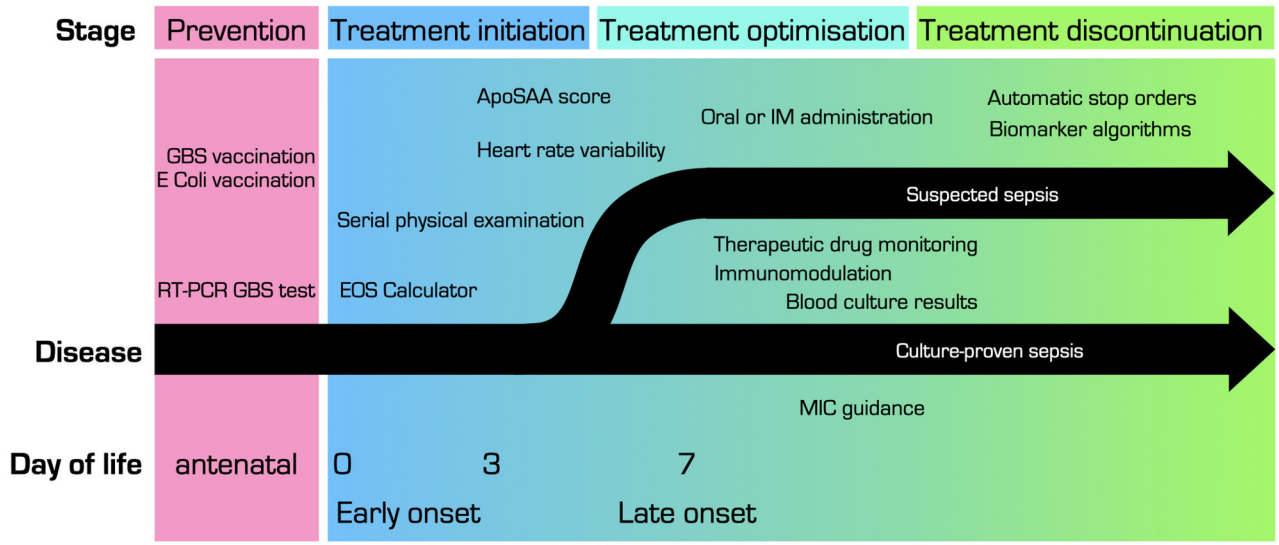


**FIGURE 2 |** Overview of opportunities for precision medicine in treatment of (suspected) neonatal bacterial infection, at different stages of disease. ApoSAA score, Serum Amyloid A (SAA) and Apolipoprotein (Apo)C2 score; *E. coli*, *Escherichia coli*; EOS, early onset sepsis; GBS, Group B streptococcus; IM, intramuscular; MIC, minimal inhibitory concentration.

neonatal EOS has been the application of point-of-care molecular testing for GBS colonization (53), which is likely to allow for quick and reliable qualification of maternal GBS colonization status and thereby facilitating more precise prophylaxis on admission. Validation and confirmation studies evaluating timing and dosage of intrapartum antibiotic prophylaxis as well workflows involving molecular testing are necessary to further improve this prevention strategy and minimize any of its adverse effects.

## Future Opportunities

### Vaccination

Vaccination is traditionally used as a preventive measure targeting the general population and applied at a universal scale. However, there is increasing interest on the use of "*precise vaccination*," targeting specific subpopulations and tailoring vaccination on a more individual level, taking into account factors such as age, sex, and disease susceptibility. Adjusting formulation, dosage and timing to patient factors could help maximize the effects of vaccination while reducing the risks (54). In the specific setting of neonates, immunization through vaccination of subpopulations of pregnant women could be an elegant manner to protect the neonate against invasive infections in the first 3 months of life, including bacterial infections. Vaccine-specific IgG can be transferred across the placenta during late-second to third trimester and provides a time window for effective and safe vaccination (55). To illustrate feasibility and relevance of this approach, safety, and efficacy has been documented for maternal pertussis vaccination which resulted in higher antibody concentrations in newborns in the first 3 months of life and a maternal vaccination program has already been implemented in several countries (56, 57). Similar efforts are ongoing for GBS and *E. coli*.

Several phase I/II trials in non-pregnant and pregnant women have evaluated the safety and tolerability of a multivalent GBS vaccine. Studies have shown the vaccine to be safe and did not report related major adverse events in vaccinated women. GBS-specific antibody responses were significantly higher among vaccinated women compared to controls (58). A phase II study reported that vaccination reduced the vaginal and rectal GBS colonization in healthy non-pregnant women (59). Only a few studies evaluated safety for the fetus and no severe events have been reported in offspring. Women are vaccinated in their third trimester and therefore toxicity for the fetus is considered to be low (60). Unfortunately, this also means that vaccination would mainly be effective to prevent invasive GBS infection in late preterm and term neonates. Earlier vaccination, during the second trimester, would be needed to protect preterm neonates, although transplacental antibody transport is reduced before the third trimester, resulting in lower anti body concentrations in general following preterm birth (61). To our knowledge, there are no phase III trials currently ongoing but maternal immunization could be a potential additional strategy to further reduce the burden of GBS infection (55, 62).

As *E. coli* infections are associated with substantial morbidity and mortality among newborns it would be tempting to evaluate whether maternal immunization, as discussed for GBS, could be beneficial for *E. coli* infections as well. Till date, trials on *E. coli* vaccination mainly focus on vaccination against Enterotoxigenic *E. coli* (ETEC), which is associated with childhood and travelers' bacterial diarrhea with a high mortality (63). A rodent study examined the use of Outer membrane protein A (OmpA) based vaccine for *E. coli*. Gu et al. were able to generate an artificial

protein (OmpAVac) which was subsequently injected in adult and neonatal mice. They report an increased specific antibody response and better survival in immunized mice, including neonatal mice (64). A first in human phase 1b randomized clinical trial evaluated the safety and immunogenicity of a bioconjugate vaccine containing the O-antigens of four *E. coli* serotypes (ExPEC4V) in healthy, non-pregnant women with recurrent urinary tract infections. Authors reported no vaccine related adverse events and elevated antibody responses were detected against all four serotypes compared to placebo (65). These studies highlight the potential for further research on *E. coli* vaccination against invasive infection, with special focus on maternal immunization and neonatal protection.

# TREATMENT INITIATION

## Current Strategies

National guidelines, taking into account both maternal and neonatal risk factors and the clinical condition of the patient, have been developed to provide support for recognition and optimization of diagnosis and treatment of neonatal infections, especially for EOS (11, 21). Much less guidance is present for suspected LOS; therapy is usually initiated when clinical signs are present. Overall, guidelines for EOS on treatment initiation show similarities: antimicrobial treatment is often initiated based on the presence of risk factors or non-specific clinical symptoms. With regard to treatment initiation, some guidelines, such as the Swiss guideline, recommend clinical observation with monitoring of vital signs every 4 h for a period of 48 h in asymptomatic neonates with risk factors while other guidelines recommend treatment initiation even in the presence of risk factors only (21, 48, 66).

Due to low specificity of mentioned risk-factors as well as clinical signs at onset of possible bacterial infection, a quest for biomarkers to assist in the decision-making regarding initiation of antibiotics has been ongoing for years. A long list of hematologic parameters, interleukins, endothelial molecules, and various other biomarkers have been or are currently being evaluated for the early detection of neonatal sepsis (67, 68). There is, in general, consensus that classic and well-researched biomarkers such as the complete blood count and C-reactive protein (CRP) are insufficient to guide the initial decision on antibiotic initiation (69). For EOS, this is largely due to physiological fluctuation of thrombocytes, leukocytes, and CRP after birth (11, 67). For LOS, low specificity as well as a delay of hours between infection and rise of biomarker levels currently limits the usability of biomarkers at moment of infection suspicion (67). Further research is needed to analyze if biomarkers, especially those reflecting inflammation early in the sepsis course, such as interleukin-6, may be used to inform precise treatment decisions beyond the start of antibiotics, such as start of adjuvant therapies, transferal to higher levels of care, or inotropic support (70).

Several developments may allow transition to a more precise risk assessment without the need for biomarker analysis as a basis for the decision to start antibiotics or not. The neonatal EOS Calculator (kp.org/eoscalc) has been developed based on a dataset of over 600,000 term and late-preterm neonates. It provides an individual quantitative risk estimate calculated from five quantitative objective risk factors at birth (exact gestational age, highest intrapartum maternal temperature, duration of ruptured membranes, and maternal GBS colonization status), and an assessment of the neonate based on objective clinical parameters (71, 72). Although several characteristics of the EOS Calculator affect the accuracy of the individual risk estimates (73), it has proven markedly useful for risk stratification. Studies have shown that its implementation is associated with a marked reduction (relative risk reduction 44%) in neonates receiving empiric antibiotics, without occurrence of adverse effects such as increases in sepsis incidence or worse clinical outcome (74, 75). Although the EOS Calculator does provide an individual risk estimate, such risk stratification remains imperfect, meaning that clinical vigilance remains mandatory even for low-risk neonates (73, 76).

An approach completely depending on the clinical vigilance is the use of serial clinical observations in term neonates. It encompasses structured and repetitive examinations of selected or all newborns by a skilled and trained nurse or physician, for the first 24–48 h postpartum (77, 78). It deliberately restricts antibiotic treatment initiation to clearly symptomatic neonates, and can reduce the rate of antibiotic treated neonates for suspected EOS to as low as 1.3% (compared to 2.9% pre-implementation) (79). Protocols for serial clinical observations differ greatly but require intensive individual medical assessment, such as hourly physical examinations. This approach has, until now, only been evaluated in a few, mostly well-staffed settings (79, 80). As a result, safety data are still limited, and the approach may not easily be implemented in settings unable to provide repeatedly assessments by clinical professionals.

For neonates admitted beyond the 1st days of life, clinicians may face similar clinical decision dilemmas if signs of a bacterial infection become present but may be explained by other factors or diagnoses. This may lead to both unnecessary treatment of uninfected neonates, as well as delayed treatment initiation in sick neonates.

## Future Opportunities

The use of physiomics such as heart rate variability (HRV) can possibly contribute to earlier infection recognition. The autonomic nervous system plays an important role in the maintenance of body homeostasis and regulates, among other processes, the beat-to-beat variability of the heartbeat (81). HRV in turn, is linked to other vital signs such as respiration and blood pressure. Sepsis, especially the presence of endotoxins, can induce autonomic dysfunction which leads to a decreased HRV, which in turn has been associated with a higher disease severity and mortality among septic patients (82, 83). Moreover, HRV is commonly used to monitor fetal condition during labor using cardiotocography and a decreased HRV can be used as a predictor for fetal distress such as intra-uterine infections (84, 85).

In preterm neonates, a decrease in HRV in combination with the presence of transient decelerations has showed to be an early predictor for sepsis and has consequently led to the development of a heart rate characteristics (HRC) monitor. This monitor can

be used to identify patients at risk for developing LOS in the next 24 h, allowing timely initiation of antibiotic therapy (86). A large randomized clinical trial showed a significant reduction in mortality among (22% relative reduction from 10.2 to 8.1%) preterm neonates in whom HRC scores were displayed to the treating physician. Authors did however report an increase in sepsis workups and days on antibiotics (87). A retrospective study in which scores were available twice daily reported a limited usability of the HRC score as many elevated scores were not related to a LOS episode (poor specificity) (88). It is known that non-infectious conditions such as medication (dexamethasone, paralytics, and anesthetics), surgery, initiation of mechanical ventilation, and bronchopulmonary dysplasia can also influence the HRC score (86, 89, 90). Although the use of HRV does not seem to be limited to preterm neonates, the HRC monitor has not yet been validated in late preterm and term neonates, nor for sepsis episodes that occur in the first 72 h of life (88, 91).

The Sepsis MetaScore (SMS) is diagnostic test aiming to diagnose sepsis based on gene expression. The SMS has been developed using a multicohort analysis and consists of an 11-gene set that can discriminate non-infectious inflammation from acute infection. It has been validated in several transcriptomic cohorts of adults and pediatric patients (92). It has recently been validated in three genome-wide expression based neonatal cohorts and has a high diagnostic accuracy for the discrimination of non-septic from septic neonates. Moreover, when combined with "traditional" biomarkers (white blood count, CRP, and neutrophil count) it improved the diagnostic accuracy of all three biomarkers, mainly because it led to a rule-out of sepsis (specificity) among low-risk patients. Moreover, and very relevant for the late preterm and term population, the SMS is capable of distinguishing neonates with suspected sepsis from those with confirmed sepsis (AUC: 0.90). Although the SMS needs to be further evaluated in prospective studies, it underscores the potential for transcriptomics to guide treatment (93).

Micro-ribonucleic acids (miRNAs) are involved in different cell processes such as cell signaling and immune activity and could therefore serve as potential biomarker for the diagnosis of sepsis. Specifically, miRNA-23b has been associated with the regulation of innate immunity and its expression is related to inflammation. In adults with sepsis, lower levels of miRNA-23b were associated with sepsis and mortality among septic patients. Within the sepsis group, lower levels were seen among non-survivors (94). This illustrates its usability for both diagnosis and severity grading of sepsis. It has therefore been evaluated in a small cohort of preterm and term neonates with EOS and LOS (95). Reduced miRNA-23b expression compared to controls was seen in both preterm and term neonates who died from EOS. Among EOS survivors, miRNA-23b expression was higher compared to controls, thus expression seems to correlate with sepsis progression. In LOS cases, miRNA-23b expression was lower in all septic neonates (both survivors and deaths) compared to non-septic controls.

Proteomic studies led to the development of the ApoSAA score, which combines serum amyloid A (SAA) and Apolipoprotein (Apo)C2. A case control study among preterm neonates showed that the ApoSAA score can differentiate non-septic infants from LOS or necrotizing enterocolitis cases (96).

Another proteomic study by Buhimschi et al. (97) revealed haptoglobin and haptoglobin-related protein immunoreactivity as a potential additional biomarker for EOS. In this study, cord blood of presumed and proven EOS patients was profiled, identifying significantly elevated levels in neonates with EOS. Further research is needed to evaluate the usability of miRNAs, the ApoSAA and haptoglobin as new biomarkers for neonatal sepsis.

# TREATMENT OPTIMIZATION

Antimicrobial stewardship programs have been developed aiming to optimize clinical outcomes while reducing the negative consequences of antimicrobial use (17). These contribute to further tailoring of antibiotic therapy and have shown to be beneficial in the reduction of unnecessary antibiotic use and prevention of antimicrobial resistance (19). Principles of these programs are *appropriate selection*, *appropriate administration*, and *timely de-escalation* (20).

## Current Strategies

The predominantly causative pathogens are the main determinant for correct choice of antibiotic therapy. For both EOS and LOS, it holds true that, at moment of infection suspicion, the possible causative pathogen of the infection and the antibiotic susceptibility test are not yet known. For EOS, empiric therapy usually consists of a combination of a penicillin with an aminoglycoside. For LOS a wider variety of combinations is used (98). Importantly, the choice of the antibiotic regimen highly depends on causative pathogens and antimicrobial resistance rates which differ substantially throughout Europe and worldwide (99). In the absence of strong evidence and in part related to differences in product availability and preferences, variability in daily practice between units, especially for LOS, is extensive (98).

After identification of a pathogen, its susceptibility for a specific antibiotic is defined by the minimal inhibitory concentration (MIC) which is the lowest antibiotic concentration needed to prevent further replication of the pathogen and is thus of importance for the determination of the dosing regimen (100). In order to facilitate precise antibiotic treatment, several pharmacological components should be considered. Drug dosing regimens have commonly been extrapolated from adult studies and practices, thus the majority of drugs prescribed to neonates are off label (101). However, neonates differ substantially from older children or adults, thereby influencing the pharmacokinetics (PK; what the body does to the drug) and pharmacodynamics (PD; pharmacological response of the body to the drug) of a drug. Simplistic extrapolation from adults and children to neonates could lead to under- (compromising efficacy) or overexposure (risking toxicity) (102). Consequently, drug dosing regimens should ideally be based on integrated knowledge concerning the disease to be treated, the physiological characteristics of the neonate, and the PK/PD of a given drug.

### Pharmacokinetics/Pharmacodynamics of Antibiotics

Efficacy of antibiotics strongly depends on the mode of action of the chosen class. This can be time-dependent killing (Time > MIC; beta-lactam antibiotics), concentration-dependent killing [maximum concentration (Cmax)/MIC; aminoglycosides] or combined time- and concentration-dependent killing [area under the curve (AUC)/MIC; vancomycin] (91). Besides these targeted effects, antibiotic exposure also results in off target effects like alterations in the gut microbiome. Intriguingly, these alterations themselves can also alter enteral drug and first pass metabolism (103).

PK compromises the process of *absorption*, *distribution*, *metabolism*, and *elimination* and both maturational and non-maturational covariates can impact a dosing regimen (*get the dose to target*). The distribution of antibiotics is driven by maturational differences in body composition (water %), by presence of disease or by treatment modalities, like extra-corporeal membrane oxygenation (104). Plasma protein concentration and binding capacity may also be of relevance for some protein bound antibiotics, like cefazolin or vancomycin. This because the fraction of time during which the free, unbound (%*f*T), antibiotic concentration is above a given MIC (%*f*T > MIC) is the efficacy target (105, 106). Subsequent elimination of antibiotics is almost exclusively by renal elimination, and to a smaller degree through metabolism or biliary elimination. The main factors involved in the development of renal function are GA, postnatal age and birth weight (107). This results in rather complex dosing regimens within the neonatal population, as reflected in different recent reviews on this topic (108, 109). Only for specific antibiotics, drug metabolism by cytochrome P450 (CYP) or glucuronidation) is involved in its clearance. Consequently, the clearance of erythromycin (CYP3A), clindamycin (CYP3A), or chloramphenicol (glucuronidation) clearance is driven by the maturational activity of these enzymes, further affected by non-maturational changes like genetic polymorphisms or disease characteristics (e.g., inflammation affects CYP3A activity) (110).

## Future Opportunities

### Therapeutic Drug Monitoring and Model Informed Precision Dosing

Therapeutic drug monitoring (TDM) is used to optimize antibiotic dosing and is especially of interest in case of a narrow therapeutic window. Moreover, it can be informative for drugs that show a large interpatient variability, as serum concentration predictions can be difficult. TDM has historically been developed to prevent toxicity. However, nowadays it is also used to guide therapy. It can be applied in drugs for which a correlation is present between serum concentrations and the pharmacological effect of the drug, thus the concentration in the target tissue. Moreover, the pharmacological effect should not be easily measurable through less invasive methods and a quantification method should exist (111).

TDM is not commonly used for dosing of beta-lactam antibiotics. They have a broad therapeutic window and are, in general, perceived to be not very toxic. However, given the increase in antimicrobial resistance and reported increase in MIC of certain pathogens, and thus narrowing of the therapeutic window in time, it could be beneficial to use TDM for other antibiotics to confirm target attainment. Moreover, most of beta-lactam antibiotics are renally cleared, and thus as previously discussed, the concentration-time profiles can be influenced by maturational and non-maturational covariates (107).

Finally, the most appropriate pharmacokinetic target remains a point of discussion. In neonates, who are perceived as relatively immunocompromised, a T > MIC of at least 40–50% of the dosing interval is recommended (112). However, on the adult ICU, targets for critically ill patients range from 100% T > MIC up to 100% T > 4 × MIC so one could question whether 40–50% is enough in neonates (113). It is important to notice that the used concentration is the free unbound concentration, which is the antimicrobial active part of the drug. This fraction varies between neonates and adults and most centers measure total concentrations, therefore a correction should be applied when interpreting the concentrations (100, 113). The relevance of this free fraction and protein binding has recently been explored for the free vancomycin AUC target to consider in neonates (105). The higher unbound vancomycin fraction in neonates can result in a lower dosing regimen.

Model-informed precision dosing (MIPD) is an obvious next step for TDM and has recently gained more attention as it may serve as a powerful tool to help individualize dosing. MIPD is a next generation dosing paradigm in which mathematical models, in combination with individually measured patient characteristics (e.g., drug concentration, genotype, organ function) and disease characteristics (e.g., pathogen susceptibility), are used to calculate the optimal dose (114). Bedside integration of combined data on exposure and effect would allow a quick/real-time individualization of dosing and target attainment (115). For neonates, this has yet been evaluated for amikacin and vancomycin. A prospective study on amikacin evaluated a model-based dosing regimen in neonates reporting optimized concentrations in almost all neonates with use of the model (116). A retrospective study on vancomycin also reported improved target attainment in neonates when using a model-based dosing approach (117).

### Routes of Administration

Antibiotics are most commonly administered intravenously to hospitalized newborns in the 1st weeks of life. It allows precise and direct drug disposition into the circulation, but it requires intravenous access. Moreover, dissolving all drugs and flushing the lines in between administrations may contribute to a fluid overload (118). Late preterm and term neonates, in general, are not dependent on central access for feeds and fluid and receive the venous access solely for the administration of antibiotic therapy. As intravenous therapy is generally only provided in hospital, this leads to prolonged hospitalization of an otherwise relatively healthy newborn.

Intramuscular administration has been evaluated in several large trials, especially in low- and middle-income countries, as an alternative in case referral to a hospital is not possible and can be an effective alternative when intravenous administration is not possible (119). A study showed that, although parents recognize

intramuscular administration to be more painful, they may still prefer this modality as to their opinion, it allows better bonding and breastfeeding compared to intravenous administration (120).

Oral administration of antibiotics is not commonly performed in newborns in the 1st months of life because of uncertainties on absorption, bio-availability and target exposure. However, several small pharmacokinetic studies have evaluated the use of oral antibiotics in neonatal infections and although absorption is slower compared to that of older children and adults and inter-individual variation is seen, target levels can be reached following oral administration (121). With regard to efficacy, several large trials have evaluated the use of an oral regimen in low- and middle-income countries. No increase in mortality or adverse outcome was reported. Unfortunately, results are not applicable to a high-income setting as pathogen distribution and the availability of diagnostics differs substantially (119, 121). A randomized clinical trial evaluating the effectiveness and safety of intravenous-to-oral antibiotic switch therapy in neonates with a probable bacterial infection is currently ongoing and results are expected by the end of 2021 (NCT03247920) (122).

### Add-On Therapies and Immunomodulation

Antibiotic therapy solely targets the causing pathogen but does not target the host' immune system and the subsequent inflammatory responses (123). Immunomodulation, targeting specific cellular and molecular processes involved in the development of neonatal sepsis, could be a very promising add-on therapy. Unfortunately, many trials have failed to show efficacy in neonatal sepsis (124, 125).

The transplacental transport of immunoglobulin G (IgG) occurs for the greater part, in the third trimester (GA $>$ 32 weeks), thus very preterm neonates are considered immunoglobulin deficient. Therefore, several clinical trials have evaluated the use of iv immunoglobulins (IVIG), both for prevention and treatment of infection in neonates. A recently updated Cochrane Review evaluating the additional benefit of IVIG in neonates ($<$28 days of age) with suspected or proven bacterial infection reported no significant difference in mortality between IVIG treated patients and placebo (126). When only looking at studies in preterm neonates (GA $<$ 37 weeks) at risk for LOS, a small, but significant reduction in the incidence of sepsis was reported (3%; number needed to treat: 33). However, no significant reduction in mortality from infection, necrotizing enterocolitis, bronchopulmonary dysplasia or intraventricular hemorrhage was seen. With regard to safety, no major adverse reactions following iv administration of IVIG were reported (127). As a clear benefit of IVIG is lacking, it may be hypothesized that pathogen-specific immunoglobulins could contribute to sepsis management. However, a clinical trial evaluating the use of anti-staphylococcal human immunoglobulins showed no significant differences in *S. aureus* and CoNS sepsis rates, nor in mortality rates, between treatment and placebo (128).

Pentoxifylline (PTX) could be a promising drug; it is a vaso-active drug, originally developed for the treatment of claudicatio intermittens in adults. It has anti-inflammatory effects that influence cytokine production possible attenuating the hyper inflammatory response associated with neonatal sepsis. Moreover, it also influences the microcirculation, which is often impaired in neonatal sepsis (129, 130). A large international randomized placebo-controlled trial in currently ongoing in which the survival of preterm neonates (GA $<$ 29 weeks) following additional treatment with PTX when LOS is suspected, is evaluated (ACTRN12616000405415). Next, and very relevant as PK studies on the use of PTX for neonatal sepsis are currently lacking, a single center dose optimization study is currently ongoing in which the optimal dose is studied (NCT04152980).

## TREATMENT DURATION

### Current Strategies

Treatment duration depends on several aspects and is ideally based on the causative pathogen. However, in many cases, cultures remain negative and therapy is continued because laboratory or clinical signs of infection remain present. In that case, guidelines recommend continuation of antibiotic therapy for 5–7 days (49). For proven bacterial infections, there appears to be little evidence for current treatment durations. For now, the questionable "magic numbers" for treatment duration are 7, 10, 14, and 21 days, depending on the cultured microorganisms. A trial for radiologically proven neonatal pneumonia (without bacteremia) did not indicate a difference in treatment success between a 4 and 7-day treatment (131). For uncomplicated GBS infections, intravenous treatment for 10 days is recommended. A retrospective analysis showed however that, in some cases, a shorter course ($\leq$8 days) is prescribed. Patients receiving a short IV course were older compared to patients receiving prolonged IV therapy. Recurrence rates were not higher in the short IV therapy group (132). A systematic review on the evidence of short vs. long duration of antibiotic treatment for neonatal bacterial infections is currently underway (133).

### Future Opportunities

In contrast to tools providing individual risk assessments to guide the initiation of antibiotics, only a few tools allow truly individualized decision making with regard to treatment duration in neonatal infections. These decisions therefore mostly rely on clinical judgment, but data from recent studies provide opportunities for improving tailored decision making. As blood cultures remain the best proxy for a definitive diagnosis (134), average time-to-positivity can be an important variable when considering (dis)continuation of treatment. Recent data show this time-to-positivity is $<$36 h for at least 94% of positive blood cultures obtained for suspected EOS, and $<$24 h for at least 68% of those (135, 136). Depending on *a priori* risk, clinical course, and infection parameters, this may facilitate discontinuation of antibiotics at these time points. Molecular techniques using DNA amplification are promising as they can detect bacterial, viral, and fungal material and have a shorter turnaround time of on average 6 h in comparison to culture-based methods. Unfortunately, at this moment, these techniques can only be used as add-on diagnostics as contaminant detection and negative results in culture positive infections have been reported. Moreover, pathogen susceptibility testing, crucial for targeted therapy, is not possible using these techniques (134).

Automatic stop orders enforce proactive decisions on continuation, which may induce more personalized decision-making, and can be highly effective in reducing unnecessary continuation of antibiotic treatment (137, 138). Such personalized decisions may be further improved if guided by combining risk stratification and age-dependent reference values for biomarkers such as procalcitonin (PCT) and or CRP (139–141). Serial low CRP values, normal PCT values, or the combination of CRP and PCT can support discontinuation of antibiotics (142). The use of PCT as guidance for antibiotic treatment in late preterm and term neonates has been evaluated in a large clinical trial (NeoPInS study) (143). The study showed that PCT-guided decision making can reduce the duration of antibiotic therapy (55 h intervention group vs. 65 h control group) in neonates treated for suspected EOS (139). The algorithm has recently made available as a mobile application (NeoPInS app; Apple app store/Android) and can be used in daily clinic as support tool in late preterm and term neonates with suspected EOS.

# A RESEARCH AGENDA TOWARD PRECISION MEDICINE

Millions of late-preterm and term neonates are born each year and are potentially at risk for bacterial infections. A "one size fits all approach" is inappropriate for this population, which is susceptible to consequences of both under- and overtreatment. The relatively low incidence of sepsis in this group is a key research challenge and calls for concerted and widespread collaborations. Aiming for a future in which precision medicine mitigates the risks of these consequences using a tailored approach, we propose the following research agenda. Specific research opportunities for this agenda are listed in **Table 1**.

## Step 1: Reaching Consensus Definitions

As mentioned before, a consensus definition of neonatal sepsis is critically lacking. The lack of agreement on the definition of what consists a "sepsis case" not only hampers clinical diagnosis, but also hinders research aiming to provide tailored approaches. The myriad of terms such as "culture-negative sepsis," "probable sepsis," or "clinical sepsis" makes comparing studies and their outcomes difficult, renders implement research findings into clinical workflow challenging, and sustains discussions that prevent research progress. Efforts that provide objective and measurable criteria to define a case of neonatal sepsis and/or define the need for (sustained) antibiotic treatment constitute a first step in progressing precision medicine, because such criteria will be highly beneficial to the development and implementation and evaluation of precision medicine tools as described in this paper.

## Step 2: Implementing Current Opportunities

In spite of the limitations of hitherto used proxy definitions, an array of current opportunities to tailor medicine for neonatal bacterial infections are readily available for clinical implementation today. For example, the EOS calculator is endorsed by academic societies and widely being implemented (22, 144). Likewise, automatic stopping orders are facilitated by most of today's electronic health care information systems. Interdisciplinary efforts are envisioned to make MIPD widely available in clinical practice (145). Despite their imperfections, careful implementation of currently available tools presents a large first step toward precision medicine and can directly impacts today's patients.

## Step 3: Addressing the Understudied Population

Relatively few clinical trials involving precision medicine have focused on late preterm or term neonates. Consequently, several of the tools developed for preterm neonates have not yet been evaluated in term neonates. Although preterm neonates are more at risk for severe disease and bad outcomes, the absolute numbers of late preterm and term neonates render these an understudied group for precision medicine, with large potential for reductions of antibiotic use, hospitalization rates, economic costs and improvements of quality of life. Validation and development of precision medicine tools should therefore regain priority, recognizing key differences from preterm neonates. Examples of these include differences in pathogen distribution, maturation-specific pharmacokinetics (volume of distribution, renal excretion), and specific immune responses (such as mild disease course of CoNS infections).

## Step 4: Leveraging New Research Fields

### Omics

The use of multi-omics is an exciting development in the field of neonatal bacterial infections. It has the potential to reveal a patients' unique disease signatures in response to a pathogen, allowing tailored therapy and disease management. However, additional clinical studies on the development and integration of these -omics derived biomarkers into daily clinic are required.

### Computational Power

Medical data analysis and decision-making can be done by humans without technology, by humans assisted by technology, or completely machine-guided. Today, this decision-making spectrum contains a myriad of possibilities including straightforward decision aiding tools based on traditional statistics, up to and beyond complex variations of machine learning and artificial intelligence (146). Improvements in computational power of recent decades allow for analysis of massive amounts of data, and leveraging this power is projected to fundamentally alter medical practice (147). Machine learning models to improve precision in neonatal sepsis management are being developed, but it will take adaptation of the medical electronic infrastructure, evaluation cycles, and scientific research to allow the promises of true clinical impact to become reality (147–149).

**TABLE 1 |** Current and future opportunities for precision medicine in neonatal bacterial infection management, with potential improvements, challenges, and specific research agenda items.

| Area of opportunity | Potential improvement | Main challenges | Research agenda |
|---|---|---|---|
| **Prevention** | | | |
| Intrapartum antibiotics | Reduced incidence of neonatal sepsis | Appropriate and timely indication | RT-PCR implementation |
| Vaccination | Reduced incidence of neonatal sepsis | Achieving effective antibody levels | Phase II/III trials |
| **Treatment initiation** | | | |
| EOS calculator | Reduced overtreatment | Local implementation and evaluation | Cluster-randomized trials; integration in electronic healthcare systems |
| Serial physical examination | Reduced overtreatment; early sepsis identification | Few large studies; labor-intensive; lack of uniform practice | Development and testing of unified approach in large studies |
| Heart rate variability | Early sepsis identification; reduced mortality/morbidity | Very few validation studies; not validated for late preterm/term neonates | Validation studies, particularly for late preterm/term neonates |
| "Omics" | Improved diagnostics | Lack of validation; integration of systems biology into clinic | Validation studies of promising omics data; development of point-of-care biomarkers derived from omics data; studies focused on clinical decision-making |
| Computational power (machine learning) | Better identification of neonatal sepsis | Data collection and processing; validating models | Improving digital infrastructure; validation and implementation studies |
| **Treatment optimization** | | | |
| Oral administration | Less invasive treatment | Few data regarding safety/efficacy | Randomized trials for oral vs. intravenous treatment |
| IM administration | Availability in low-resource settings or in absence of intravenous access | Reducing pain; pharmacokinetic/pharmacodynamic uncertainties | Randomized trials for IM vs. intravenous treatment |
| Immunomodulation | Improved treatment efficacy: less mortality/morbidity | Limited knowledge on mechanism and efficacy | Randomized clinical trials |
| Therapeutic drug monitoring/model-informed precision dosing | Optimal pharmacological effect for individual | Lack of reliable/validated models | Model development and prospective validation |
| MIC guidance | Effective treatment | Lack of PK/PD data for neonates | PK/PD studies for (preterm) neonates |
| **Treatment duration** | | | |
| Automatic stop orders | Reducing overtreatment | Changing clinical paradigm | Quality improvement initiatives |
| Biomarker algorithms | Reducing overtreatment; better identification of sepsis | Limited or variable reference limits for biomarkers; limited sensitivity | Studies combining clinical parameters and multiple biomarkers; machine learning approaches |
| Blood cultures | Reducing overtreatment | Obtaining adequate volume; real-time blood culture reporting | Studies reporting time-to-positivity, Studies researching blood volume sensitivity; studies evaluating additional detection techniques |
| **General** | | | |
| Neonatal sepsis definition | Reliable and clinically relevant diagnosis | Defining criteria for organ dysfunction; defining long-term outcomes | Systematic reviews on organ dysfunction |
| Researching understudied populations | Improvements for relatively large population | Low sepsis incidence | Large cohort studies in late preterm and term populations |

## CONCLUSIONS

The global burden of suspected neonatal infections remains high in both preterm and term neonates. This results in a high antibiotic exposure in the first weeks of life. The heterogeneity of disease, together with increasing evidence with regard to the negative effects of antimicrobials and emerging resistance rates, ask for a holistic disease approach and improved treatment strategies. Precision medicine is a promising development involving improved stratification of neonates at every stage of management, thereby facilitating precise balancing of under- and overtreatment.

Key challenges in finding that balance consist of selective but timely administration of antibiotics to those who need treatment, and discontinuation or de-escalation of antibiotic therapy when possible. Fortunately, continuous effort on the development of prediction tools has shown to be beneficial and led to a reduction of antibiotic prescriptions. Further research on potential biomarkers, using omics, could lead to a combined risk stratification tool. Moreover, in case of a true infection, those tools would give insight in the patient specific immune signatures triggered by the interaction of the host and the causative pathogen and possibly even predict disease severity, thereby allowing early, targeted initiation of supplemental therapy next to antibiotics.

In addition, therapy should be safe and effective and one should be well aware of the negative side-effects of treatment such as microbiome perturbations. As the host-pathogen interaction ("disease signatures") is unique, treatment should be guided using biomarkers and TDM instead of being standardized for all patients. Add-on therapies such as immune modulation should be considered based on these disease signatures and should be administered timely and only when indicated.

To achieve these objectives in a field with scattered but promising developments, there is a need for focused and concerted research efforts. For this, we propose a research agenda (**Table 1**) with distinct implementation and development opportunities toward a reality of precision medicine in neonatal bacterial infections. This agenda is certainly not exhaustive, but may serve as guidance in upcoming research efforts and can be adapted with complementary and promising developments.

In conclusion, the field of precision medicine is an exciting development offering many opportunities for better management of neonatal bacterial infections. Some tools, especially decision-making tools and algorithms are readily available for implementation, whereas other tools such as therapeutic drug monitoring and the use of *omics* still require further development or validation. Research may focus on the late preterm and term population and how they respond to infection early in life as this group remains relatively understudied. Finally, in the near future, advances in data science and analysis are likely accelerate developments in precision medicine for neonatal bacterial infections.

## AUTHOR CONTRIBUTIONS

FK and NA wrote the first version of the manuscript. KA and RK supervised the writing of the first draft. GT-S, AR, and IR critically reviewed and rewrote the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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# Outcome of Very Premature Newborn Receiving an Early Second Dose of Surfactant for Persistent Respiratory Distress Syndrome

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**Background:** Infants presenting respiratory distress syndrome (RDS) not responding to surfactant often receive a second instillation. Few studies evaluated the consequences of this second administration. This study aimed at determining the outcome of infants presenting persistent RDS and receiving an early second dose of surfactant.

**Methods:** Infants below 32 weeks' gestation who received a second dose of 100mg/kg of surfactant within the first 72 h of life, were retrospectively involved in this 42 months' study. They were matched to two controls receiving a single dose of 200mg/Kg based upon gender and gestational age.

**Results:** 52/156 infants receiving two doses (Group 2-doses) were significantly more often SGA [22 (42%) vs. 21 (20%) $p = 0.04$] and outborn [29 (56%) vs. 13 (12%) $p = 0.001$]. They had received antenatal corticos teroid therapy less often [26 (50%) vs. 89 (86%) $p = 0.001$] and presented more severe RDS based upon FiO2 level, oxygenation index and radiography. Group 2-doses survival was lower (65.4% vs. 79.6 % $p < 0.1$) but surviving infants did not have different morbidity than controls.

**Discussion:** Premature newborn receiving a second dose of surfactant had adverse antenatal characteristics, presented more severe RDS and only partially responded to the first dose. Outcomes of surviving infants who received 2 doses of surfactant were comparable to others.

**Keywords: premature, two doses surfactant, neonatal respiratory distress syndrome, outcome, mortality**

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## KEYNOTES

- Newborn receiving a second dose of surfactant had adverse antenatal characteristics, presenting with more severe respiratory distress syndrome initially and only partially responded to the first dose.
- Infants mortality was higher when they had received 2 doses of surfactant.
- Outcomes of surviving infants who received 2 doses of surfactant were comparable to others.

## INTRODUCTION

Respiratory distress syndrome (RDS) is a progressive respiratory failure caused primarily by a deficit of pulmonary surfactant and a structural immaturity of preterm infants' lungs. It is a major cause of morbidity and chronic lung disease. According to the latest European recommendations considering the generalization of antenatal corticosteroids (1), prophylactic administration of surfactant in preterm infants is no longer a valid option. Early administration of surfactant is now recommended for preterm infants who need intubation and for very preterm infants whose mothers did not receive antenatal corticosteroids. A fraction of inspired oxygen (FiO2) of 30% or more after 2 h of continuous positive airway pressure (CPAP) may be predictive of CPAP failure and should indicate surfactant replacement therapy (2).

More recent approaches to deliver surfactant while minimizing invasive ventilation have been proposed (3): less invasive surfactant administration (LISA) (2) or similar procedure [MIST: minimally invasive surfactant therapy (3)], using an intra tracheal catheter or a feeding tube during CPAP without intubation and mechanical ventilation. However, in case of persistent RDS after a first dose of surfactant, the management remains controversial and the use of a second dose of surfactant is a common but poorly defined practice in timing and dosing. A second dose of surfactant has been suggested as an option when encountering difficulties in weaning from mechanical ventilation, with chest X-rays showing persistence of RDS few hours after the first dose. Studies have used an ultrasound score to better identify the need for a second dose of surfactant (4–6).

In 1993, Halliday et al. (7) compared high and low dose surfactant regimen for the treatment of RDS and showed that the high dose regimen (total dose of 600 mg/kg) of Poractant alfa was not superior to the low dose one (total dose of 300 mg/kg). On the other hand, according to Dunn et al. in1990 (8), the administration of several doses of Beractant seemed more effective, but the optimal dosage and time interval between doses were not clearly defined. Of note, this study was carried out on more mature newborn (30–36 weeks of gestation). In a Cochrane meta-analysis, Soll et al. (9) demonstrated that a multiple dose regimen of surfactant, rather than a single dose, further reduced the risk of pneumothorax (RR 0.51, 95% CI 0.3–0.88) and was associated with a tendency toward a reduction in mortality. Another study by Speer et al. (10) showed a decrease in mortality and pneumothorax in neonates receiving 3 doses (400 mg/kg) of Poractant alfa.

All above studies, conducted at a time when antenatal corticosteroids were not yet routinely used, showed decreased rates of pneumothorax and a tendency toward a reduction in mortality in children who received more than one dose of surfactant for persistent RDS after the first dose.

Figueras Aloy et al. (11) worked on defining an optimal timing of the administration of the second dose of surfactant. The 57 newborn included in their study received a second dose of surfactant at either 2 or 6 h of life. Results showed a moderate improvement in premature infants of <1,000 g having received the second dose at 2 h of life.

The official recommendation guidelines state that "an additional dose of 100 mg/kg may be administered 6 to 12 h after the first dose to neonates with persistent signs of respiratory distress and remaining on ventilatory support. The cumulative total dose should not exceed 400 mg/kg. "(12).

Few studies on the administration of two doses of surfactant are available and do not define a target population. We still do not really know whether a second dose of surfactant instillation is indeed beneficial. Many questions remain unanswered regarding the time delay between the first and second dose, the correct dosage to be administered and the criteria required for its administration. The aim of our study was to determine primarily the survival then secondary the short-term outcomes of very premature newborn presenting with persistent RDS who received a second dose of exogenous surfactant. Other secondary outcomes were to characterize this population of premature newborn and compare it to the infants who only received one dose of surfactant over the same period of time, in order to determine criteria to select which newborn could benefit from the administration of a second dose of surfactant.

## MATERIALS AND METHODS

Very premature infants were involved in a monocentric retrospective cohort study in the level 3 maternity hospital of Nancy from November 1st, 2013 to April 30th, 2017 using infants' medical records. All infants born before 32 weeks of gestation and who received two doses of surfactant were included in the study. This study was approved by the Commission Nationale Informatique et Libertés and recorded under the number R2018-09.

Infants who presented congenital heart disease or major congenital malformations were excluded.

The primary outcome measure was survival. Secondary outcome was short-term morbidity of preterm neonates who received a second dose of surfactant for persistent RDS after a first dose of 200 mg/Kg of Poractant alfa. A favourable evolution was defined by survival and a length of hospitalization below the 3rd quartile of the hospitalisation duration of newborns receiving a single dose of surfactant.

Perinatal data included obstetric and neonatal information: gestational age (GA), calculated with date of last menstruation and first trimester ultrasound; birth weight; gender; single or multiple pregnancy; mode of delivery; antenatal corticosteroids; small for gestational age (SGA) defined by a birth weight less than the 10th centile of the weight expected for their gestational age, evaluated by Fenton growth chart (13); premature rupture of membranes (PRM); 1-min and 5-min Apgar score; umbilical cord pH; early onset sepsis; ventilator settings; timing and dosage

**Abbreviations:** BPD, bronchopulmonary dysplasia; CPAP, continuous positive airway pressure; FiO2, fraction of inspired oxygen; GA, Gestational age; HMD, hyaline membrane disease; Paw, airway pressure; Pep, positive expiratory pressure; PtCO2, transcutaneous pressure in CO2; RDS, Respiratory distress syndrome; PRM, premature rupture of membranes; SGA, small for gestational age.

of the first and second dose of surfactant; severe RDS was defined by a grade greater than or equal to grade 3 radiologically.

We also recorded all additional respiratory treatments: Doxapram, caffeine, inhaled NO, and corticosteroids.

Then, we evaluated morbidity and mortality (events and dates) by the incidence of death, patent ductus arteriosus (PDA) requiring treatment, necrotizing enterocolitis (NEC) as defined by Bell (14), retinopathy of prematurity (ROP) stage 2, 3 and higher (15), late onset sepsis, pneumothorax, severe intraventricular haemorrhage defined by a grade of 3 or 4 (16), duration of mechanical ventilation and bronchopulmonary dysplasia (BPD). BPD was defined as mild when the infants required 21% of FiO2 at 36 weeks of gestation, as moderate when they required oxygen with FiO2 $<$ 30%, and severe when they required oxygen with FiO2 $\geq$ 30%. (17).

## Statistical Analysis

Fifty-two patients received 2 doses in the cohort of 400 very premature infants born during the study period. Two controls per patient, paired for sex and gestational age, were chosen for the analysis. Thus, 156 patients were evaluated in this study.

Normally distributed data are presented as mean values with SD; non-normally distributed data are presented as medians with range or Inter Quartile Range (IQR). A Chi2 test or Fisher exact test was used when appropriate for categorical variables. For continuous variables not normally distributed, we used the Mann-Whitney *U*-test in comparisons.

As the FiO2 values after the first dose of surfactant have non-normal distributions and as our aim was more to identify subgroups of subjects than to establish an equation for the whole sample, we did not use multivariate regression models because of the instability that would have affected some coefficients. We chose a strategy allowing us to hierarchize variables and identify subgroups of subjects with respect to the SGA, Maturation, Outborn and FiO2 1h after the first dose of surfactant. We used a Chi-squared automatic interaction detector (CHAID) method. The IBM SPSS statistic software has extended CHAID algorithms to handle target or predictor variables even if they are categorical, ordinal or continuous. An alpha level of 0.05 was chosen as a significant difference. All analyses were performed with SPSS IBM Statistics V25.

# RESULTS

From November 1st, 2013 to April 30th, 2017, 52 out of 400 newborns born before 32 weeks of gestation (13%) receiving early surfactant eventually had two instillations within the first 72 h of life (Group 2-doses). Gestational age was 27.0 $\pm$ 1.9 (Mean $\pm$ SD); range (24–31.6) WGA and birth weight 972 $\pm$ 337g. These children were matched on sex and term of birth with 104 children who received a single dose of surfactant over the same period (Group 1-dose).

Newborns' adaptation to extra uterine life, evaluated on cord pH value ($p = 0.18$) and 1-min and 5-min Apgar score ($p = 0.15$; $p = 0.16$), was not significantly different between the two groups (**Table 1**).

**TABLE 1** | Perinatal characteristics of the neonates.

| | Group 1-dose (104) | Group 2-doses (52) | p |
|---|---|---|---|
| Males, *n* (%) | 54 (52) | 28 (54) | 0.82 |
| Gestational age, mean, (sd, range), weeks | 27.4 (1.9, 24–31.7) | 27.2 (2.0, 24–31.6) | 0.44 |
| Birth weight, mean (sd), | 982.5 (338.1) | 953.8 (336.1) | 0.46 |
| SGA, *n* (%) | **21 (20)** | **22 (42)** | **0.004** |
| Prenatal corticosteroids, *n* (%) | **89 (86)** | **26 (50)** | **0.001** |
| Incomplete steroid maturation, *n* (%) | **12 (13)** | **11 (48)** | **0.001** |
| Outborn, *n* (%) | **13 (12)** | **29 (56)** | **0.001** |
| Caesarean section, *n* (%) | 54 (52) | 28 (53) | 0.82 |
| Premature rupture of membranes, *n* (%) | 32 (30) | 21 (40) | 0.23 |
| Multiple pregnancy, *n* (%) | 23 (22) | 19 (37) | 0.05 |
| Mfi *n* (%) | 15 (28) | 30 (29) | 0.97 |
| Apgar score (median, IQR:1/5 min) | 3(2,6)/6(5,7) | 3(1,5)/6(5,7) | 0.15/0.16 |
| pH at birth, mean (sd) | 7.30 (0.10) | 7.26 (0.15) | 0.18 |

*SGA, small for gestational age; pH, potential hydrogen; MFI, maternal-foetal infections. Bold values are statistically significant.*

In Group 2-doses, infants were significantly more often SGA [22 (42%) vs. 21 (20%) ($p = 0.04$)] and outborn [29 (56%) vs. 13 (12%) ($p = 0.001$)] than in Group 1-dose. They had received antenatal corticosteroids therapy less often [26 (50%) vs. 89 (86%) ($p = 0.001$)] and often incompletely [11 (48%) vs. 12 (13%) ($p = 0.001$)] (**Table 1**). The mean time of administration of the second dose of surfactant (100 mg/kg dosing) was 15 h 30 min $\pm$ 42 min of life; range (3-64) h.

## Primary Outcome

The survival was lower in Group 2-doses (65.4 vs. 79.6 %, $p = 0.049$) (**Figure 1**).

Median duration of hospitalization for the surviving infants in Group 1-dose was 77.5 days with an interquartile range (IQR) = (59; 97). In Group 2-doses, the median duration of hospitalization was 67 days, and 20 surviving infants (58, 9 %) had a duration of hospitalization lower than or equal to 97 days, the 3rd quartile of Group 1-dose duration of hospitalisation.

For these infants with a favourable evolution, mean gestation age was 28.7 (25.2–31.0) weeks of gestation, mean birth weight was 1224g (880-1610g), SGA was present for 6 of them, and half of them were outborn. There were 7 multiple pregnancies and 8 children received antenatal corticosteroids, including 4 partial courses.

## Secondary Outcomes

### Respiratory Status Before Surfactant Administration

A significant difference was found both for the oxygenation index (Paw x Fio2/PaO2), ($p = 0.008$) and Fi02 ($p = 0.001$) which were higher in Group 2-doses (**Table 2**). Newborn infants in Group 2-doses had significantly more severe HMD. Surfactant

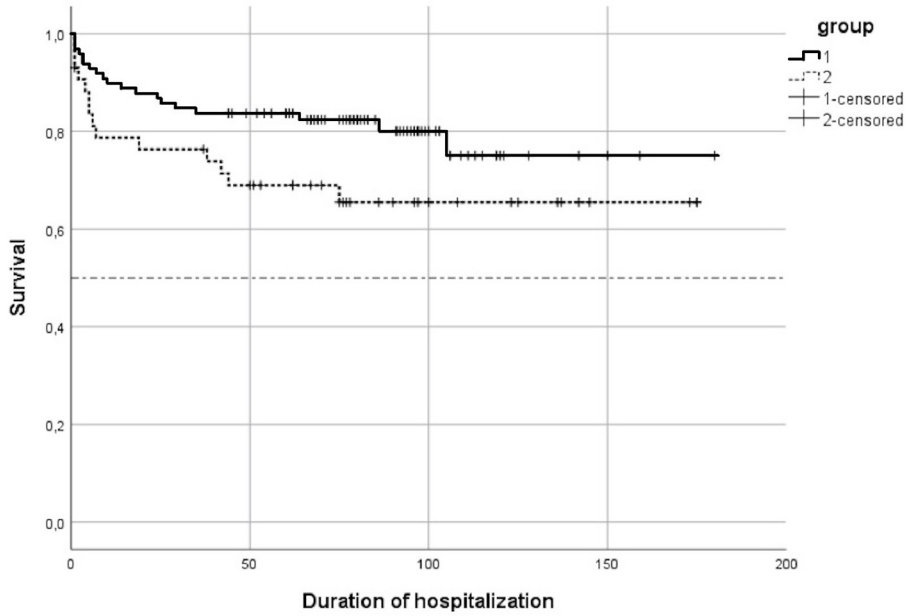


**FIGURE 1 |** Survival curve.

**TABLE 2 |** Respiratory criteria before surfactant administration.

| | Group 1-dose (104) | Group 2-doses (52) | *P* |
|---|---|---|---|
| Severe hyaline membrane disease, *n* (%) | **42 (41)** | **43 (84)** | **0.001** |
| Fi02 | **0.5 (103) [0.3; 0.7]** | **0.7 (50) [0.59; 0.8]** | **0.001** |
| Time of the first dose (hour since birth) | **1.5 (103) [1;2]** | **1 (51) [1;2]** | **0.02** |
| Oxygenation index | **8.28 (73) [3.8; 13.5]** | **10.6 (34) [7.9; 13.8]** | **0.008** |
| Respiratory rate (cycles/min) | 48.5 (102) [40; 50] | 50 (47) [45; 55] | 0.10 |
| Pep in cm h 20 | 4 (99) [4; 4] | 4 (47) [4; 4] | 1 |
| tcPCO2 in mmhg | 51 (91) [44; 56] | 51 (35) [44: 60] | 0.58 |
| Conventional mechanical ventilation, *n* (%) | 99 (96) | 46 (92) | 0.28 |
| Dosage of the first dose mean (mg/kg) | 200 (103) [200; 200] | 200 (52) [200;200] | 1 |
| Airway pressure in cm h 20, median (*n*) [IQR] | 8.2 (74) [7.7; 9] | 8.3 (39) [7.5; 9.1] | 0.63 |

*PEP, positive expiratory pressure; tcPCO2, transcutaneous pressure in CO2; PAW, airway pressure; PaO2, pressure in O2. Bold values are statistically significant.*

**TABLE 3 |** Respiratory criteria after surfactant administration.

| | Group 1-dose (104) | Group 2-Doses (52) | *p* |
|---|---|---|---|
| Fi02 | **0.23 (102) [0.21;0.29]** | **0.31(48)[0.26;0.53]** | **0.001** |
| Respiratory rate (cycles/min) | **40 (101) [30; 50]** | **50 (46) [40; 55]** | **0.001** |
| tcPCO2 in mmhg | **43 (94) [38.8; 49]** | **47 (39) [43: 59]** | **0.001** |
| Oxygenation index | **3.28 (79) [2.5; 4.5]** | **5.2 (35) [3.3; 7.4]** | **0.001** |
| Pep in cmh 20 | 4 (90) [3; 4] | 4 (42) [4; 4] | 0.98 |
| Conventional mechanical ventilation, *n* (%) | 90 (87) | 43 (90) | 0.69 |
| Airway pressure in cm h 20, median (n) [IQR] | 8.0 (80) [7.0; 9.1] | 8.1 (40) [7.4; 9.0] | 0.66 |

*PEP, positive expiratory pressure; tcPCO2, transcutaneous pressure in CO2; PAW, airway pressure; PaO2, pressure in O2. Bold values are statistically significant.*

was administered earlier in Group 2-doses (1 h after birth *vs.* an hour and a half in Group 1-dose, $p = 0.02$) (**Table 2**).

## Respiratory Outcome After the First Dose of Surfactant

After the first dose of surfactant, FiO2 decreased significantly in both groups, with a more important decrease in the two-doses group who started with a significant higher FiO2 level (0.23 vs. 0.31, $p = 0.001$). Oxygenation index also decreased more in Group 2-doses (3.28 vs. 5.20, $p = 0.001$). There was no statistically significant difference in airway pressure ($p = 0.66$) and positive expiratory pressure ($p = 0.98$) between the two groups (**Table 3**).

Infants receiving two doses of surfactant had significant higher transcutaneous PCO2 [43 vs. 47 mmHg ($p = 0.01$)] and respiratory rate [40 in Group 1-dose vs. 50 in Group 2-doses ($p = 0.001$)] (**Table 3**).

FiO2 level > 0.3 1 h after the first dose of surfactant, was associated with a sensitivity of 0.67 and a specificity of 0.76 for receiving a second dose, by ROC curve analysis with an area under the curve (AUC) (0.95 CI) = 0.778 (0.702–0.855) (**Figure 2**).

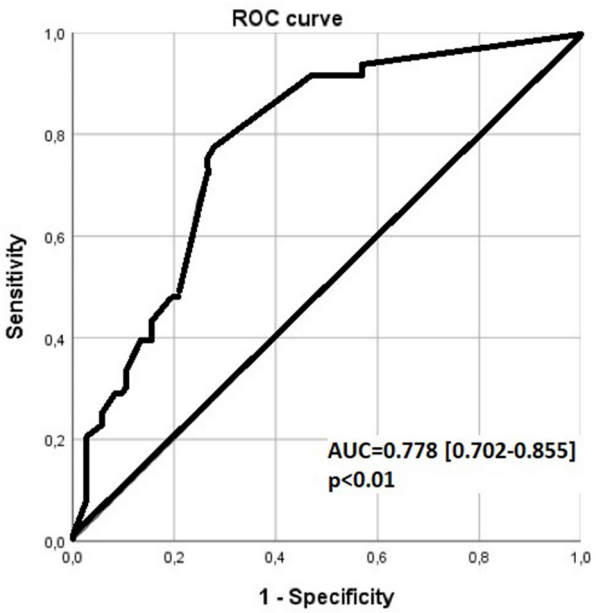


**FIGURE 2 |** FiO2 level for a second instillation of Surfactant.

**TABLE 4 |** Neonatal outcome.

| | Group 1-dose (104) | Group 2-Doses (52) | *p* |
|---|---|---|---|
| Sepsis, *n* (%) | 59 (57) | 29 (55) | 0.88 |
| Severe bpd, *n* (%) | 39 (38) | 19 (37) | 0.87 |
| Bpd, *n* (%) | 40 (39) | 12 (25) | 0.09 |
| Patents ductus arteriosus, *n* (%) | 42 (40) | 29 (58) | 0.04 |
| Severe intraventricular hemorrhage, *n* (%) | 10 (9) | 7 (13) | 0.59 |
| Rop, *n* (%) | 25 (24) | 18 (36) | 0.36 |
| Nec, *n* (%) | 10 (10) | 6 (11) | 0.78 |
| Doxapram treatment, *n* (%), | 53 (51) | 23 (46) | 0.56 |
| Caffeine treatment, *n* (%) | 90 (86) | 39 (76) | 0.12 |
| Corticosteroid treatment, *n* (%) | 42 (40) | 23 (46) | 0.51 |
| Pneumothorax, *n* (%) | 2 (2) | 4 (8) | 0.09 |
| Mechanical ventilation duration (days), med (n) [Q1; Q3] | 5 (104) [1;11.8] | 6 (52) [4;15.7] | 0.03 |
| O2 duration (days), med (*n*) [Q1; Q3] | 52.5 (104) [29.8;76] | 41.5 (48) [7.3; 74.8] | 0.31 |
| Hospitalization duration (days), med (*n*) [Q1;Q3] | 77.5 (98) [59;97] | 67 (43) [19;100] | 0.31 |
| Hospitalization during the first year | 1 (76) [0;2] | 1 (27) [0;2] | 1 |
| Mortality, *n* (%) | 21(20) | 18(35) | 0.05 |

*BPD, bronchopulmonary dysplasia; ROP, retinopathy of prematurity; NEC, necrotizing enterocolitis.*

### Morbidity

Bronchopulmonary dysplasia rates were not different between the two groups ($p = 0.88$). Neither were other neonatal morbidities such as late-onset sepsis, cerebral intraventricular haemorrhage or ventricular dilatation, necrotising enterocolitis, and retinopathy of prematurity (**Table 4**).

Mean duration of mechanical ventilation was significantly lower in the one-dose group (5 days vs. 6 days, $p = 0.03$) (**Table 4**).

Based on CHAID decision tree, outborn ($p = 0.0001$) is the most discriminant variable among the 4 variables that were significant in bivariate analysis (**Table 1**: SGA, Maturation, Outborn and FiO2-1) analysed together to explain Group 2-doses association (**Figure 3**). For the inborn infants (outborn = 0), the second most discriminant variable is FiO2 1 h after first dose instillation ($p = 0.013$). We may notice that 57% of Group 2-doses infants were outborn and for the 23 inborn infants of Group 2-doses, a cut of FiO2-1 at about 0.23 concerned 91% of them.

### Characteristics of the Infants Who Died

**Table 5** shows the characteristics of the infants who died vs. those who survived in Group 2-doses. Infants who died also had incomplete corticosteroids maturation [12 (38%) vs. 11 (13%) infants ($p = 0.003$) respectively] and their adaptation to extra uterine life was worst in the group of dead infants as illustrated by significantly lower Apgar scores (**Table 5**).

Respiratory settings were also significantly different, with a maximal FiO2 of 0.73 in the group of infants who died vs. 0.55 ($p = 0{,}002$), and an higher OI (**Table 5**). The timing of the first instillation of surfactant was not different between the infants who eventually died (14 h and 15 min of life) and the surviving infants (16 h and 30 min of life in average). Characteristics of dead infants in each group were compared and presented in **Table 5**. The only significant difference between the two groups was the absence of antenatal administration of corticosteroids for 44% in Group 2-doses vs. 14% in Group 1-dose ($p = 0.03$).

## DISCUSSION

In this study we observed that the survival rate was significantly lower in the group receiving two doses of surfactant. This observation is in agreement with a recent study by Cosal et al. (18) who found a mortality of 26 vs. 35% in our study. Few publications are available with survival data for infants who received two doses of surfactant but, these infants were sicker with more severe RDS to start with. Thus, a poorer outcome was expected. The need for multiple doses of surfactant could potentially reflect severe underlying respiratory immaturity and associated gas exchange challenges in these extremely premature infants (12). In the study by Cosal and al (18) newborns had a lower GA and they may have received more than two doses of surfactant, while two was a maximum in our study.

The median length of stay for the 98 surviving infants who received a single dose of surfactant was 77.5 days with an upper quartile at 97 days. The median length of stay for infants who received two doses of surfactant was 67 days, 62% of them staying less than or 97 days. Therefore, we may consider that 62% of the infants receiving two doses of surfactant had a short term favourable outcome comparable to the infants who received one dose only. To the contrary of Cosal and al (18) we did not observe

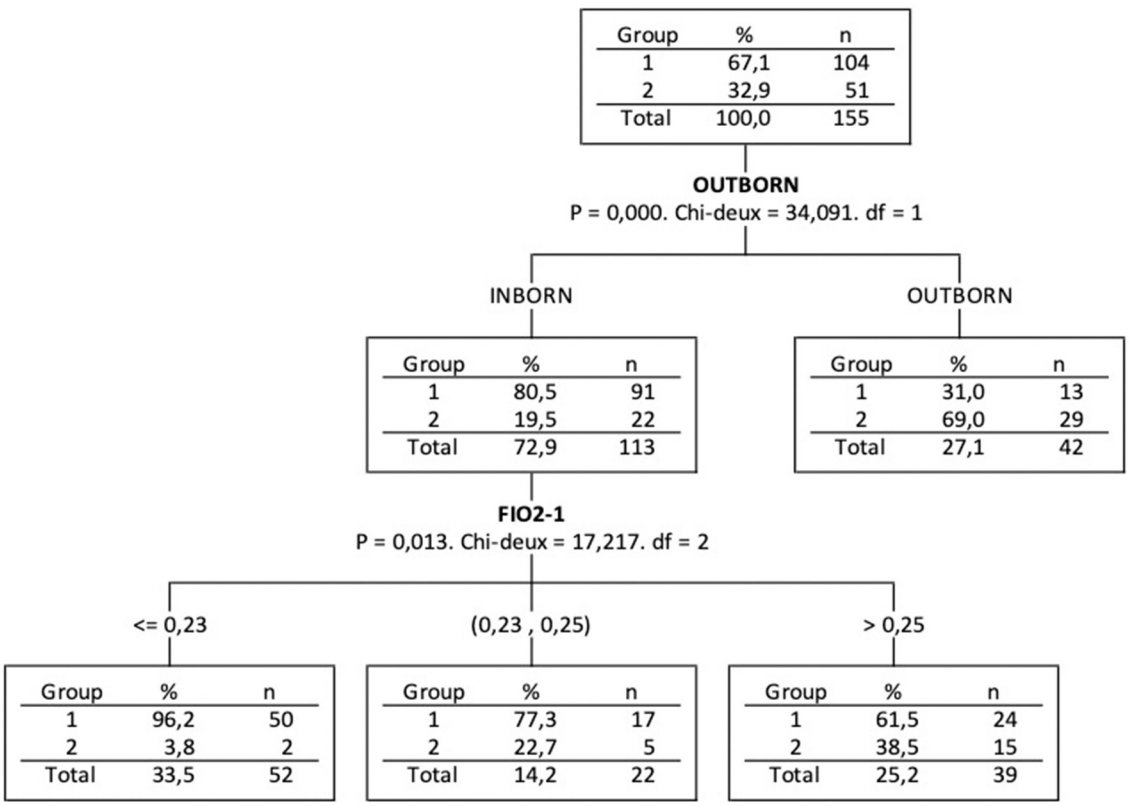


**FIGURE 3 |** Decision Tree by CHAID method.

**TABLE 5 |** Perinatal characteristics of neonates comparing the dead and the living preterms in the group who received two doses of surfactant and comparing the dead in the two groups.

| | **DEAD Group 2-doses (18)** | **DEAD Group 1-dose (21)** | **SURVIVING GROUP 2-Doses (34)** | $p_a$ | $p_b$ |
|---|---|---|---|---|---|
| Gestation age, mean, (range), WK | 25.7 (24–28.8) | 26.2(24.3–31.5) | 27.8(24.3–31.5) | 0.001 | 0.48 |
| Birth weight, mean (range), g | 785 (395–1,270) | 820.1 (510–1,250) | 1,043 (510–1,665) | 0.01 | 0.69 |
| Apgar score 1 and 5 min | 3 [1-5] | 3.5 [1-5] | 4 [2–6] | 0.032 | 0.28/0.19 |
| Median, [IQR] | 6 [2.3–7] | 6.0 [4.5–7] | 6 [5.5–7] | 0.018 | |
| Oxygenation index (paw x fio2 / pao2), mean (range) | 16.2 (8.47–46) | 12 (3.7–53) | 12 (3.7–53) | 0.037 | 0.18 |
| Maturation by corticosteroids *n* (%) | 11 (61) | 3 (14) | | | 0.03 |

*$p_a$, comparison of dead in Group 2-doses vs. surviving in Group 2-doses.*
*$p_b$, comparison of dead Group 2-doses vs. dead Group 1-dose.*

an increase in major morbidities such as ROP, pneumothorax, HIV or BDP in our population. This may be related to the lower GA in Cosal study suggesting that these morbidities are more related to the level of immaturity than to the instillation of a second dose of Surfactant when needed.

In our study, the evolution of the infants was similar in both groups in of terms of comorbidities and outcome. The duration of mechanical ventilation was one day longer in the group that received two doses which is not clinically significant with regards to the severity of the initial respiratory disease. Also, the duration of hospitalization was similar in both groups. So was the number of re-hospitalizations during the first year of life.

Over 42 months, 13% of preterm infants below 32 weeks of gestation (WGA) who had received a first early dose of surfactant had a second instillation within the first 72 h of life in our NICU. All patients were intubated shortly after birth. Early use of intubation may be explained both by the severity of the respiratory distress syndrome and by the large number

of outborn infants whose airways needed to be secured for transportation to the level III NICU. Premature infants receiving a second dose of surfactant presented more antenatal criteria of bad prognosis and more severe RDS which is consistent with the study of Katz et al. (19).

In our study, infants who received a second dose of surfactant were significantly more often SGA or born from multiple pregnancies. They were more often outborn, received less antenatal corticosteroid therapy and when they did, it was more often incomplete. These results are consistent with the literature as a complete antenatal corticosteroid therapy would improve short and long term respiratory prognosis of the newborns (20).

As compared to infants who received only 1 dose of surfactant, infants in Group 2-doses did not differ significantly with respect to gender, method of delivery and the rate of premature rupture of membranes. Newborns' adaptation to extra uterine life, evaluated on the cord pH value and on 1-min and 5-min Apgar score, did not appear to differ between the two groups. These results suggest that perinatal anoxia was not associated to the administration of a second dose of surfactant. FiO2 requirements were significantly higher for the infants who eventually received a second dose of surfactant. But, the two groups did not differ significantly with respect to ventilator settings (respiratory rate, mean pressure, positive expiratory pressure) and tcPCO2. It would seem logical that infants with a more severe radiological respiratory disease and higher FiO2 requirements would also require more aggressive ventilation but it was not the case in our study.

All infants had received a first dose of 200 mg/kg of poractant alfa (Curosurf®). This recommended dosing is associated with a reduction of the need for a second dose of surfactant according to Singh and al (21). The timing of administration was slightly but significantly different between the two groups, Group 2-doses having received surfactant 15 min earlier in average. This difference may be explained by the frequent absence or incomplete antenatal corticosteroid therapy in Group 2-doses leading to a greater severity of RDS urging the use of surfactant. However, an impact of this difference of 15 min on the infant outcome is unlikely.

According to our study, a criterion that may indicate the need for administering a second dose of surfactant could be the oxygenation index which seemed to be a good indicator associated with RDS severity. Also, the oxygen requirements were significantly higher to start with (FiO2 of 0.7 vs. 0.5, respectively). Finally, RDS was significantly radiologically more severe in Group 2-doses. These three indicators appear to be appropriate to predict the severity of RDS and the need for a second dose of surfactant when the first dose is not rapidly successful. Indeed, there is also a difference regarding the response to the first dose of surfactant, despite it was administered with the same method and at the same dosage. One h after the administration of surfactant, FiO2 and OI were still significantly higher in the two doses group. So was the PCO2 level, despite a greater respiratory rate.

The mean delay of administration of a second dose of 100 mg/kg of surfactant was about 15 h and 30 min of life in average. In the literature there is no clear recommendation about the time of administration of the second dose of surfactant. Studies by Figueras et al. and Koskal et al. compared two groups who received a second dose 2 h vs. 6 h after the first one. They showed that the group with the shortest delay between the two doses presented greater respiratory improvement (11, 22). These results are consistent with our data suggesting that a shorter time could be recommended for the infants who are non-responders, as early as 1 h after the first dose in our study. The usual recommendation to give a second dose 6 to 12 h after the first one (12) is broad and may reflect the absence of appropriate data. To follow these recommendations, one could suggest considering 6 h as the very last limit after the first dose for indicating a second dose of surfactant, in case of persistent RDS.

In the studies allowing two doses, including ours, the response to the first dose of surfactant was significantly lower. The physiopathology of this lesser response remains unclear. Because infants needing a second dose had a significantly lower gestational age, less antenatal steroids, and more patent ductus arteriosus, lung immaturity leading to more vulnerability to barotrauma could be responsible of the lesser response to the first dose of surfactant. The study by Speer et al. describes a significant inflammatory process inducing lesions of the alveolar-capillary unit, allowing proteins of the serum to pass into the alveolar lumen and decreasing the effectiveness of the surfactant. An early repeated dose of surfactant might then help to reduce and prevent somehow this inflammation (23). One of the limitations of our study is the fact that it was a retrospective study. Because the prevalence of a second dose of surfactant is rather low (13%) the power of the study is low despite this was a retrospective study of all cohort of patients. Anyhow, we did not observe any significant difference for morbidity in the survivors. Thus, our study suggests that giving a second dose of surfactant seems indeed rather relevant. Another limitation is the large number of intubated children, the intubation criteria in our units should be re-evaluated.

## CONCLUSIONS

The administration of a second dose of surfactant in very preterm infants is difficult to predict. Nevertheless, our study showed that outborn birth was the main indicator of the need for a second dose. Physicians should also be alert in case of the absence of appropriate antenatal maturation, multiple pregnancy, and SGA. Some other indicators associated with an increased risk of a lesser response to a first dose of surfactant have been shown, such as a high oxygen requirement with an elevated oxygenation index and a high grade of severity on chest radiography or lung ultrasound. This observation could help to closely monitor these infants and administrate earlier a second dose of surfactant improving the overall outcome.

Thus, one could suggest that an FiO2 level $> 0.3$ 1 h after a first dose of surfactant, associated with persistent signs of RDS, might be a guide to support a second dose of surfactant. Further studies are needed to determine more precisely the time of administration of a second dose of surfactant in case of persisting RDS after a first early dose.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

## AUTHOR CONTRIBUTIONS

EG, AW, and J-MH contributed to conception and design of the study. EG organized the database and wrote the first draft of the manuscript. EA performed the statistical analysis. All authors contributed to the article and approved the submitted version.

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REVIEW
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# Precision Medicine in Neonates: A Tailored Approach to Neonatal Brain Injury

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Despite advances in neonatal care to prevent neonatal brain injury and neurodevelopmental impairment, predicting long-term outcome in neonates at risk for brain injury remains difficult. Early prognosis is currently based on cranial ultrasound (CUS), MRI, EEG, NIRS, and/or general movements assessed at specific ages, and predicting outcome in an individual (precision medicine) is not yet possible. New algorithms based on large databases and machine learning applied to clinical, neuromonitoring, and neuroimaging data and genetic analysis and assays measuring multiple biomarkers (omics) can fulfill the needs of modern neonatology. A synergy of all these techniques and the use of automatic quantitative analysis might give clinicians the possibility to provide patient-targeted decision-making for individualized diagnosis, therapy, and outcome prediction. This review will first focus on common neonatal neurological diseases, associated risk factors, and most common treatments. After that, we will discuss how precision medicine and machine learning (ML) approaches could change the future of prediction and prognosis in this field.

**Keywords: personalized medicine, brain injury, intraventricular hemorrhage, stroke, newborn, preterm, artificial intelligence, precision medicine**

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## INTRODUCTION

Despite enormous advances in neonatal care to prevent neonatal brain injury and future neurodevelopmental impairment, predicting long-term outcome in neonates at risk for brain injury remains difficult. Parents and families of newborns admitted to the NICU with brain injury inevitably face many unknowns. In the initial period after birth, their first question will usually be: "Will my baby survive?" immediately followed by other fundamental questions such as: "What kind of future can we expect for our child? And for us as a family?" or "Will my baby be able to walk? Will he/she go to school?"

Currently, prediction of outcome is based on developmental milestones measured at specific ages. Early prognosis is based on CUS, MRI, EEG, and/or general movements assessment (GMA) assessed during follow up visits. These methods' predictive power is based primarily on population data reflecting the general outcome in similar children. Thus, predicting the outcome in a specific individual (personalized prediction medicine) is not yet possible and is urgently needed. Estimating the most accurate prognosis, as early as possible, is essential to adequately inform the child's family and begin intervention therapy even before the onset of clinical symptoms,

particularly given that the brain's plasticity is highest in the first few months after birth. An individualized approach to neonatal brain injury and neurologic-oriented precision medicine is warranted for fragile neonates, not only for outcome prediction but also for preventing or reducing neonatal brain injury and supporting decision-making. Perinatal conditions leading to brain injury in the neonatal period include **hypoxia-ischemia, arterial ischemic stroke, and intraventricular hemorrhage** and especially its complications [post-hemorrhagic ventricular dilatation (PHVD) and periventricular venous hemorrhagic infarction (PVHI)], primary causes of neonatal mortality, and life-long disabilities such as epilepsy and cerebral palsy (1, 2). It has become clear that there is a need for individual and precise information on the spectrum of risk factors, symptoms, early detection, type, and location of brain injury to design/initiate effective therapeutic and supportive strategies.

In a NICU, medical professionals are continuously trying to obtain as much information as possible on the patients in their care. Education and experience provide them with the skills to make the right decisions. However, the patient load is high. Furthermore, the human mind can only recall the outcome of the most recent or complicated case. Computer programs can approach human cognitive tasks. Thus, a possible approach to fulfill the needs of modern neonatology is developing new tools for a precision medicine approach based on large databases, and machine learning (ML) applied to neuromonitoring and neuroimaging data and genetic analysis and assays measuring multiple biomarkers (omics). A synergy of all these techniques and the use of automatic quantitative analysis could give clinicians the possibility to provide patient-targeted answers to parents' questions. Artificial intelligence can mimic human experience-based-learning with ML supervised by experts. ML learns from past experiences, identifies trends and patterns in data, and uses it to build a model or algorithm. These algorithms can be used afterwards to make predictions on new data as a supportive-decision making tool. ML models can be created from data where the outcome is known (supervised learning). Also, ML can be used to identify patterns in data without previous knowledge (unsupervised learning).

This review will first focus on common neonatal neurological diseases such as perinatal hypoxia-ischemia, perinatal ischemic stroke and intraventricular hemorrhage and their risk factors and most common treatments. Afterwards, we will focus on how precision medicine and ML approaches might accurately identify infants who will develop HIE and cerebral palsy. We will primarily discuss the newest (and with highest predictive value) clinical, neurophysiological, neuroimaging, and "omics" techniques, that in our opinion, could change the future of prediction and prognosis in this field. However, we are aware that the present review cannot be comprehensive of all the techniques in the field, therefore we chose to focus on a limited list where the first steps are already taken toward a more individualized neonatal care and a better prediction.

## Hypoxic-Ischemic Encephalopathy

Hypoxic ischemic encephalopathy (HIE) is characterized by a disturbed neurologic function in the perinatal period, manifesting with an abnormal level of consciousness, seizures, respiratory insufficiency, and depressed tone and reflexes (3). Currently, the only effective treatment to reduce death or severe long-term neurological impairment is therapeutic hypothermia, which led to an increase in survival rate, with a persistent rate of death and disabilities around 16–30% (4, 5). However, timing of intervention is a significant factor in improving outcome and treatment efficacy (6).

Multiple mechanisms are involved in brain injury pathogenesis, such as hypoxia-ischemia, inflammation, excitotoxicity, and oxidative stress (7). The degree and extent of injury and individual vulnerability depends on sex, genetic background, maturational age, and the extent of brain injury and the degree of brain development of particular regions at the moment of insult (8, 9). Antenatal conditions such as maternal infection/inflammation, intrauterine growth restriction *in utero* hypoxia can also influence and modulate vulnerability to brain injury (7). Furthermore, different stages of brain injury can be recognized, and, for each stage, different mechanisms are involved. This information is critical to program therapeutic interventions (10, 11). Recent findings and ongoing studies, using ML-based on big data and -omics approaches, suggest that by combining clinical, neurophysiological, neuroimaging, and metabolic/(epi)genetic data, it might be possible to identify infants who will develop NE and cerebral palsy accurately, shortly after birth (12, 13). This would allow early initiation of therapy. However, these methods are currently not yet available at the bedside.

## Perinatal Arterial Ischemic Stroke

Perinatal arterial ischemic stroke (PAIS) is a relatively common (birth-prevalence in term and near-term newborns ranges from 6 to 17/100,000) (14) and a severe neurologic disorder affecting primarily term infants (15). The actual treatment is supportive; however, neuroprotective approaches have been developed and are currently under evaluation in clinical trials. Among them, therapeutic hypothermia, erythropoietin, and stem cell therapy showed promising results in pre-clinical and pilot studies (16–18).

Sex (male), obstetrical conditions (first pregnancy, caesarean section), and perinatal complications such as perinatal hypoxia, and foetal/neonatal inflammatory state, are most commonly associated with neonatal stroke (19). In general, most studies emphasize the role of maternal/fetal infection/inflammation (20). Inherited or acquired prothrombotic status contributes minor to the PAIS (21). The cumulative perinatal risk factors increase the incidence dramatically (22). Other conditions, such as bacterial meningitis, hypoglycemia, and congenital heart disease, may also be involved as risk factors in PAIS development (23).

Few studies using ML have attempted to obtain reproducible automatic segmentation of the stroke lesion volumes, mainly in adults (24). A comparative study evaluating different segmentation (simple vs. complex ML) methods shows that high-level ML methods lead to significantly better segmentation results compared to the relatively simple classification methods. However, none of the methods could achieve results in the range of the human observer agreement (24). Thus, more studies

are needed in this field since segmentation can help quantify the size and location of injury to test the efficacy of therapies and prognosis.

## Intraventricular Hemorrhage (IVH), Periventricular Hemorrhagic Infarction (PVHI) and Post-hemorrhagic Ventricular Dilatation (PVHD)

Intraventricular hemorrhage (IVH) and its severe complications: PVHI and PVHD, are common conditions after premature birth and are frequently associated with mortality and adverse long-term neurodevelopmental outcome (25). Regarding treatment, IVH prevention bundles such as delayed cord clamping, minimal handling, midline head position, limiting the number of infusions, and frequent multidisciplinary assessments have emerged as essential tools for reducing IVH morbidity (26–29).

Most relevant risk factors are lower gestational age, absent antenatal steroid treatment, low Apgar scores, pneumothorax, early sepsis, inherited thrombophilia, and the use of inotropic drugs during the first days of life (30, 31). However, recently Tortora et al. (32) suggested that the congenital variation in the vascular architecture of subependymal veins might play a role in the pathogenesis of IVH, especially when other risk factors affecting the cerebral circulation occur. Another recent study demonstrated that the presence and expression of specific vascular endothelial growth factor (VEGF) genetic phenotypes were associated with higher incidence rates of IVH in extremely preterm newborns (33). Regarding the possibility to apply ML to diagnose or prevent consequences of IVH in preterm infants early, one study attempted to determine whether ML techniques would be able to identify specific clusters of risk factors with different probability estimates for severe neonatal morbidity (including IVH) in preterm infants, with promising results (34). However, there is still much room for improvement, and further studies on this field are needed before clinicians will be able to use these tools in daily practice. A first, fundamental step can be to build a big publicly available dataset of clinical data, CUS and MRI images, neurophysiological and biochemical/genetic data by which deep ML models can find more generalized features to improve their performance.

## THE ROLE OF PRECISION MEDICINE FOR "BRAIN ORIENTED CARE"

Advances in neonatal care, specifically "brain oriented care," particularly the use of therapeutic hypothermia for the treatment of hypoxic-ischemic encephalopathy, paved the way for neuroprotection in newborns at risk for brain injury. A multidisciplinary team for "brain-oriented care" is warranted in the NICU to optimally implement such treatments (26) and provide tailored care. This team should include pediatric neurologists, neonatologists, and "brain-oriented" specialized nurses (26). Furthermore, specific protocols should be combined with neuroimaging (MRI) and neuromonitoring [video multi-channel EEG and amplitude-integrated EEG, near infrared spectroscopy (NIRS)] (**Figure 1**). Moreover, laboratory support

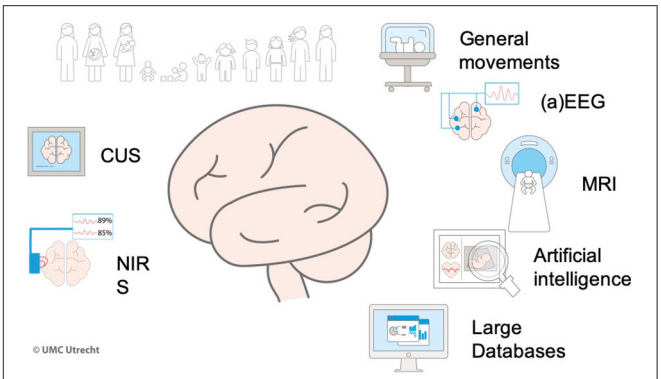


**FIGURE 1 |** Precision medicine for brain-oriented care.

for biomarkers, genetic and metabolic tests, and data scientists to analyze big data providing rapid algorithms for diagnosis and *ad hoc* treatments should be available. Dissemination of knowledge and research personnel and facilities is also warranted. Furthermore, genuinely personalized medicine is unlikely to be realized without the use of artificial intelligence (AI) (35) and ML. In neonatal neurology, ML is used to prevent brain injury from the continuous assessment of vital signs (36). Fairchild et al. (37) used a heart rate characteristic index (HRC index) from the first 28 days after birth in preterm infants and related this to neurodevelopmental outcome. They found an abnormal HRC to correlate with acute brain injury.

Much effort was made to develop automated seizure detection. When tested, all algorithms showed clinically relevant detection rates (38). Doyle et al. (39) and Malarvilli and Mesbah (40) used heart rate variability to detect seizures. Their model showed relevant results with sensitivity and specificity above 80%. Karayiannis et al. (41) designed a trained neural network that was able to distinguish seizures from random infant movements based on video images. In the term infant, encephalopathy severity can be classified based on the EEG signal. Several attempted to grade the degree of abnormality in the EEG of a neonate with hypoxic-ischemic encephalopathy (42). The ML models were suitable as a clinical decision support tool to predict outcome after hypoxic-ischemic encephalopathy (43). Furthermore, evaluating the human connectome and its relation to normal and abnormal development in preterm and term infants is virtually impossible without ML (44). AI and ML are developing fields of research and will be introduced in clinical practice. Moreover, the interpretation of MRI images of term and preterm infants using ML can possibly individualize the outcome prognosis. ML understanding of EEG patterns can potentially guide medical treatment and the use of sedation. EEG and vital parameter analysis can explore sleeping patterns in preterm babies. The potential of automatically warning a caregiver when a baby is sleeping makes true personalized developmental care possible (45, 46).

The combination of clinical and ML tools trained on combined datasets of MRI, EEGs, clinical and biochemical/genetic data would hopefully help clinicians in

providing all the treatments and support to mitigate the long-term effects of brain injury through the use of "brain oriented-care" during the whole admission and after discharge in a follow-up program.

Particularly, many efforts have been put into implementing MRI, EEG, GMs, Hammersmith Infant Neurological Examination (HINE) training for clinicians worldwide. However, achieving high education and gain experience in all these techniques is very hard in small centers. Thus, providing a decision support tool derived from ML algorithms can help every physician in decision-making, hopefully, a relevant improvement in long term outcome.

## MRI

The use of magnetic resonance imaging (MRI), a non-invasive neuroimaging method, has revolutionized our knowledge of structural alterations to normal neural development leading to neurological impairment later in childhood (47). MRI can provide detailed info *in vivo* of the fetal and neonatal brain that cannot be obtained in any other imaging modality, helping clinicians define specific risk factors for neonatal brain injury (48).

Standard sequences (T1 and T2-weighted images) provide anatomic detail of the developing brain. They can detect brain injury and lesions linked to common neurological neonatal diseases: hypoxic-ischemic brain injury, perinatal arterial ischemic stroke, IVH-PVHI, infections of the central nervous system, and congenital cerebral malformations. Furthermore, advanced MRI sequences can be used in specific conditions to assess: brain metabolism (MR spectroscopy), the presence of hemosiderin (susceptibility-weighted images), microstructural integrity (diffusion tensor imaging), acute ischemic injury (diffusion-weighted images), cerebral veins and arteries (magnetic resonance angiography and venography), brain perfusion (arterial spin labeling), and function (resting-state functional MRI). Moreover, quantitative approaches can measure brain volumes of all different regions, quantify microstructural integrity and cortical development (47, 48), that are otherwise difficult to quantify by eye. *Ad hoc* protocols and specific methodologies have been developed in order to address the methodological challenges of the newborn population such as: sensitivity to motion, small brain size, different soft-tissue contrast and incomplete maturation of brain structures (47). Thus, the use of neonatal specific MRI post-processing tools is essential in order to obtain reliable results (47).

Recently, newly developed ML techniques have been applied to earlier acquired neonatal MRI databases to predict cognitive scores at 4 years (49). Similar techniques have been used to predict cognitive, and motor development in preterm infants based on the microstructure of white matter regions measured using diffusion tensor imaging (DTI) correlated with the Bayley Scales of Infant-Toddler Development (BSID-III), as well as to predict neurological outcome in patients with neonatal encephalopathy based on connectivity networks (50–54). Moreover, deep learning–methods based on neonatal MRI and brain segmentation analysis have successfully automated classification of impaired brain maturation in full-term infants born with congenital heart disease and have provided insight into the pathogenesis of cerebellar dysplasia (55, 56). However, to optimize and refine the prognostic value of quantitative MRI techniques, it would highly be recommended to use standardized protocols, imaging modalities, and scan timing across centers (57).

## Quantitative MRI Techniques and Outcome Prognosis

Quantitative brain MRI aims to offer objective and reliable measures of brain structure, function, and brain connectivity, in the normal and abnormal brain. The main aims of quantitative neonatal MRI are: the development of automatic algorithms for images interpretation (58), the detection, measurement, and characterization of "subtle" brain abnormalities/injuries (59, 60), and prediction of behavior, cognitive and motor long term outcome based on sophisticated algorithms (61). Quantitative MRI analysis is based on the use of multiple software packages capable of drawing together neuroimaging data processing routines from across, linking them together to implement end-to-end processing and analytic solutions. These solutions not only lead to detailed mathematical and statistical results but also help to improve the reproducibility of measurements and reduce the post-processing duration (62).

Using ML approaches, quantitative analysis of brain morphometry showed significant deviations between different groups of preterm infants (with or without brain injury, extremely/moderately preterm) compared with full-term infants (47). A study comparing visual vs. quantitative MRI assessment of the thalami in infants with HIE showed that both approaches are needed since visual assessment alone can underestimate injury (63).

Automatic methods for brain volume and cortical morphology quantification, early as well as term equivalent age MRI, were good predictive tools of both motor and cognitive outcome at 2–3 years (64, 65). Furthermore, quantitatively assessed volume and location (frontal, parietal and temporal) of white matter injury, measured from MRIs, were predictive of motor outcome, while only frontal injury was predictive of cognition in a large group of preterm infants (66).

Diffusion MRI quantitative measures have also been related to later behavioral development in infants at risk for brain injury. Using automatic voxelwise analyses of DTI showed that WM microstructure in full-term newborns correlates with neurodevelopmental outcome at 2-years (67). Another study on neonatal connectome (detected using deep learning approaches) at birth showed its predictive value on the 2-years cognitive outcome in both full-term and preterm infants (68), with connections involving the frontal lobe being the most important for classification. Smyser et al. (69), using a multivariate pattern analysis on resting-state functional MRIs from preterm infants compared to term controls, were able to estimate birth gestational age, and thus, brain maturity, with an accuracy of 84%.

Therefore, MRI advanced techniques provide direct information on brain morphology, structural brain connectivity, microstructural integrity of both gray and white matter, and also on cerebral function (47), giving indirect insights into molecular and cellular impairment in relation to brain injury. Thus, ML application to neonatal MRI, combined with other clinical, behavioral, and electrophysiological (see next paragraph) markers, can play an essential role in early diagnosis and prediction of neonatal brain injury and long-term impairment.

## CRANIAL ULTRASOUND (CUS)

Ultrasound is a neonatal neuroimaging technique with several advantages over other neuroimaging techniques: it is considered less burdensome to the patient, requires no transport (e.g., to the MRI unit), or sedation, it can be performed at the bedside with acceptable disturbance to the infant. It can be initiated directly after birth and repeated if necessary (70). Ultrasound is seen as complementary to MRI because it still lacks several important neuroimaging features such as quantitative tissue analysis US. Furthermore, CUS is operator dependent, has a limited field of view, and variability across the quality of ultrasound machines. However, ultrasound technology developments are rapid, and ultrasound techniques such as elastography, ultrafast Doppler, contrast-enhanced ultrasound, and functional ultrasound are examples of techniques finding their way in routine neonatal care (71–75). Early and serial neuroimaging can provide valuable information about the timing and evolution of neonatal brain lesions in (pre-)term infants and enables visualization of (a-)typical brain maturation (76).

Trained ultrasonographers, using modern ultrasound systems, can detect most neonatal hemorrhagic and ischemic brain lesions and major congenital as well as maturational anomalies (77). The use of different and higher frequency transducers (allowing submillimeter resolution) and additional acoustic windows (e.g., the mastoid fontanel) improved visualization, resulting in a more reliable detection of abnormalities (78). Doppler sonography of neonatal brain vessels enables the evaluation of intracranial blood flow velocities and the patency of both arteries and veins (e.g., to diagnose sinovenous thrombosis, arterial vessel occlusions) (78). Modern ultrasound machines have advanced Doppler modes, allowing visualization and quantification of low flows (1–2 cm/s) in small vessels (100–200 $\mu$m) (79).

### ML in Cerebral Ultrasound Techniques

Both 2D and 3D ultrasound measurements are useful to study (a-)typical fetal and neonatal brain growth (80). For example, using 3D ultrasound measurements, ventricle volumes can be calculated to evaluate PHVD (81, 82). Machine learning can be applied to classify fetal brain ultrasound images as normal or abnormal, to detect non-typical brain growth, and detect general and focal brain injury (e.g., IVH) on neonatal CUS (83). ML is very effective in ultrasound analysis by modeling complex multidimensional data (84).

Ultrasound elastography is a relatively new technique that calculates tissue stiffness and is used to study (ab-)normal neonatal brain development. Two types of elastography are frequently used in neonatal CUS studies: 1. strain elastography (using external compression) and 2. shear wave elastography (using applied acoustic energy). Contrast-enhanced neonatal CUS (CE-CUS) is another promising technique to study microvasculature and cerebral vascular autoregulation in infants at risk for brain injury (e.g., infants with HIE, infants with congenital heart defects) (85, 86). Contrast-enhanced neonatal CUS uses injection of gas-filled microbubbles to study blood flow. CE-CUS also allows targeted (localized) medication delivery, which has potential future use for localized drug delivery in the brain (87). Another fast-developing ultrasound technique that holds promise for neonatal care is ultrafast doppler (UFD). Perinatal brain injury is commonly associated with inadequate brain perfusion, and UFD can be used to study microperfusion in detail (72). Combining continuous UFD with EEG could unravel the relationship between cortical electrical activity and perfusion (e.g., infants with HIE and seizures) (71).

Because of the large amount of data that the above-discussed ultrasound techniques generate, the integration (registration) with other imaging- and neuromonitoring techniques, and the observer dependence, ML will play a significant role in the future of neonatal CUS. ML will be needed to design clinical decision support algorithms that take several individualized variables into account.

## THE USE OF EEG/aEEG FOR PRECISION MEDICINE

Newborns with vital instability or at risk of serious morbidity are admitted or transferred to the NICU, where vital parameters such as heart rate, blood pressure, oxygen saturation, and other measures are closely monitored. Additionally, brain function monitoring is essential. EEG can monitor brain function, giving continuous, long-time, and high-resolution data on cortical function. Thus, EEG is a crucial tool for precision medicine and a tailored approach to neonatal brain injury. EEG can be useful for precise diagnosis, evaluation of treatment efficacy, and prognosis. However, interpreting conventional EEG presents significant challenges to clinicians, and the most prominent current limitation is the need for expertise in the interpretation of EEG traces (88, 89). Thus, most NICU currently use the filtered and time-compressed EEG trace (aEEG). aEEG is a non-invasive, inexpensive, bedside tool that evaluates the brain functional status of the newborn, with a relatively easier interpretation based on background patterns recognition. This technique is a powerful tool for the prediction of neurodevelopmental outcome in both preterm and term neonates.

In the last decade, the automatic classification of EEG/aEEG has been developed (90). Different automatic algorithm classifications of background patterns, sleep-wake cycling, and seizure detection have been investigated using machine learning approaches (90). This paves the way for future incorporation of these algorithms in the daily neuromonitoring of newborns at

risk. The first positive results were obtained to predict adverse seizure-related outcomes in critically ill children, albeit in a small number of patients (91). Recently, an ML algorithm for neonatal seizure recognition, ANSeR, was investigated in a randomised controlled trial and was found to be safe and able to detect neonatal seizures. However, it did not yet improve the identification of individual neonates with seizures (38).

## EEG/aEEG in HIE and Stroke Patients

Term infants with NE need continuous monitoring of brain function using aEEG/EEG. The visual interpretation of the background pattern is a useful tool to monitor the recovery of cortical activity after HI injury (92, 93). Mainly, the normalization of the EEG after HI injury correlates with the outcome at 2 years of age. This process goes through different recovery steps from almost no electrical activity at the time of injury to the increasing number of bursts, toward a more continuous EEG with the appearance of sleep-wake stages (92, 94–96). For more personalized, brain-oriented care, visual EEG interpretation requires high expertise, and the evaluation incorporates multiple EEG characteristics such as continuity, amplitude, frequency, symmetry and synchrony, presence of sleep stages, and clinical information regarding gestational and postnatal age, differential diagnosis, administration of sedatives (94). This high expertise is not always continuously available in the NICU. Thus, the development of real-time, automated EEG analysis algorithms could be very valuable to assess cortical brain activity for clinical management, treatment evaluation, and prognosis. An important attempt was performed by Stevenson et al. (94) who developed a method for automatically grading the degree of EEG abnormality in neonates with HIE. EEG signals were post-processed based on EEG automated classification of abnormalities and assigned to one of four long-term EEG grades, resulting in 83% of EEG correct grading from 54 neonates. Lofhede et al. (90) managed to achieve 100% correct classification when separating burst suppression EEG from all other EEG patterns and 93% true positive classification when separating quiet sleep from the sleep stages in term infants. Burst suppression (BS) has also been associated with poor outcome (97), allowing for the analysis of interburst intervals (IBI) to be used as a feature that can assess the recovery of the infant's brain. New machines for cerebral functional monitoring incorporate automatic and real-time IBI calculation algorithm (IBI%), making it available for the daily clinical management of these infants.

Furthermore, as already stated, the presence of seizures can be an indicator for neurodevelopmental outcome, as they can be caused by HIE (98) or perinatal stroke and can be detected through EEG data or clinical observation (92, 99–102). Automated seizure detection algorithms (SDA) are being developed with a reasonable performance compared to human expertise (103, 104) and with the advantage of being more objective, capable of analyzing long EEG recordings with low false detection rates and low missed seizures rates (105). Yet, currently available SDAs show significant limitations since seizures can be of short duration, low amplitude, and possibly migrate from channel to channel, with large intra and interpatient variability of seizure morphology and repetitive patterns (106). Furthermore, there is a high number of artifacts both of biological or technical origin mimicking seizures, that in combination with the low incidence of seizures and the wide range of normal rhythmic background activity (varying across gestational ages and post-natal ages) can reduce the power in seizure detection rates and increase the number of false-positive detections (107).

Analysis of the newborn's sleep-wake cycle (SWC) can also provide helpful insights on outcome in infants with brain injury (108). Regular SWC can distinguish those with proper brain integrity from those with HIE (109), both in full-term (96) and extremely preterms (110). Recent work suggested that decreased EEG delta-frequency power and longer periods of quiet sleep, and lower sleep-wake state entropy were also predictive of worse neonatal neurobehavioral scores (110). Despite many publications regarding ML and the development of automatic EEG/aEEG algorithms, there are still studies failing to establish the long-term predictive value of early aEEG/EEG characteristics in neonates (111).

A possible solution to allow a more comprehensive picture of the brain and thus yield more consistent, personalized, and reliable results should be the use of a combination of different measurements of brain dynamics, such as aEEG/EEG, NIRS, and MRI, together with the clinical neurologic examination. Nowadays, only a few studies on newborns have focused on the use of combined early aEEG/EEG and other cerebral monitoring techniques for the prediction of future outcome, with some promising results (112–116).

## EEG/aEEG Concerning IVH-PVHI

Preterm infants are at risk for peri/intra-ventricular hemorrhage, especially during the transition phase, with associated adverse outcomes such as death or neurodevelopmental delay (117). Cerebral functional monitoring is essential to monitor preterm brain function during the first postnatal days. aEEG/EEG is the only effective bedside tool available in the NICU (118, 119).

In extremely preterm infants, EEG/aEEG develops through to full term age showing increasing continuity of the background patterns, appearance of specific transient waveforms typical of prematurity, and the appearance of sleep-wake cycling (120). Assessing the infants' EEG recording can give insights into individual brain maturation in relation to GA and postnatal age (PNA), and serial recordings can help determine the timing and severity of brain injury and, thus, outcome prognosis in this high-risk group (120). Therefore, cerebral functional monitoring (CFM) using aEEG/EEG is critical for diagnosis, prognosis, and treatment in the newborn period (120). ML approaches have been used to analyze several clinical factors in 230 very preterm infants to predict the risk of intracerebral hemorrhage with good predictive ability achieved with different combinations of clinical and laboratory parameters (121). However, the developed models need to be tested further in new larger datasets before being used in the clinics.

Regarding neonatal seizures in preterm infants, these are a distinctive sign of neurological dysfunction in early life, and diagnosis is always challenging in this group. Clinical

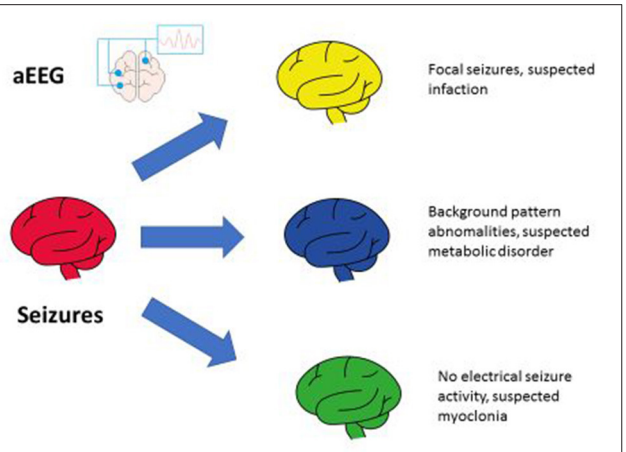


**FIGURE 2 |** aEEG for neonatal seizures.

features, when present, can often provide valuable clues about etiology. However, the majority of neonatal seizures are subclinical. Conventional video EEG and aEEG represents the gold standard for diagnosis, but ∼15% of patients will require more sophisticated algorithms for diagnosis, including metabolic and genetic screening (110, 122, 123) (**Figure 2**). Currently, the standard recommendation is to monitor all neonates at high risk for seizures with long-term video-EEG (124) and to develop brain-oriented NICUs where neonatologists and pediatric neurologists would collaborate for early diagnosis and *ad hoc* treatments based on electroclinical phenotypes and etiology (125). Further steps should be taken in this direction in a multicenter/multicultural approach.

## NEAR INFRARED SPECTROSCOPY (NIRS)

Near-infrared spectroscopy (NIRS) is a bedside technique that can provide valuable continuous information on neonatal cerebral blood flow, cerebral blood volume, and oxygen consumption (126–130). NIRS monitoring can help evaluate the balance between tissue oxygen delivery and consumption, allowing assessment of brain perfusion in critically-ill infants. Several studies have shown correlations between cerebral NIRS data and different neonatal conditions (e.g., anemia, hypotension, patent ductus arteriosus, hypoxia, hypocarbia, sepsis, HIE, stroke). The SafeBoosC studies have examined steering treatment on guideline-driven cerebral $rSO_2$ monitoring in extremely premature infants to improve clinical outcomes (131). Cerebral NIRS monitoring has now become a useful addition to other monitoring tools in several neonatology departments. NIRS monitoring has been incorporated in several multimodal neuromonitoring approaches to assess neonatal cerebral functioning in the past decade (132). For example, NIRS is used to study cerebral vascular autoregulation, in which machine learning is applied to unravel the complex interactions between blood pressure, NIRS, and EEG data (114, 133–135). ML has several uses in NIRS data analysis such as artifact detection and correction, the quantitative evaluation of deep and shallow tissue layers, to analyze the high-frequency raw NIRS data signals to study in beat-to-beat variations within the NIRS signals and to cope with the large amount of data when multiple NIRS optodes are applied to the neonatal head (136, 137). We believe that with the help of ML, NIRS will be part of the multimodal neuromonitoring of infants at risk for brain injury to diagnose injury and steer treatment to prevent further injury and optimize neurodevelopment.

## GENERAL MOVEMENTS ASSESSMENT (GMs)

Assessment of general movements (GMs) is a neurodevelopmental biomarker and evaluates the presence and quality of spontaneous movements originating in the brainstem (88). GMs begin in fetal life and are useful to build neural connectivity between motor and sensory systems. The presence of specific movement patterns such as cramped-synchronized at term equivalent age, together with the absence of fidgety movements at 3–5 months, are predictive of the development of cerebral palsy and other developmental problems (138). GMs has a sensitivity of 98% for cerebral palsy (CP) prediction and represent, together with neonatal MRI (86–89% sensitivity) and the HINE (90% sensitivity), the best predictive tool for detecting cerebral palsy before 5 months' of age and as early as by 3 months (139–142). Limitations to the broader use of GMs evaluation is the lack of trained clinicians and its subjective nature. Recently, an attempt to a more objective and cost-effective alternative based on the automatic video-based assessment of GMs has been made (143–145). In the paper by Orlandi et al. (144) retrospective videos were evaluated using automatic analysis, and GMs were classified as typical or atypical using different classification algorithms. This retrospective study showed up to 92% accuracy in predicting CP. More effort should be made in this direction to support clinicians in early diagnosis and treatment.

## HAMMERSMITH INFANT NEUROLOGICAL EXAMINATION (HINE)

As previously mentioned, HINE can predict the development of cerebral palsy before 5 months of age with 90% sensitivity. There is evidence that the congruent combination of abnormal GMs trajectory, abnormal MRI and low HINE score is even more accurate than the individual technique alone (139, 140). The use of HINE plays a role also in the determination of severity of disability, a very important matter for parents or caregivers. Severity of motor outcome is difficult to predict before 2 years of age due to the rapid brain growth and re-organization in response to external stimuli and therapy. Thus, developing of motor skills but also the inconstant and changing presence of hypertonia (140), outcome prediction should always be discussed cautiously and based on standardized examinations. In particular, the following HINE cutoff scores predict the probable severity of motor outcome before 2 years of age: 50–73 Indicates likely unilateral CP (i.e., 95–99% will walk), • <50 indicates likely

bilateral CP. Furthermore, a score <40 at 3–6 months indicates the high chance of walking inability (142).

## LARGE DATABASES

Many preterm infants are affected by the same neonatal neurological incidents. However, their internal variation in inflammatory response, environmental expositions, and (epi)genetics can influence the etiology and treatment response. Identification of individual risk factors and pathophysiological reactions can lead to targeted interventions. The use of electronic health records (EHR) has created vast amounts of clinical data on infant treatment. Utilizing the knowledge extracted from this data has the potential of providing individualized treatment plans (36). Large databases consist of a combination of individuals. These databases provide an insight into the epidemiology of neonatal disease with trends over time and the distribution of risk factors (146). Neonatal research networks, such as the NICHD and Vermont Oxford network, are collaborative networks that combine data from different hospitals. They can combine relevant information on relatively rare diseases affecting the newborn infant using large numbers. Shankaran et al. (147) described a cohort of 4,216 infants to assess post-hemorrhagic dilatation outcomes in extremely preterm infants. They were able to identify several predictors of neurodevelopmental impairment or death, such as surgery for retinopathy of prematurity, even though the incidence in a general NICU would only be a few cases.

Even more significant numbers can be obtained using nationwide databases. Most of this database research is limited to general mortality and morbidity trends for extreme preterm birth (43). Matsushita et al. (148) used the Neonatal Network of Japan database to identify risk factors for epilepsy at 3 years of age in VLBW infants. As only 1.7% of the cohort developed epilepsy, it would have been almost impossible to identify clinically relevant risk factors in a smaller cohort. Technological innovations make it possible to combine different information sources and provide information on a more detailed level. Clinically collected data from EHR with well-regulated, international, and privacy proof unrestricted access for researchers, such as the MIMIC-III and AmsterdamUMCdb database, and the increased availability of raw trial data could bring about a revolution in research on preterm neonates (149–152).

## (Epi)GENETICS AND OMICS—FUTURE

The future concept of personalized medicine will be based on the idea that by using individual genetic/metabolic information, scientists may ensure the most appropriate treatments to the right patients—thus, "the right drug, at the right dose for the right person" (153). Genomics and epigenetics, i.e., the interaction between the genome and the environment, are changing the concept of clinical medicine, and this is particularly true in the field of neonatal neurology. Neonatologists and pediatricians have the unique chance to ensure that young patients derive maximal benefit from these new technologies.

In a recent study, epigenetic changes measured in blood leucocytes and analyzed using AI/ML techniques appeared to predict cerebral palsy accurately and provide crucial information on the pathogenesis of long-term disability (12).

Both genomics and epigenetics will provide clinicians new insights into the biological basis of health and disease (154). This will also lead to the sometimes-challenging choices of both the clinicians and the patients/families. Furthermore, understanding the mechanisms through which the environment exerts changes on genome expression will give new possibilities for new treatments by modulating gene expression and should be further investigated (155). In a not far-off future, knowledge of patients' genomes will help improving diagnosis and, through informed prediction of individual drug metabolism and responsiveness, the individualized selection of therapies.

Metabolomics can also provide valuable information for outcome prediction. Metabolites offer a unique signature potentially usable to predict neonatal diseases and evaluate disease progression and treatments' effect (156–158).

Metabolomic analysis performed in cord blood predicted the development of NE with an AUC of 0.67, with lactic acid and alanine as primary metabolite predictors for NE. When metabolomic analysis results were combined with clinical data, the AUC rose to 0.96 (13). Moreover, urinary metabolic spectra of extremely preterm infants early after birth were associated with moderately to severely abnormal cortical grey matter and white matter abnormalities at MRI performed at term equivalent age MRI (158). A growing number of studies had been published on this subject since metabolomic has the advantage of being rapid and non-invasive. Thus, metabolomics could be useful for monitoring early cellular injuries and cell death during perinatal insults. Therefore, it can pave the way for the early preventive measure to improve the neurodevelopmental outcome of the affected newborns.

## CONCLUSION

The machine learning approach will provide more detailed information using AI for MRI, CUS, EEG, NIRS, and GM/HINE. An algorithm combining all techniques might give the best decision support tool for defining risk factors for brain injury or impaired brain development, therefore enabling better treatment and long-term outcome. In IVH, PAIS, and brain injury after HIE, MRI can improve personalized prognosis and treatment plan. EEG and aEEG provide more information on the brain than just a background pattern and seizure activity. Together with the HINE, GMs are the best predictive tools for early detecting cerebral palsy, and automatization of GMs classification can increase the rate of early diagnosis.

However, for the utilization of this potential, more expertise and the dissemination of knowledge is essential. Machine learning has the prospective of alleviating the task of bringing all the pieces of knowledge together. With the increasing amount of data on the infant in the NICU, it will become nearly impossible to interpret all these variables for a clinician and use it for the benefit of the individual patient. Artificial intelligence

can fill (part of) the gap of knowledge and interpretation. Especially when genetics, epigenetics, biomarker research, and metabolomics will provide us with even more variables in the near future. As the era of AI, -ethics, and –omics is approaching, we must consider the Ethics. Will all babies benefit equally from precision medicine? Most efforts to personalised medicine require a high resource setting. MRI, continuous monitoring with ML interpretation, whole-genome sequencing, and even fully equipped NICU are not available in most parts of the world. Furthermore, to make an individual treatment plan and risk assessment, many assumptions are taken into account. They hold the risk of bias and even discrimination. Attempts to personalise treatment plans must include careful ethical consideration; therefore, it should eventually be considered a decision support tool. Careful monitoring of infants in the perinatal period can potentially identify and improve neonatal brain injury treatment. "Precision medicine toward personalised care" is the aim for the near future.

## AUTHOR CONTRIBUTIONS

MT, DV, and MB: conception/design, literature review, manuscript draft and review, critical review, and approval of final manuscript. JD: literature review, manuscript draft and review, critical review, and approval of final manuscript. All authors contributed to the article and approved the submitted version.

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