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REVIEW ARTICLE

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Knowledge gaps in late-onset neonatal sepsis in preterm neonates: a roadmap for future research

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Late-onset neonatal sepsis (LONS) remains an important threat to the health of preterm neonates in the neonatal intensive care unit. Strategies to optimize care for preterm neonates with LONS are likely to improve survival and long-term neurocognitive outcomes. However, many important questions on how to improve the prevention, early detection, and therapy for LONS in preterm neonates remain unanswered. This review identifies important knowledge gaps in the management of LONS and describe possible methods and technologies that can be used to resolve these knowledge gaps. The availability of computational medicine and hypothesis-free-*omics* approaches give way to building bedside feedback tools to guide clinicians in personalized management of LONS. Despite advances in technology, implementation in clinical practice is largely lacking although such tools would help clinicians to optimize many aspects of the management of LONS. We outline which steps are needed to get possible research findings implemented on the neonatal intensive care unit and provide a roadmap for future research initiatives.

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IMPACT:

- This review identifies knowledge gaps in prevention, early detection, antibiotic, and additional therapy of late-onset neonatal sepsis in preterm neonates and provides a roadmap for future research efforts.
- Research opportunities are addressed, which could provide the means to fill knowledge gaps and the steps that need to be made before possible clinical use.
- Methods to personalize medicine and technologies feasible for bedside clinical use are described.

INTRODUCTION

Neonatal sepsis is one of the main causes of death and morbidity in preterm neonates worldwide.[1–3] Preterm neonates are especially vulnerable for acquiring late-onset neonatal sepsis (LONS). The incidence of LONS, defined as sepsis onset >72 h after birth, is up to 20% among very low birth weight neonates (VLBW; birth weight <1500 g) with 40% of those with a birth weight <750 g having more than one LONS.[2,4] Despite extensive research efforts in the past decades, many preterm neonates do not recover from LONS or survive with risk of neurodevelopmental impairment.[5,6]

Strategies to optimize care for preterm neonates with LONS are likely to improve survival and outcome, but many important questions on how to improve the prevention, detection, and therapy of LONS in preterm neonates remain unanswered. This review focuses on identifying knowledge gaps in the management of LONS, which could provide important information to improve the outcome of preterm neonates suffering from LONS. We recognize the importance of a clear definition of neonatal sepsis for further research efforts and easy comparison of study results, but this will not be within the scope of this review as it is currently still under debate.[7–9]

Figure 1 shows the conceptual framework for neonates at risk for LONS and the most important knowledge gaps. Below, we will focus on these knowledge gaps for the management of LONS, illustrate their importance, and suggest a roadmap for future research efforts.

PREVENTION OF LONS

In many neonatal intensive care units strategies for preventing LONS are implemented, such as hand hygiene protocols, wearing face masks, and care bundles to reduce catheter-related LONS. Below we describe supplements and drugs that could benefit prevention of LONS (summarized in Table 1).

Probiotics

Delayed bacterial colonization and an abnormal gut microflora have been observed in VLBW neonates. This might lead to interrupted integrity of the intestinal barrier, causing bacteria to translocate into the bloodstream.[10] With the aim to prevent this, prophylactic use of probiotics was introduced. There is conflicting evidence on the impact of probiotic treatment on the

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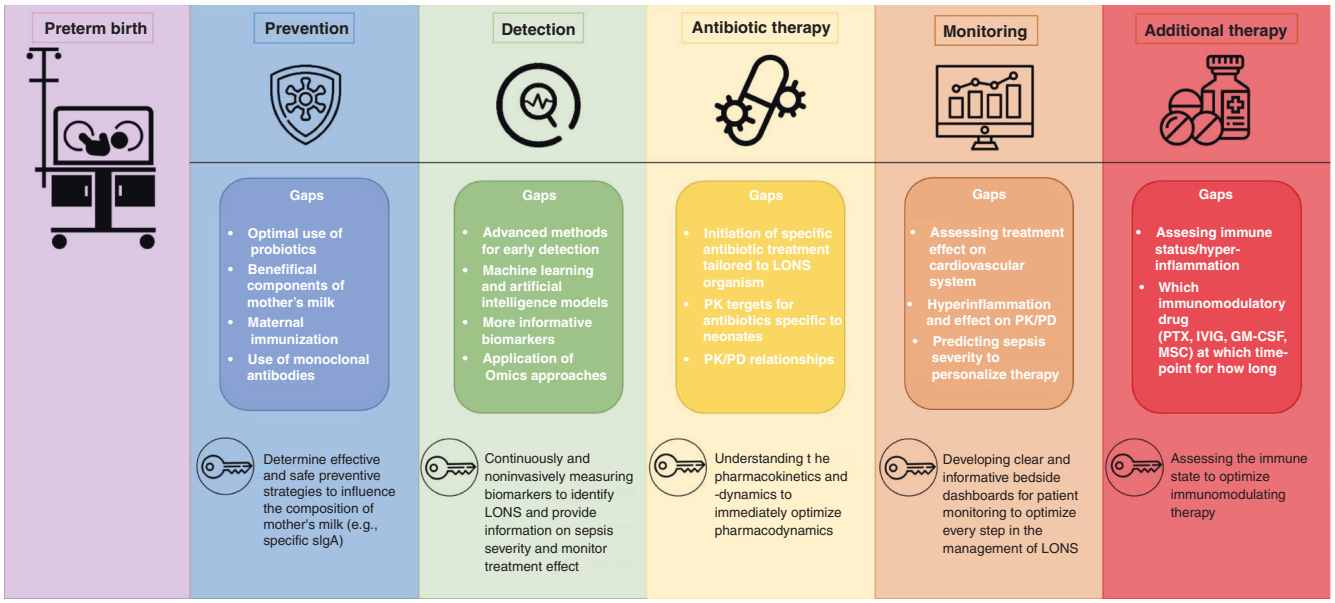

Fig. 1 Conceptual framework for preterm neonates at risk for LONS—knowledge gaps. This framework shows opportunities for optimizing components of LONS management and key objectives (keys) that need to be addressed. LONS late-onset neonatal sepsis, PK/PD pharmacokinetics/pharmacodynamics, PTX pentoxifylline, IVIG intravenous immune globulin, GM-CSF granulocyte–macrophage colony-stimulating factor, MSC mesenchymal stromal cells.

development of LONS. A recent meta-analysis of 67 trials in 15,712 patients showed a reduced overall mortality and incidence of NEC in VLBW neonates, but the risk reduction for culture proven sepsis was non-significant. At the same time the authors acknowledge that there was large heterogeneity in the included studies.[11] Another meta-analysis of 37 randomized controlled trials (RCT) showed that probiotics only decreased the risk of acquiring LONS in mother's milk fed preterm neonates.[12] Both analyses conclude that only probiotic mixtures, not single-strain products, were effective in reducing LONS incidence.[11,12] This was supported by a recent network meta-analysis by Chi et al., which showed that different combinations of pre- and probiotics could be beneficial for different neonatal endpoints.[13]

Knowledge gaps and opportunities for future research. In the future, more focus should go to clarifying the relationship between probiotic supplementation and feeding practices in preterm neonates. Additional research should also focus on type of probiotic microorganisms, dose, starting time of and duration of administration to make the available evidence more comparable. The recent network meta-analysis could be used as a starting point for strain combinations tot test.[13]

Mother's milk and donor milk

Feeding preterm neonates with their own mother's milk has a lot of benefits including decreased rates of LONS, necrotizing enterocolitis (NEC), and improved neurodevelopmental outcomes.[14–17] If mother's milk is unavailable, donor human milk (DHM) can be used as substitute, with several limitations. Unfortunately, the process of pasteurization of DHM, when performed, destroys or significantly decreases the concentration of many of the essential protective molecules.[18–21] Feeding DHM instead of formula did not significantly affect mortality or frequency of LONS.[22,23] However, DHM feeding might still be more beneficial compared to formula feeding as a meta-analysis showed DHM compared to formula feeding resulted in a decreased risk of developing NEC.[23] More recently, research groups have been focusing on the specific effects of colostrum which is very rich in protective immune components.[24,25] However, due to its low quantity, administration via a feeding tube is difficult and extremely preterm neonates can be intolerant to feeding. Oromucosal administration of very low quantities every 3 h has been associated with higher sIgA and lactoferrin concentrations in a group of preterm infants.[24] Currently, the first study on the effect of colostrum on neonatal outcome is ongoing.[26]

Knowledge gaps and opportunities for future research. It is important to understand which components of human milk are essential for the protective effect against LONS. Next, processes of preparation and storage of DHM need then to be adapted to retain these essential components and subsequently the adapted DHM should be evaluated in patient studies. Also, beneficial components that reduce LONS incidence may be used to improve formula feeding. Additionally, the potential benefits of early colostrum administration needs to be further studied in RCTs.

Maternal immunization

As IgA is transferred in mother's milk, it might be also interesting to think about immunizing mothers with the pathogens found in routine skin and rectal swabs. Several studies on the protective role of breastmilk in women vaccinated during pregnancy identified a high amount of vaccine-specific sIgA in the breastmilk samples up to several weeks postpartum.[27–29] Consequently, a lower incidence of respiratory illness with fever episodes in young infants of influenza-vaccinated mothers was reported.[28,29] The potential of neonatal immunization against Gram-negative bacteria has also been described, either a maternal vaccination to pregnant woman, via breastmilk and directly to the neonate.[30]

Knowledge gaps and opportunities for future research. Protection offered to the newborn via secretory antibodies present in mothers milk support need for robust immunization strategies that include pregnant women. Effective protection of pregnant women may translate to dual protection to both mother and the infant. Maternal vaccination has already been shown to protect the neonate from severe infections and currently represents the best preventive option against various pathogens. Development of vaccination strategies in pregnant women with imminent preterm labor or possibly soon after preterm birth could improve

Table 1. Knowledge gaps in pharmacological and immunological therapy to prevent and treat LONS and the associated opportunities to close these.

Therapeutic agent	Component of sepsis management	Knowledge gaps	Opportunities
Probiotics	Prevention	Effect of type of feeding, type of microorganism/mixture, starting time, dose, and duration of administration	Studies according to feeding type, studies evaluating different microorganisms and regimens
Mother's milk	Prevention	Components in breastmilk that are essential for the protective effect; effect of pasteurization on human donor milk	Study different preparation techniques for human donor milk, adapt mother's milk by treating or supplement mothers
Maternal immunization	Prevention	Timing and effect of maternal immunization on specific secretory IgA in mother's milk for potential causative microorganisms of LONS	Adapt secretory IgA patterns to possibly support the immune system of preterm neonates
Lactoferrin	Prevention	Optimal dosing regimen, types of lactoferrin (human vs bovine lactoferrin), long-term effects	Larger studies with longer follow-up
Pagibaximib	Prevention	Correct dose; exact effect on the reduction of sepsis if used for prevention, as well as for treatment	Study dose–effect relationship in preterm animals, investigate potential benefit in the treatment of staphylococcal infections
Targeted antibiotics	Treatment	Lack of effect markers, lack of neonatal PK target, high inter-individual variability	MIPD, routine therapeutic drug monitoring, more research into effect biomarkers
Pentoxifylline	Treatment	Best time to start therapy unknown, best dose unknown, duration of therapy unknown	Dose finding trials, efficacy trials with different time points to start treatment
Mesenchymal stromal cells	Treatment	Information on safety, correct dosage, underlying working mechanism and possible patient selection	Might bear potential but must be studied in larger trials

MIPD model-informed precision dosing, *LONS* late-onset neonatal sepsis.

prevention of LONS by enhancing the immune system of the neonate. These strategies should be evaluated in prospective, preferably randomized, studies.

Lactoferrin

Lactoferrin is a major iron-binding glycoprotein in mature human milk with direct antimicrobial and anti-inflammatory effects.[31] In several small RCTs the oral supplementation of bovine lactoferrin (bLF) was associated with a decrease of LONS incidence, although studies were small with high risk of bias and possibility of publication bias was noted.[32,33] Until now, however, no adverse effects of bLF in neonates have been reported.[33] Larger, already ongoing studies will hopefully provide more data on the effectiveness of lactoferrin supplementation.

Knowledge gaps and opportunities for future research. Next to a potential interaction between probiotics and bLF, which might warrant further study, optimal dosing regimens, types of lactoferrin (human vs bLF), and their effects on long-term outcomes need to be determined.[22]

Monoclonal antibodies

The use of monoclonal antibodies to modulate the neonatal immune system to prevent LONS is interesting. Most evidence to prevent staphylococcal sepsis in preterm neonates has been reported on pagibaximab. In several smaller studies, a good safety and tolerability profile was demonstrated; however, no effect on LONS was established so far.[19,34,35] Pagibaximab is a humanized mouse chimeric monoclonal antibody directed against lipoteichoic acid (LTA), a major cell wall component of Gram-positive bacteria, which promotes staphylococcal phagocytosis.[36] The ability of two other antistaphylococcal immunoglobulins, Altastaph and INH A-2, to augment the neonatal immune system to prevent infections has also been studied although not recommended yet.[37] A new promising humanized immunoglobulin SpAKKAA-mAb bound with high affinity, blocked IgG binding to protein A, and provided protection against *S. aureus* sepsis in neonatal mice.[38]

Knowledge gaps and opportunities for future research. Further research should focus on exploring the development and use of monoclonal antibodies to prevent LONS. Phase 1–3 trials are required for the transition to neonates. The pharmacokinetic (PK) knowledge on monoclonal antibodies and immunoglobulins in preterm neonates may highly benefit from the approach by Malik et al.[39] who described the PK profile of monoclonal antibodies in preterm neonates using a physiologically based pharmacokinetic (PBPK) model, based on the reported PK of pagibaximab and palivizumab, as well as immunoglobulins. This enables a prediction of the disposition of other as well as new monoclonal antibodies and immunoglobulins in preterm infants.

EARLY DETECTION OF LONS

Timely initiation of adequate antibiotic and supportive therapy is critical to improve survival of patients suffering from LONS.[40] In most cases of LONS the first clinical symptoms are subtle and non-specific (e.g. instability of feeding, temperature, or breathing), but the course can be fulminant and leading to death within a few hours.[41,42]

Physiological parameters

Ideally, non-invasive methods should be able to predict the onset of LONS. Up until now, several physiological parameters (physiomics) have been investigated, of which heart rate variability (HRV) is the most widely used. Multiple mathematical computations of the heart rate can be used to monitor changes in HRV.[43,44] Changes in HRV are associated with the onset of LONS and HRV monitoring has been shown to reduce mortality in VLBW neonates in a large randomized controlled trial.[45] However, the specificity is low, which may lead to more blood cultures taken and an increased use of antibiotics.[46,47] It was already shown that adding cross-correlation of SpO_2 with HRV, or respiratory rate and body temperature, increased the predictive value for occurrence of LONS and NEC, although specificity remained low.[48,49] Predictive ability improved when adding physiological parameters

to baseline characteristics and conventional risk factors of LONS.[50] Also, adding ECG-derived estimates of neonatal movements adds information on top of HRV.[51]

Knowledge gaps and opportunities for future research. Despite the common knowledge that vital signs change in patients with LONS, predictive monitoring is not used widely in the NICU due to limited predictive ability (low specificity). Future research should focus on improving specificity by combining multiple physiological parameters and adding new non-invasive continuously measured physiological parameters such as skin conductance, changes in microcirculation, and peripheral pulse rate variability. The rapid progress in the development of electronics will produce other non-invasive techniques observing the infants that may outperform those currently available. Combining non-invasive signals with rapidly available (serum) biomarkers is a promising scenario to investigate.

Using today's technology of big data mining combined with machine learning (ML) and artificial intelligence (AI) approaches[52–56] will most likely allow to characterize those with changing physiology indicative of LONS. Data incorporated in ML/AI models include electronic health record data, vital sign analysis, administered therapy, and rapidly available biomarkers. Unfortunately, in adult ICU most ML and AI models remain in prototyping environment and are not tested in bedside settings.[57] Challenges lie in data management (sample size, privacy regulations, model generalizability, standardized data formats), development of models (ensuring data quality, preprocessing of data, data labeling, measurement errors), and implementation in clinical workflow (lack of insight in model development limiting possibility of validation studies, concerns for patient safety).[57–59] Developed predictive algorithms should be prospectively validated, ideally in randomized controlled trials, for their effect on short- and long-term outcome. To improve model performance and generalizability, initiatives should be employed to share high-frequency vital sign datasets with paired outcome data including a harmonized definition of sepsis in order to give researchers the possibility to develop predictive models with the biggest dataset possible.

Serum biomarkers

"Classical" biomarkers. Many studies investigating single biomarkers or a combination of several biomarkers to diagnose or rule out LONS have been performed without establishing an adequate solution for clinical use.[60–66] In addition, the dynamics of many biomarkers in response to LONS as well as the different phases of a neonates immune response have not been fully understood.[67] It seems unlikely that a single biomarker will have sufficient predictive value to accurately diagnose or rule out LONS. This emphasizes the need for profiles of multiple biomarkers to identify the status of infections as well as the neonatal immune response in order to improve diagnostic ability.[68] A solution could be found in *-omics approaches*.

Systems biology; -omics approaches. Relatively novel *systems biology* approaches such as proteomics (simultaneous determination of large numbers of proteins), transcriptomics (RNA transcripts), and metabolomics (cellular metabolites) seem promising to detect the changing (patho)physiology of a preterm neonate during LONS.[69] Mass spectrometry (MS) and liquid chromatography techniques have made high-throughput determination of -omics platforms possible.[70–72] Several studies have investigated the use of -omics profiles in diagnosing LONS, yielding promising results.[73–83] Several biological metabolic pathways, like glucose metabolism, oxidative stress, and the fatty acid pathway,[77–80] have been shown to be involved in LONS and are promising targets to investigate. In the largest proteomic study so far including data from 258 predominantly preterm neonates,[74] biomarker discovery was performed and subsequently, a composite score (ApoSAA score) was constructed. Then, prospectively validated in a single center setting, the ApoSAA score showed high specificity and sensitivity (0.84 and 0.89, respectively).[74] However, the ApoSAA score remains to be evaluated in a multi-center setting. A gene-expression-based risk score (Sepsis MetaScore, comprised of 11 gene-expression levels) showed a high diagnostic yield (AUROC 0.92–0.93 in three separate centers) but remains to be prospectively validated.[83] These studies illustrate that despite promising results, the route taking research results to the bedside is challenging.

Knowledge gaps and opportunities for future research. Future studies should define biomarker cut-off levels specific for (small) ranges of gestational and postnatal age as biomarker levels are affected by both.[22] New biomarkers should be studied that rapidly change in a short time-period, because they are more likely to provide a high predictive value for diagnosing or excluding LONS. Because sepsis is a complex immunological process, it is likely that the predictive value of multiple pro- and anti-inflammatory biomarkers yields more information than a single biomarker, and should be assessed in age (both post-menstrual and postnatal) and weight specific groups of neonates. Large and well-defined patient populations are needed for discovery of novel -omics biomarkers as well as confirming prospective studies are needed for clinical implication.

In general, predictive models are not likely to consistently show absence of false-negative results, which has the highest clinical concern because of the potential consequences of delayed treatment of LONS. Therefore, a combination of biomarkers with a high positive predictive value could serve as alert to medical staff to consider (early) antibiotic treatment or additional testing.

From -omics research approaches to the bedside. Although hypothesis-free approaches are promising and will likely yield interesting results, the road to bedside implementation is long. Research efforts will have to be made to minimize the amount of blood needed for these -omics approaches as well as making them available 24/7 with a rapid turnover time. For both proteomics and metabolomics technical advances show promise in minimizing equipment size and blood sample volume for bedside implementation, when the appropriate proteomic and/or metabolomic targets have been identified.[84–87] As *-omics* approaches currently are only used in research settings, biobanking of biological specimens and international collaboration will be critical for success.

Furthermore, future research should also focus on continuously measuring informative biomarkers using new techniques such as sensors and micro-dialysis devices,[88] and measuring biomarkers in other, freely available body materials such as urine, saliva, or the microbiome of the skin.[89] In order for these tests to have an impact on clinical decision-making and neonatal outcome such biomarkers sets should be made available as rapid onsite tests. Another option may be measuring volatile organic compounds (VOC). VOCs have been shown to change several before clinical LONS and NEC diagnosis and are specific to causative bacteria.[90] An eNose in an incubator could continuously analyze exhaled air and can be an addition to early detection of LONS.[91,92]

ANTIBIOTIC THERAPY

Empirical antibiotic therapy

During the initial treatment phase, if sepsis is suspected, it is of utmost importance to assure efficacy of treatment.[10,93] Usually, empirical antibiotic therapy is initiated covering the most common causative microorganisms.[94] Upon determination of the causative microorganism and its antibiotic sensitivity, the antibiotic therapy can be narrowed with specific antibiotics or stopped if the blood culture remains negative and the clinical

Table 2. Knowledge gaps in detection, treatment, and monitoring in late-onset neonatal sepsis and the associated opportunities to close these.

Subject	Component of sepsis management	Knowledge gaps	Opportunities
Detection methods	Detection	For classical blood cultures, the result takes up to 48 h; high rate of culture-negative sepsis	Explore predictive ability new algorithms including multiple physiological parameters and/or chemical biomarkers; machine learning and artificial intelligence to improve early detection of LONS
Serum biomarkers	Detection	No perfect biomarker exists	Study the complex interaction between biomarker and pathogen in neonates using -omics approaches; assess predictive ability of a combination of biomarkers
-Omics to bedside	Detection	Clinical application is limited due to small sample sizes and lack of validation	Larger patient populations for discovery of novel -omics biomarkers and establishment of profiles indicative of LONS
Dosing antibiotics	Treatment	Multiple models based on small studies with slightly different results; collected PK data are too sparse to make good inferences on the PK; large unexplained variability in the PK remains in models	Data sharing and model-based meta-analysis to bundle all available evidence and get to a best-practice dosing; use clinical trial simulation to calculate power and determine sampling time points for clinical trials
MIC breakpoints	Treatment	MIC breakpoints have been defined for adults	Define MIC breakpoints in neonates
Assessing the immune response	Monitoring	Biomarker dynamics over time and in relation to immunological phase of sepsis unknown	Combining knowledge of the developing immune system of the neonate, inflammatory state, causative pathogen, and drug concentrations to assess whether a change in a biomarker reflects an upcoming change in the patient's condition
In vitro/ in vivo models	Monitoring	Development of the neonatal immune system not completely understood	Preclinical studies in high-throughput in vitro/ in vivo models to understand neonatal immune response, its dynamics and timing of immune-modulating interventions

MIC minimally inhibitory concentration, *PK* pharmacokinetics, *LONS* late-onset neonatal sepsis.

signs do not fit sepsis.[10,94] Unfortunately, many blood cultures remain negative although sepsis cannot be ruled out clinically, inevitably leading to unnecessary use of antibiotics. This has been associated with an increased risk of NEC and death in patients receiving large amounts of antibiotics,[95] and the emergence of multidrug-resistant Gram-negative bacteria.[96]

Knowledge gaps and opportunities for future research. Instantly available information on the causative microorganism would limit unnecessary use of antibiotics. This is critical for effective treatment and to prevent formation of more resistant strains. One way to go forward with this could be the use molecular diagnostics such as PCR or MALDI-TOF MS, a mass spectrometry technique on blood specimens after laser exposure, which have been shown to lead to quicker and more sensitive results, but with limited specificity.[62,97] In the future the added value of these techniques in patients with negative cultures needs to be investigated to reduce the use of antibiotics in culture-negative sepsis. Also, biomarkers to detect resistant microorganisms and enabling adapted empiric antibiotic therapy are needed (Table 2).

Personalized antibiotic therapy

Ideally, we want to give the most specific, narrow, and optimally dosed antibiotic therapy, individualized for each patients with suspected LONS, and avoid empirical broad-spectrum antibiotic therapy. This would reduce unnecessary use of antibiotics and the accompanying risk of side effects and resistance. To accomplish personalized antibiotic treatment in LONS patients, rapidly available information on the causative microorganism is needed. Conventional blood culture methods are clearly too slow.[98,99]

Knowledge gaps and opportunities for future research. Future research should focus on the development of bedside molecular diagnostics in order to facilitate personalized antibiotic treatment. Information from classical serum biomarkers (e.g. C-reactive protein (CRP), interleukin-6 (IL-6)) provide limited information in adults, but this has not been investigated in neonatal populations.[100–103] Analysis of the entire metabolome or proteome has great potential to find predictive biomarkers for the causative microorganism of LONS.[104–106] However, it could be that only a subgroup of patients is suitable for personalized antibiotic treatments, based on their biomarker profiles predicting a causative microorganism with a high certainty. Possibly some patients do not need antibiotic therapy at all. If reliable biomarkers can be established, adapted antibiotic therapy needs to be investigated in a sufficiently powered RCT in order to assess safety and efficacy.[107]

PKs of antibiotics

Due to the lack of available PK data, it has long been a challenge to understand the factors driving the drug levels in septic neonates.[108] Fortunately, this has drastically improved in the last decade due to more sensitive laboratory equipment that enabled quantification of drug levels in small plasma volumes and advances in PK modeling and simulation.[109] Nowadays, the most commonly used antibiotics have been labeled for neonatal age and PK data are available.[110] The most relevant antibiotics for the treatment of LONS are known to be predominantly eliminated renally.[111] It may therefore be expected that determinants of antibiotic clearance are similar for most of these drugs. These determinants reflect prenatal (birth weight, gestational age) and postnatal maturation (postnatal age, current body weight).[112,113] Nevertheless, different existing models for one antibiotic often result in slightly different dosage advice. The reasons for these difference relate to include the inclusion of different patient

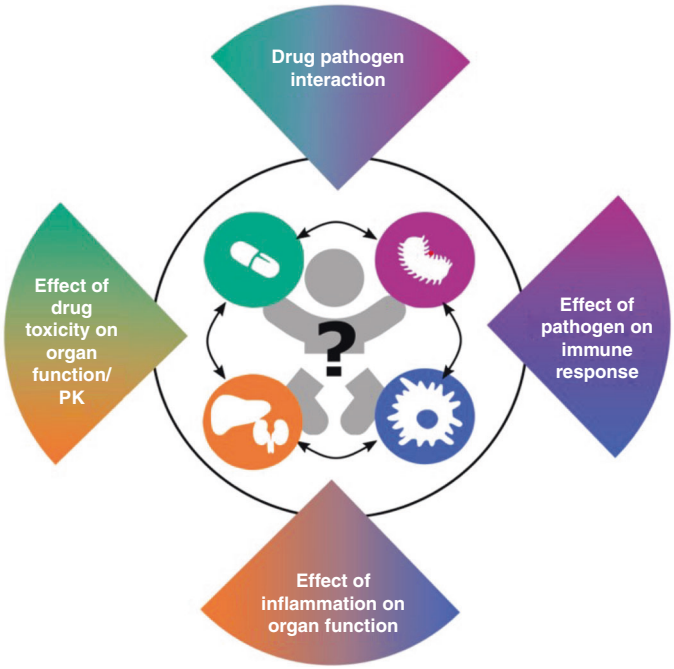

Fig. 2 Schematic representation of the complex interplay of different factors that complicate quantifying treatment effects in preterm neonates with late-onset sepsis. Pink: causative pathogen, blue: immune system, orange: organ function, green: drug, adapted from https://ascpt.onlinelibrary.wiley.com/doi/epdf/10.1002/cpt.2194.

populations, small cohorts and different PK targets as these have not yet been clearly defined for neonates (Table 2).

Knowledge gaps and opportunities for future research. Future research should therefore focus on pooling all available data on one drug[114] or performing model-based meta-analyses,[115] which will result in more uniform and more reliable dosage advice. In addition, more studies are needed to identify the appropriate PK targets in neonates. In case of performing new PK trials, clinical trial simulations prior to the actual start of a study can help to choose the most informative blood sampling time scheme to ensure the study is sufficiently powered for PK analyses.[116]

Determining the right dose for antibiotics

Minimal inhibitory concentration. To determine the most adequate dose for the antibiotic therapy of LONS, the minimal inhibitory concentration (MIC) is often used. This concentration describes the lowest concentration at which bacterial growth is inhibited in vitro.[117] However, the resulting MIC breakpoints that define whether a pathogen is susceptible are based on PK, pharmacodynamics (PD), and outcome data in adults and not in children, let alone neonates. Differences in neonatal immune response leading to altered bacterial killing patterns might lead to a need for a higher MIC to effectively kill the bacteria. This becomes even more relevant if the microorganism has a high MIC.[118] Furthermore, the elimination and distribution of drugs in neonates is different from older children and adults.[119] Even when reaching similar plasma concentrations in the blood by means of individualized dosing, the concentration at the site of infection might vary. This concentration, will, in turn impact the immune response, the pathogen load, and possible biomarkers for diagnosing LONS and/or monitoring treatment effect (see Fig. 2).

PK/PD relationships. For different classes of antibiotics, different MIC-related PK/PD indices, relating PK parameters to efficacy, have been defined depending on the mechanism of action. These include concentration-dependent killing and time-dependent killing and a combination of the two.[93] For example, vancomycin shows time- and concentration-dependent killing that is best described by the area under the curve (AUC)/MIC. In clinical practice, AUC/MIC is assessed by using the trough concentration as a surrogate for AUC.[120] However, due to their significantly lower clearance in neonates and other differences in PK, a sufficient AUC/MIC ratio can be achieved aiming for lower trough concentrations.[121] Moreover, the choice of dosing interval and daily dose leading to the target AUC has a great impact on the corresponding trough concentration.[122] This underlines that the adult PK target cannot simply be extrapolated to neonates and that more research into suitable PK targets for neonates is needed.

Knowledge gaps and opportunities for future research. In absence of targets determined for neonates, model-informed precision dosing (MIPD) bears the potential to initiate antibiotic treatment with the optimal individual dosage. MIPD captures drug, disease, and patient characteristics in modeling approaches and can be used to perform Bayesian forecasting and dose optimization (Table 2). Despite all reported PK models, this has hardly been implemented yet.[123] Depending on the antibiotic drug, this may imply to start with a loading dose for an immediate adequate exposure and quick onset of effect. Subsequently, therapeutic drug monitoring (TDM) can be used to further optimize individual exposure which should aim for the most evidence based target so far: AUC. Due to the observed large inter-individual difference in PK in preterm neonates, TDM is of additional value with respect to preventing toxicity as well as assuring treatment efficacy.[124] One good example for this could be beta-lactams as recent studies have shown that in neonates with sepsis therapy failure, as little as 20% of the benzyl penicillin concentrations were within the target range.[125]

MONITORING SEPSIS COURSE

Assessing treatment effect

LONS may compromise the cardiovascular system, leading to reduced cardiac output and/or disturbed microcirculation, but also other organ systems. Adequate biomarkers to judge effectiveness

of LONS management would significantly improve clinical care; knowing you are on the right track even before the patient shows clinical recovery, potentially prevents overuse of supportive therapy and unnecessary switches to broad-spectrum antibiotics. An ideal biomarker to assess treatment effect should have a high predictive value and a fast response to changes in the disease state and easily available. Preferably, the biomarker changes before the clinical condition of the patient improves or deteriorates and is measured non-invasively.[105,126] Conventional methods for assessing the hemodynamic status during sepsis, such as blood pressure, urine output, and heart rate, seem inaccurate and unreliable.[127,128] More objective physiological biomarkers of the (micro) circulation could be used during disease monitoring. Repeated assessment of cardiac output measurements, obtained by (neonatologist performed) echocardiography, may also provide objective physiological biomarkers that could be used during for disease status monitoring.[129,130] However, these measurements require intensive training and there is a lack knowledge regarding the targets to aim for. Furthermore, assessment is limited to central blood flow, while end-organ (micro)circulation is not measured.[129,130] To this end, near-infrared spectroscopy or transcutaneous assessment of capillary flow might be used to monitor regional perfusion, but again targets are lacking and are likely to be individual.[131–134]

Knowledge gaps and opportunities for future research. Future research should develop methods that enable us to measure end-organ blood flow or microcirculation to improve hemodynamic assessment during sepsis, which could facilitate tailored management. Objective measuring tools are needed that generate easy interpretable results with targets available that need to be pursued. Once established, prospective studies should be performed to evaluate whether these tools improve neonatal outcome, when implemented and targeted for.

Hyperinflammation

The preterm neonates immune system is immature and relies on a less well developed innate immune system, making preterm neonates more vulnerable to infections.[135–139] Studies in neonatal cord blood show that the neonatal immune system responds differently to bacterial antigens at birth, compared to adults, which results in different cytokine responses, e.g. lower levels of tumor necrosis factor-α (TNF-α) and higher levels of IL-6.[137,140] These differences can result in a dysregulated pro-inflammatory response to infection, sometimes also called "cytokine-storm".[141] Such an inflammatory response can cause damage to different organ systems[126] which can, in turn, affect the PKs of drugs (see Fig. 2). Depending on the causative pathogen, neonatal immune response will vary. Cell models comparing the response to different aerobe and anaerobe Gram-positive and Gram-negative bacteria in umbilical cord cells and adults cells indicate specific differences in the immune response for different pathogens.[142]

Knowledge gaps and opportunities for future research. Means to identify the different stages of inflammation in preterm neonatal sepsis are of paramount importance to increase clinical understanding and potentially guide therapy. It is likely that the level of biomarkers reflecting the immune response are affected by complex host–pathogen–drug interactions.[143] Many factors can determine the level of such a biomarker: (1) the developing immune system of the neonate, (2) the inflammatory state, (3) the causative pathogen, and (4) administered drugs (see Fig. 2). Research on immune response biomarkers increases the understanding of the neonatal immune response and its dynamics, and should aim to define (immune) biomarkers that can be clinically used for treatment response and for PK/PD of drug treatment. The neonatal immune response could relatively easily be studied in in vitro or high-throughput animal models of sepsis.[144] Up until now animal models of sepsis rarely yielded new biomarkers or therapeutic agents that have been successfully used in clinical practice.[145] A promising new candidate model is zebrafish larvae. The immune system of the zebrafish closely resembles the human immune system.[146] In zebrafish models, larvae can be infected under controlled conditions and the immune response can be observed for different pathogens and maturational statuses. Zebrafish already have been successfully exposed to pathogens relevant for neonatal sepsis, which has already resulted in the identification of factors necessary for the establishment and spread of the infection.[144] Furthermore, the emergence of -omics approaches enables researchers to map and understand physiological changes during sepsis. Rapid onsite omics approaches could be used to monitor the inflammatory response during the course of sepsis, which would enable individualized management according to the patient's functional immune status.[147]

Prediction of severity of sepsis

During the sepsis course, some patients will develop severe sepsis symptoms with need for inotropes or mechanical ventilation and risk of mortality, while others may not. Identification of patients with oncoming severe sepsis could enable personalized monitoring and possible additional interventions to prevent subsequent clinical deterioration. A recent study showed that commonly used chemical biomarkers, such as IL-6, PCT, and CRP, may provide information on who will have a severe LONS.[148] Cut-off values for the biomarkers were calculated to be used in clinical practice as a warning signal for subsequent clinical deterioration. As one may recognize, the severity of illness is also related to the underlying causative microorganism (Gram-negative bacteria); thus, biomarkers establishing the causative organism rapidly after LONS diagnosis are likely to show overlap with predicting severe LONS. In adult sepsis it has been shown that PCT levels differ according to specific causative organism[101] and therefore could potentially be used to predict the microorganism even before the result of the blood culture. However, the discriminatory power of PCT as a sole biomarker is too low to guide therapeutic decisions.[101]

Knowledge gaps and opportunities for future research. Future research should focus on identifying patients with severe sepsis and a developing hyper-inflammatory syndrome in order to facilitate personalized monitoring (e.g. inserting peripheral arterial line) and additional therapy (e.g. immune-modulating treatment) in the early stages of sepsis, thereby going from reactive management towards forward-looking management. Early prediction of the causative organism by (set of) biomarkers might be one way to facilitate this, where the focus should lie on identifying a set of biomarkers rather than a single biomarker. Another potential biomarker that could provide information on sepsis severity could be a biomarker reflecting bacterial load. Prospective studies are needed to investigate which biomarkers could be of value in clinical practice.

ADDITIONAL THERAPY

Next to antibiotic therapy, several studies in neonates have focused on additional therapies consisting of a variety of immune-modulating intervention, up until now evidence remains sparse (knowledge gaps and opportunities are summarized in Table 1).[149] Evidence for the most interventions, such as IVIG, lactoferrin, and granulocyte–macrophage colony-stimulating factor, remains inconclusive.[10,33,94,150–153] When considering additional therapies, it is important to adapt an individual approach, since the immune response varies over time and the timing of immunomodulatory therapy is critical.[154]

Pentoxifylline (PTX)

PTX is a phosphodiesterase inhibitor, originally registered for intermittent claudication in adults,[155] which suppresses the production of TNFα and other inflammatory cytokines and prevents their subsequent effects.[156–159] Furthermore, PTX has beneficial effects on endothelial cell function, coagulation, and microcirculation in preterm neonates with LONS.[160–163] A recent meta-analysis of six studies showed that PTX is associated with a decreased mortality during hospitalization,[164] which makes PTX a promising candidate for the adjunctive treatment of LONS. A larger study to confirm these effects and study long-term outcome is currently ongoing (ACTRN12616000405415). Unfortunately, available data on PKs of PTX and its active metabolites in neonates are limited. Most clinical studies of PTX in neonates used the same dosage of 5 mg/kg/h during 6 h.[157,158] PTX dosages of 5 mg/kg/h during 12 h led to increased plasma levels and were well tolerated in seven preterm neonates.[158,165] However, the most effective dosage and target concentration as well as timing in preterm neonates still need to be determined (dose per kilogram per day, duration of infusion and treatment). In vivo animal models might help define optimal timing and dosing, which subsequently could be validated in a clinical trial.

Mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) are non-hematopoietic multi potent stromal cell that can be isolated from various tissues, such as umbilical cord tissue. MSCs may reduce multi-organ dysfunction and improve survival in sepsis by several mechanisms, which include reducing the inflammatory response, vascular injury, and bacterial load.[166–169] A phase-I trial in nine adults with sepsis showed that infusion of MSCs seemed safe.[170] Although MSC-treatment seems promising in neonatal animal models,[171] information on safety, correct dosage, underlying working mechanism, and possible patient selection is missing in neonatal sepsis. Clinical trials are needed to assess safety, feasibility, effectiveness, and to determine the effective dosage.

Timing of additional therapy

The host immune response in sepsis varies in the same patient over time[172] and gestational age and postnatal age, but also sex, affect the immune responses.[173–175] Therefore, mapping the immune status for each individual patient during the course of sepsis would enable to select patients that might benefit from immunomodulatory treatment at a certain time point during the disease process (see "Hyperinflammation").[176]

Knowledge gaps and opportunities for future research. Depending on the immune state of the patient, immune-modulating drugs may benefit the patient, while other patients may suffer from adverse effects if the drug is administered at an inappropriate time point because of its potentially harmful interference with the developing immune system.[176] For example, giving pentoxifylline in a late stage of severe sepsis has adverse consequences in a swine model of sepsis.[177] However, knowledge regarding sepsis phases in preterm human neonates is still largely lacking.[22] Future research should aim to understand immune response mechanisms and study how and when the immune response should be modulated to improve LONS outcome. As this is complicated to study in preterm neonates, in vitro experiments and/or animal models[146] should be explored first before clinical application is feasible. As a final step, an individualized management based on immune status of the status should be prospectively compared with a group-based approach ideally in a randomized controlled trial.

PERSONALIZED MEDICINE IN CLINICAL PRACTICE

Ideally all information from a patient comes together in real-time bedside "dashboard" of the patients clinical status. This dashboard can incorporate physiological data, patient characteristics (e.g. GA, PNA, sex), clinical data (e.g. previous diagnoses and therapies), biomarkers, and metabolomic and proteomic profiles. Using supervised ML models, all these characteristics could be able to predict a causative organism, stratify the patient according to risk, provide information on immune status, and detect if the patient is responding to the initiated therapy.[178] An optimal (additional) treatment and monitoring strategy tailored to the individual patient could be recommended. By continuously extracting patient (physiological) data and additionally determined biomarkers, the data driven "dashboard" model could update treatment and monitoring strategies and provide additional suggestions for LONS management if needed.[69,179] Development of predictive modeling dashboards will require harmonized and large-scale databases, fed by multicentre collaborations, if possible including proteomics and/or metabolomics data.[180]

A ROADMAP FOR FUTURE RESEARCH

In conclusion, the research field of LONS, is entering an exciting new era. The availability of computational power, computational medicine algorithms, and hypothesis-free -omics approaches give way to building bedside feedback tools to guide clinicians in personalized management of LONS. However, important knowledge gaps still need to be addressed. First, we need to identify biomarkers, which ideally can be measured continuously and non-invasively or in low fluid volumes, to identify preterm neonates with LONS as early as possible and provide information on the severity of LONS. This will enable personalized therapy and monitoring of treatment effect. Second, means of assessing the immune state of a preterm neonate are likely to harness important information to provide optimal immune-modulating therapy at the right time. Third, we need to improve our understanding and utilization of neonatal pharmacology (pharmacokinetics and pharmacodynamics), which may provide means to immediately optimize treatment and supportive strategies. Last, we need to develop clear and informative bedside dashboards, incorporating biomarker information, ML, and AI and monitoring to help clinicians optimize every step in the management of LONS in preterm neonates and improve outcome.

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AUTHOR CONTRIBUTIONS

H.R.T. and S.V. designed and directed the manuscript. H.T., S.V., S.K., K.F. and R.F. performed literature searches and drafted the document. I.K.M.R., S. H.P.S. and H.K. reviewed and revised it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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The authors declare no competing interests.

CONSENT STATEMENT
No patient consent was required.

ADDITIONAL INFORMATION
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