Late-onset neonatal sepsis: recent developments

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ABSTRACT

The incidence of neonatal late-onset sepsis (LOS) is inversely related to the degree of maturity and varies geographically from 0.61% to 14.2% among hospitalised newborns. Epidemiological data on very low birth weight infants shows that the predominant pathogens of neonatal LOS are coagulase-negative staphylococci, followed by Gram-negative bacilli and fungi. Due to the difficulties in a prompt diagnosis of LOS and LOS-associated high risk of mortality and long-term neurodevelopmental sequelae, empirical antibiotic treatment is initiated on suspicion of LOS. However, empirical therapy is often unnecessarily used with unnecessary broad-spectrum antibiotics and a prolonged duration of treatment. The increasing number of multidrug-resistant Gram-negative micro-organisms in neonatal intensive care units (NICU) worldwide is a serious concern, which requires thorough and efficient surveillance strategies and appropriate treatment regimens. Immunological strategies for preventing neonatal LOS are not supported by current evidence, and approaches, such as a strict hygiene protocol and the minimisation of invasive procedures in NICUs represent the cornerstone to reduce the burden of neonatal LOS.

EPIDEMIOLOGICAL AND CLINICAL ASPECTS OF LOS

The onset of LOS is most frequently defined at 72 h after birth, a cut-off time point considered to adequately differentiate LOS from EOS in terms of the spectrum of causative pathogens (table 1). As demonstrated in table 2, the incidence of LOS is inversely associated with birth weight (BW). Similarly, 36.3% of neonates with gestational age (GA) <28 weeks had at least one episode of LOS, as compared with 29.6%, 17.5% and 16.3% of moderately preterm (GA of 29–32 days), late preterm (GA of 33–36 weeks) and term infants. Apart from immaturity, other well-recorded risk factors for LOS include the long-term use of invasive interventions, such as mechanical ventilation and intravascular catheterisation, the failure of early enteral feeding with breast milk, a prolonged duration of parenteral nutrition, hospitalisation, surgery and underlying respiratory and cardiovascular diseases.

INTRODUCTION

Neonatal sepsis contributes substantially to neonatal morbidity and mortality, and is an ongoing major global public health challenge. According to the onset of age, neonatal sepsis is divided into early-onset sepsis (EOS) and late-onset sepsis (LOS). EOS reflects transplacental or, more frequently, ascending infections from the maternal genital tract, whereas LOS is associated with the postnatal nosocomial or community environment, with the peak incidence reported to be between the 10th and 22nd day of life. Since the early 1980s, epidemiological studies have observed a general reduction in EOS, probably due to advances in obstetric care and the use of prophylactic intrapartum antibiotics to prevent infections caused by Group B Streptococcus. Meanwhile, the incidence of LOS has increased in parallel with the improved survival of premature infants, especially in those with very low birth weight (VLBW), indicating the role of hospitalisation and long-term nosocomial or community environment.

The microbial characteristics of LOS are of primary importance in guiding clinical antiseptic practice, and strategies to prevent and treat neonatal LOS may, in turn, influence the pattern of LOS pathogens. An up-to-date and thorough understanding of the epidemiology and management of neonatal LOS may help to reduce the burden of this disease.
The combination of multiple biomarkers, such as the total availability and a reasonable price. Up to now, no single biomarker is considered to be sufficient for the diagnosis of LOS. However, this ‘gold standard’ testing method is time-consuming and may produce false positive results as well as false negative results, which can be attributed to the difficulties in discriminating a true CONS infection from sample contamination. A timely and accurate diagnosis of LOS is of utmost importance, given the mortality rate and long-term adverse outcomes associated with LOS.

**NEW APPROACHES TO DIAGNOSE NEONATAL LOS**

Blood culture remains as the definitive tool for neonatal sepsis. However, this method is time-consuming and may produce false results as well as false negative results, which can be attributed to the difficulties in discriminating a true CONS infection from sample contamination. A timely and accurate diagnosis of LOS is of utmost importance, given the mortality rate and long-term adverse outcomes associated with LOS.

The inherent limitations of blood culture technique have given impetus to an extensive search of biomarkers for diagnosing neonatal sepsis. To qualify as an ideal biomarker, many criteria need to be satisfied, such as a small blood volume, high sensitivity and specificity, high positive and negative predictive values, short laboratory turnaround time, 24 hours bedside availability and a reasonable price. Up to now, no single biomarker has been identified to fulfill most, if not all, these criteria. The combination of multiple biomarkers, such as the total number of neutrophils, immature to total neutrophil ratio and C-reactive protein (CRP) holds promise to enable a fast and accurate diagnosis of LOS. Sequential detection of CRP may help to rule out microbial infections in a timely manner, facilitating an early cessation of antibiotic treatment.

Recently, molecular-based methods have emerged as promising diagnostic tools for neonatal sepsis. PCR, a technology based on the extraction of microbial DNA from blood samples and the subsequent sequencing or hybridisation of species-specific gene regions, is widely investigated for the detection of micro-organisms. Furthermore, real-time PCR which focuses on the temporal measurement of fluorescent signals generated in each amplification cycle, has been explored to monitor the microbial load and rapidly target specific micro-organisms in clinical specimens. Compared with the conventional culture technology, PCR technologies yield results with a higher sensitivity, a much smaller sample volume and less laboratory turnaround time. Recently developed PCR-based diagnostic platforms are highlighted by a low contamination rate, with DNA extraction, multiplex PCR and detection of PCR products performed in a closed system. This design can help to differentiate potential contamination from true positive cases, particularly for the detection of CONS, since CONS from the patients, nurses taking the blood sample and laboratory personnel may cause contamination. Another inspiring development in the field of molecular assays is microarray, which is characterised by the hybridisation of clinical samples on a glass or silicon slide preloaded with an array of protein or nucleic acid products. This technology allows us to simultaneously detect pathogens, microbial virulence and even the host immune response profile. Although highly sensitive and specific, microarray cannot replace the conventional method of culture in the isolation of pathogens and the subsequent detection of antibiotic-resistance profile.

The requirement for special instruments and highly trained staff is also one limitation that needs to be addressed.

Clinical signs of neonatal LOS are generally regarded as non-specific and inconspicuous. Recent studies show that monitoring physiological data constantly displayed in neonates is a promising method to predict proven or clinical sepsis. The greatest advance in this field is the monitoring of heart rate characteristics (HRC), and the rationale is that reduced variability and transient decelerations in heart rate, partly mediated by...
PREVENTION OF NEONATAL LOS

Given that the treatment of sepsis does not always protect infants from the risk of long-term neurodevelopmental impairments, the best strategy is to prevent rather than to treat LOS.28 So far, adherence to infection control protocols remains to be the cornerstone of LOS prevention. By implementing bundles of evidence-based strategies, namely hand hygiene, full-barrier precautions, 2% chlorhexidine skin antiseptics, avoidance of the femoral route and prompt removal of unnecessary catheters, combined with cultural and behavioural support, the Matching Michigan initiative resulted in a remarkable 47.3% decrease in the rate of bloodstream infections from central venous catheters in 19 paediatric ICUs in England.29 Standardised catheter care bundles used among 24 Ohio NICUs were also shown to be effective by reducing 20% of LOS.30 Because nearly one-third of LOS were not associated with intravascular catheters, improvement may require other preventive measures, such as the use of prenatal steroids, reduction of assisted mechanical ventilation, early application of continuous positive airway pressure, early surfactant administration and optimal feeding strategies.31 Additionally, nationwide surveillance systems can contribute to the reduction of neonatal LOS by providing ongoing surveillance data for quality management and benchmarking between institutions.32

Of note, interdisciplinary collaborations at the interface of microbiology and immunology have recently inspired new strategies to prevent neonatal LOS.

Probiotics

Gut microbiome is the complex and dynamic population of several hundred bacterial species colonising in the gut. It has been increasingly recognised as an essential ‘organ’ of newborns in delivering nutrients, regulating epithelial maturation and developing innate immune defence against infections.33 34 Vaginally delivered term infants are usually colonised by anaerobes, such as Bacteroides spp. and E. coli within days of birth, followed by a predominance of Bifidobacterium spp. and Lactobacilli spp. when breast feeding is initiated.35 Caesarean section, prolonged antibiotic use, an extended stay in the healthcare environment and formula feeding can disturb the normal colonisation process.36 VLBW infants were shown to have delayed colonisation of normal bacterial species, as well as less microbial diversity in the intestinal tract due to their frequent exposure to the above-mentioned risk factors.36 37 An abnormal gut microbiota may compromise the integrity of intestinal barrier, causing bacterial translocation into the bloodstream.38 CONS were identified to be a predominant species in the stool of VLBW neonates and were closely associated with LOS independent of the presence of indwelling devices, corroborating the role of gut as an important source of potential pathogens causing sepsis.39 40 The rationale of using probiotics, therefore, is to normalise the gut microbiome with exogenous microorganisms which commonly comprise Bifidobacterium and Lactobacillus spp.41 The potential benefits of probiotics conferred on the host are summarised in Table 3. Although theoretically promising, the use of probiotics in clinical trials has revealed inconsistent results with regard to the prevention of nosocomial sepsis, and meta-analyses showed that probiotics did not significantly reduce the incidence of sepsis as compared with the controls.41 42 The lack of effect may be largely due to the heterogeneity among trials in terms of probiotic administration protocol (strains, dosage, frequency and duration), and more studies are required to determine the efficacy and safety of probiotics in infants.37 41

Table 3 Theoretical mechanisms of currently explored feeding strategies to prevent neonatal LOS

<table>
<thead>
<tr>
<th>Explored strategy</th>
<th>Theoretical mechanisms</th>
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<tbody>
<tr>
<td>Probiotics27 41</td>
<td>† the intestinal mucosal barrier to prevent the translocation of bacteria</td>
</tr>
<tr>
<td></td>
<td>† immunoglobulin A mucosal responses</td>
</tr>
<tr>
<td></td>
<td>† modulation of host immune reactions to microbial products</td>
</tr>
<tr>
<td>Early enteral trophic feeding44 46</td>
<td>† eneral nutrition and gut maturation</td>
</tr>
<tr>
<td></td>
<td>† the establishment of healthy gut microflora</td>
</tr>
<tr>
<td></td>
<td>† gut mucosal immunity</td>
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<tr>
<td>Lactoferrin28 38</td>
<td>† antimicrobial effect by iron chelation</td>
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<tr>
<td></td>
<td>† immunomodulatory function through cytokine production</td>
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<td></td>
<td>† the formation of reactive oxygen species</td>
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LOS, late-onset sepsis.
Early enteral trophic feeding with breast milk

The initiation of enteral feeding is often delayed in VLBW neonates due to the concern that early enteral feeding may not be tolerated and may be implicated in the pathogenesis of necrotising enterocolitis ( NEC). However, the lack of enteral feeds may hinder the functional maturation of the gastrointestinal tract, and the prolonged use of parenteral nutrition is associated with an increased risk of systemic infection due to the impairment of immune cell functions. Trophic feeding, also referred to as minimal enteral feeding and priming feeding, is generally defined as an enteral intake of breast milk and/or formula, with a small volume of up to 24 mL/kg/day. This strategy attempts to overcome the absence of enteral stimulation while exerting minimal stress on the immature gastrointestinal system. Early trophic feeding, initiated within the first few days of birth, has shown benefits in the prevention of nosocomial infections without an increased risk of intestinal complications. Breast milk, apart from its abundant nutrients, also contains secretory antibodies, immune cells, lactoferrin (LF) and prebiotics which can stimulate the growth of beneficial gut flora. Therefore, breast milk has been given priority over formula in the introduction of enteral trophic feeding due to its benefits on the promotion of neonatal immune functions. It is demonstrated that human milk feeding started within the first 72 h after birth was associated with an approximately threefold reduction in the risk of LOS. Despite numerous studies, multiple factors of the feeding protocol, such as the time of initiation, method of administration and advance rate still remain controversial, and further trials are needed for protocol optimisation.

**Lactoferrin**

LF, a major protein in human milk, performs multiple functions as an important component of innate immune defence against infections (table 3). Bovine lactoferrin (BLF) has been shown to significantly decrease the incidence of neonatal LOS as compared with placebo controls. When combined with probiotics, BLF further enhanced its prophylactic effect on LOS, emphasising the synergistic action of LF and other antimicrobial agents. However, there was a small number of preterm infants included in these trials, and further studies are warranted to fully assess the effectiveness and safety of LF in neonates by addressing its optimal dosage, duration of treatment and possible combination with probiotics. The prophylactic use of LF cannot be recommended as routine yet.

**Immune replacement therapy**

The immune system of neonates, especially the most immature ones, is characterised by a low neutrophil storage pool and rapid exhaustion of bone marrow reserve during sepsis. As a consequence, neutropenia may ensue. Additionally, an inadequate transplacental transport of maternal immunoglobulin G results in a prolonged immunoglobulin deficiency at birth, which is further aggravated during the first month in life. Based on this knowledge, immune replacement therapies were widely explored in the hope of correcting the immune deficiencies and thus preventing neonatal infections. Colony-stimulating factors (CSF), such as granulocyte CSF and granulocyte-macrophage CSF, are cytokines that promote the proliferation and antimicrobial function of neutrophils, monocytes and macrophages. However, there was no significant difference in sepsis-free survival between the intervention group and placebo group (table 4). Intravenous immunoglobulins (IVIG), which can enhance opsonic activity, complement activation, antibody-dependent cytotoxicity and neutrophil phagocytosis, showed no prophylactic effect on neonatal sepsis. It is noteworthy, that IVIG treatment of neonates with suspected or proven sepsis also failed to reduce the mortality in a large multicentre trial. Moreover, INH-A21, a specific antistaphylococcal immunoglobulins against *Staphylococcus aureus*, demonstrated no significant effect to prevent neonates against sepsis despite its theoretical value.

**Table 4 Trials for prevention of late-onset sepsis in very low birth weight neonates**

<table>
<thead>
<tr>
<th>Trial of example</th>
<th>Birth year of cohort</th>
<th>Therapy</th>
<th>No. of infants</th>
<th>Outcome</th>
<th>Results (intervention vs control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune replacement therapy</td>
<td>Carr et al</td>
<td>2000–2006</td>
<td>GM-CSF</td>
<td>139 intervention: 141 control: Sepsis-free survival rate</td>
<td>66.9% vs 74.5%, difference: −8%, 95% CI −18 to 3</td>
</tr>
<tr>
<td>Kuhn et al</td>
<td>2002–2006</td>
<td>G-CSF</td>
<td>102 intervention: 98 control: Sepsis-free survival rate</td>
<td>73% vs 67%, p=0.42</td>
<td></td>
</tr>
<tr>
<td>Fanaroff et al</td>
<td>1988–1991</td>
<td>IVIG</td>
<td>1204 intervention: 1212 control: Incidence of sepsis</td>
<td>15.5% vs 17.2%, RR: 0.9, 95% CI 0.75 to 1.08</td>
<td></td>
</tr>
<tr>
<td>Delonge et al</td>
<td>2004–2006</td>
<td>INH-A21</td>
<td>994 intervention: 989 control: Incidence of sepsis</td>
<td>27% vs 29%, p=0.2</td>
<td></td>
</tr>
<tr>
<td>Manzoni et al</td>
<td>2007–2008</td>
<td>BLF alone</td>
<td>152 intervention: 168 control: Incidence of sepsis</td>
<td>5.9% vs 17.3%, p=0.002</td>
<td></td>
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<tr>
<td>BLF plus LGG</td>
<td>151 intervention: 168 control: Incidence of sepsis</td>
<td>4.6% vs 17.3%, p=0.001</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Skin care with antiseptics</td>
<td>Quach et al</td>
<td>2009–2013</td>
<td>CHG bathing</td>
<td>195 intervention:</td>
<td>Sepsis rate decreased in the period of CHG bathing (6.00 vs 1.92/1000 CVC-days; adjusted RR, 0.33, 95% CI 0.15 to 0.73)</td>
</tr>
</tbody>
</table>

* *Bifidobacterium infantis, Streptococcus thermophilus and Bifidobacterium lactis.*
†Grouping of neonates was based on the presence of sepsis.
‡The study used a before-and-after quasi-experimental design.
BLF, bovine lactoferrin; CHG, chlorhexidine gluconate; CVC, central venous catheter; GM-CSF, granulocyte-macrophage colony-stimulating factor; IVIG, intravenous immunoglobulins; LGG, Lactobacillus rhamnosus; G-CSF, granulocyte colony-stimulating factor; RR, relative risk.
The failure of immune replacement therapies in the prophy-
laxis of neonatal sepsis suggests that neonatal immunity is not
simply immature, but rather specifically regulated for the early
stage of postnatal life, and LOS should be managed based on
the immunological specificities of neonates.

Skin care with antiseptics
Neonates receiving intensive care, especially VLBW infants, are
prone to be colonised by pathogenic organisms from the hos-
pital environment. An immature skin barrier and frequent
exposure to skin-disrupting procedures further contribute to
their vulnerability to nosocomial pathogens. Antiseptics have
been shown to reduce skin colonisation of pathogens. A meta-
analysis on the use of Chlorhexidine gluconate (CHG),
however, yielded no conclusive benefits of CHG bathing on
sepsis prevention (pooled relative risk: 0.65, 95% CI 0.40 to
1.05). It should be noted that there was a high between-trial
heterogeneity with regard to CHG concentration, bathing fre-
quency and patients’ baseline characteristics. Despite a scarcity
of reported adverse events, CHG cleansing and bathing is asso-
ciated with risks of skin irritation and toxic effects following the
systemic absorption. Other concerns are that CHG may raise
skin pH in infants, thereby disturbing the physiological acidic
milieu established on neonates’ skin within days of birth. This
so-called ‘postnatal acid mantle’ is important for metabolic
activities of keratinocytes and the development of normal skin
microflora. CHG bathing may remove vernix caseosa, the bio-
logical functions of which include mechanical barrier protection
and thermoregulation, as well as antimicrobial and immunomo-
dulatory properties. Furthermore, CHG may eliminate commensal
bacteria on the skin and result in a microflora dominated by pathogenic organisms, predisposing the infant to infections. So far, CHG bathing has not been recommended for routine use in neonates due to a lack of data on safety and efficacy.

ANTIBIOTIC TREATMENT OF LOS
Due to the potential negative outcomes associated with missed
dose cases, empirical antibiotic treatment is initiated on suspi-
cion of LOS. An ideal choice of antimicrobial agents is to cover
the most common pathogens without providing selection pres-
sure for antibiotic resistance. Currently, the recommended
first-line therapy is flucloxacillin (or ampicillin) combined with
gentamicin. Recent national surveillance data from UK showed that the vast majority of organisms isolated from LOS
blood samples (95%–97%) were susceptible to gentamicin+
flucloxacillin and gentamicin+amoxicillin/penicillin, suggesting that the current guideline for empirical therapy is adequate and
most LOS cases can be appropriately treated by narrow-
spectrum antibiotics.

This may hold true for UK and some Western countries. However, in many countries and regions of the world, a different pattern of causative micro-organisms has been
identified, and the first-line antibiotic regimen is required to
be tailored to the local pathogenic epidemiology. The increas-
ing number of multiresistant strains, especially in developing
countries, is a serious matter of concern. As demonstrated by a
study of four Asian units, 37% of all Gram-negative organisms
were resistant to gentamicin and approximately one-third were
resistant to both gentamicin and third-generation cephalospor-
ins. Similarly, 14.7% of all Gram-negative organisms isolated
in a hospital in Kuwait were resistant to gentamicin, and a high
rate of resistance to cephalosporin (41.8%) was observed. A
possible explanation is that alternatives to the choice of antibio-
tics are diverse in these regions and frequently incorporate
third-generation cephalosporin, such as cefotaxime, in disregard
of the recommended regimen. It is alarming that approxi-
mately 20% of neonatal units in UK and the Republic of Ireland
use a cephalosporin, as shown by recent data. Another concern
is the increasingly resistant CONS, and the optimal trough
vancomycin concentration has been increased from 5 μg/mL to 10 μg/mL in order to sustain an effective vancomycin therapeutic range against CONS. The application of broad-spectrum antibiotics is alerting due to its
close association with multidrug-resistance, and it has been reit-
erated that the spectrum of antibiotics used for empirical therap-
ies should be as narrow as possible.

Apart from the selection of antibiotics, duration of treatment
is another important factor to consider in empirical antibiotic
therapies. A prompt cessation of antibiotics is generally war-
ranted if blood culture yields negative results after 36–48 h, and
the infant shows no subsequent clinical evidence of sepsis or
other neonatal infections. Although appropriately cautiously,
this practice still leads to unnecessary antibiotic exposure among
many infants, since blood cultures are positive in only 5%–10% of suspected cases. It is alerting that antibiotics are overused in patients who did not actually develop LOS, leading to a
higher risk of NEC or death (OR: 2.66, 95% CI 1.12 to 6.3) among them as compared with patients receiving no or limited antibiotics. Since the antibiotic treatment of culture-proven
sepsis is imperative and unavoidable, minimisation of empirical
therapy in infants who have not actually developed sepsis or
other neonatal infections contributes substantially to patients’
well-being and may help to contain microbial susceptibility to anti-
biotic treatment.

Given the increasing resistance rate of pathogens and rela-
tively slow development of novel antimicrobial agents, antibiotic
stewardship programme (ASP) is designed and implemented to
optimise antibiotic therapy. Major strategies include: (1) performing prospective audits with interven-
tions and feedbacks; (2) cooperating with local microbiology
and infection control staff to regularly monitor the adequacy of antibiotic regimens, because the pattern of causative pathogens and antibiotic resistance profile may change over time and vary
geofraphically; (3) avoiding unnecessary use of broad-spectrum antibiotic in proven infections; (4) reducing antibiotic admin-
istration at the start of life and ensuring the cessation of empirical antibiotic treatment when negative blood culture results are
obtained; (5) educating antimicrobial prescribers and document-
ing their compliance with the guidelines. To date, ASPs have
shown positive impact on the quality of antibiotic use, with
microbiological outcomes improved in 75% of the studies.

CONCLUSION
The advance in neonatal intensive care medicine is a double-
edged sword, with improved survival of neonates on one side
and an increased rate of LOS on the other. The pathogen
pattern of neonatal LOS changes with time and over regions, and
should be regularly re-evaluated to guide the management of
LOS. Despite all efforts, an early and accurate diagnostic tool
for neonatal LOS is yet to be found. In the current empirical
antibiotic regimen, ASPs should be implemented to avoid
unnecessary usage of broad-spectrum antibiotics and a
longer-than-needed duration of treatment. Up to now, the best
strategy to treat neonatal LOS lies in prevention. Besides strict
adherence to established infection control protocols and less
invasive interventions in neonatal intensive care, current
evidence shows that early feeding with breast milk is a promising
measure to effectively prevent neonatal LOS.

Review

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