Neonatal Infectious Diseases

Evaluation of Neonatal Sepsis

Andres Camacho-Gonzalez, MD, MSc[a,*], Paul W. Spearman, MD[a], Barbara J. Stoll, MD[b]

KEYWORDS

• Neonatal sepsis • Immature immunity • Early and late onset disease
• Biologic markers • Treatment

KEY POINTS

- The adoption of Centers for Disease Control and Prevention guidelines for intrapartum antibiotic prophylaxis to reduce vertical transmission of Group B streptococcus (GBS) resulted in an 80% decrease in neonatal GBS sepsis.
- Nonetheless, GBS and *Escherichia coli* remain the most common causes of early-onset sepsis in neonates.
- Coagulase-negative staphylococci are now the most common cause of late-onset neonatal sepsis, particularly in low birth weight infants.
- Among commonly used biomarkers, limited studies suggest that serial C-reactive protein levels and serial assessment of immature:total neutrophil counts provide the best negative predictive value for neonatal sepsis.
- No biomarker to date provides a good positive predictive value for neonatal sepsis.
- Newer biomarkers and broad-based and real-time polymerase chain reaction have demonstrated promise in the early detection of neonatal sepsis, but further study is required to determine if they will be useful in clinical practice.
- Among recent interventions to prevent neonatal sepsis, the use of fluconazole prophylaxis in very low birth weight infants is the only intervention that has shown repeated efficacy in multiple trials.
- Other interventions to prevent neonatal sepsis, such as antistaphylococcal monoclonal antibodies and lactoferrin administration, show early promise but require larger studies to determine real-world efficacy.

[a] Division of Pediatric Infectious Diseases, Emory Department of Pediatrics, Children's Healthcare of Atlanta, Emory University, 2015 Uppergate Drive, Suite 500, Atlanta, GA 30322, USA; [b] Department of Pediatrics, Children's Healthcare of Atlanta at Egleston, Emory-Children's Center, 2015 Uppergate Drive, Suite 200, Atlanta, GA 30322, USA
* Corresponding author.
E-mail address: acamac2@emory.edu

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EPIDEMIOLOGY OF NEONATAL SEPSIS

Neonatal sepsis remains a feared and serious complication, especially among very low birth weight (VLBW) preterm infants. Neonatal sepsis is divided into early-onset and late-onset sepsis, based on timing of infection and presumed mode of transmission. Early-onset sepsis (EOS) is defined by onset in the first week of life, with some studies limiting EOS to infections occurring in the first 72 hours that are caused by maternal intrapartum transmission of invasive organisms. Late-onset sepsis (LOS) is usually defined as infection occurring after 1 week and is attributed to pathogens acquired postnatally. Risk factors for neonatal sepsis include maternal factors, neonatal host factors, and virulence of infecting organism (**Table 1**).

In the United States, widespread acceptance of intrapartum antibiotic prophylaxis (IAP) to reduce vertical transmission of Group B Streptococcal (GBS) infections in high-risk women has resulted in a significant decline in rates of EOS GBS infection.[1] Overall, it is not believed that IAP has resulted in a change in pathogens associated with EOS; however, some studies among VLBW preterm infants have shown an increase in EOS caused by *Escherichia coli*.[2] A recent study done by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN) estimated the overall incidence of EOS to be 0.98 cases per 1000 live births, with increasing rates in premature infants.[3] Studies with stratification of disease burden by gestational age and race have shown that black preterm neonates have a significantly higher incidence of neonatal sepsis as compared with the rest of the population, accounting for 5.14 cases per 1000 births with a case fatality rate of 24.4%.[4]

Despite efforts to detect GBS colonization during pregnancy and provide appropriate GBS prophylaxis to colonized mothers, not all cases of early-onset GBS are prevented and GBS continues to be the most common cause of EOS in term neonates. Sepsis caused by *E coli* has increased in recent years, mainly affecting preterm newborns weighing less than 2500 g at birth, and is considered the most common cause of EOS in this weight group. *E coli* is frequently associated with severe

Table 1
Risk factors for the development of neonatal sepsis

Source	Risk Factor
Early-onset neonatal sepsis	Maternal Group B streptococcal colonization Chrorioamnionitis Premature rupture of membranes Prolonged rupture of membranes (>18 h) Maternal urinary tract infection Multiple pregnancies Preterm delivery (<37 wk)
Late-onset neonatal sepsis	Breakage of the natural barriers (skin and mucosa) Prolonged indwelling catheter use Invasive procedures (eg, endotracheal intubation) Necrotizing enterocolitis Prolonged use of antibiotics H_2-receptor blocker or proton pump inhibitor use
Neonatal[a]	Prematurity • Decreased passage of maternal immunoglobulin and specific antibodies • Immature function of immune system

[a] Increases the risk for both early-onset and late-onset neonatal sepsis.

infections and meningitis and it has become the leading cause of sepsis-related mortality among VLBW infants (24.5%).[4] Together, GBS and *E coli* account for about 70% of cases of EOS in the neonatal period.[5,6]

Rates of LOS are most common in preterm low birth weight infants. Studies from the NICHD NRN report that approximately 21% of VLBW infants weighing less than 1500 g, developed 1 or more episode of blood culture–confirmed LOS, with rates inversely related to gestational age (GA) (58% at 22 weeks GA and 20% at 28 weeks GA).[7,8] Intrapartum antibiotic prophylaxis has not had an impact on rates of LOS.[1,9] VLBW preterm infants are at particular risk for LOS in part because of prolonged hospitalization and prolonged use of indwelling catheters, endotracheal tubes, and other invasive procedures. Several studies have documented rates of LOS from 1.87 to 5.42, with decreasing rates as birth weight increases.[6,7] Coagulase-negative staphylococci (CoNS) have emerged as the most commonly isolated pathogens among VLBW infants with LOS.

DEVELOPMENT OF THE IMMUNE SYSTEM AND INCREASED RISK OF NEONATES TO INFECTIONS

The development of the immune system entails a number of changes that occur during the first years of life. Neonates, especially preterm infants, are relatively immunocompromised because of immaturity of the immune system, as well as decreased placental passage of maternal antibodies. Here we highlight some of the components of the neonatal immune system that are immature and contribute to increased susceptibility to serious bacterial, fungal, and viral infections.

Innate Immune System

The innate immune system produces an immediate immunologic response and is capable of doing this without previous exposure to a specific pathogen. Recognition of pathogens occurs by identification of conserved biologic regions known as pathogen-associated molecular patterns (PAMPs). Recognition receptors, such as TOLL-like receptors, NOD-like receptors and RIG-like receptors, identify and respond to PAMPs with the production of cytokines and proinflammatory responses that activate the adaptive immune system.[10] Studies comparing neonatal and adult innate immune functions show that neonatal cells have a decreased ability to produce inflammatory cytokines, especially tumor necrosis factor (TNF) and interleukin (IL)-6.[11] In addition, they induce IL-10 production, which in itself is capable of inhibiting synthesis of proinflammatory cytokines.[12] Neutrophil and dendritic cell functions are also reduced; neutrophils show a decreased expression of adhesion molecules, as well as a decreased response to chemotactic factors,[13,14] and dendritic cells have a decreased capacity of producing IL-12 and interferon (IFN) gamma. The overall reduction in cytokine production in neonates also results in decreased activation of natural killer cells.[15] Impairment of the innate immune system leads to an increased susceptibility to bacterial and viral infection in this population.

Adaptive Immune System

The adaptive branch of the immune system is designed to eliminate specific pathogens. In newborns, the adaptive immune system slowly increases its function toward an adultlike response, minimizing the otherwise overwhelming inflammatory response that would occur when infants transition from a sterile to a colonized environment.[16] Decreased cytotoxic function (strong T-helper 2 polarization with decreased IFN-gamma production), lack of isotype switching, and overall immaturity and decreased memory (because of limited pathogen exposure at time of birth), reduce the neonate's

ability to respond effectively to infections.[17–20] For example, the reduction of cell-mediated immunity increases the risks of infections caused by intracellular pathogens, such as *Listeria*, *Salmonella*, herpes simplex virus (HSV), cytomegalovirus, and enteroviruses.

Transplacental passage of maternal immunoglobulin G (IgG) is inversely related to gestational age and limits the functional ability of the neonate to respond to certain pathogens.[21,22] Minimal IgG is transported to the fetus in the first trimester, whereas fetal IgG rises in the second trimester from approximately 10% at 17 to 22 weeks' gestation to 50% at 28 to 32 weeks' gestation.[23,24] Thus, preterm infants lack adequate humoral protection against a number of infant pathogens, whereas term infants will often be protected against most vaccine-preventable neonatal infections through transplacental passage from the mother's serum. Histologic studies have also demonstrated that the marginal zone of the spleen is not fully developed until 2 years of age, increasing the infant's susceptibility to encapsulated bacterial infections (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*).[25] Finally, transfer of IgA, IgG, cytokines, and antibacterial peptides present in human milk may be compromised, especially in premature babies. The lack of secretory IgA decreases the ability of the neonate to respond to environmental pathogens.[26]

Complement

Complement levels increase with increasing gestational age, but are only about 50% of adult levels at term. Reduced complement levels are associated with deficient opsonization and impaired bacterial killing. Although both pathways seem to be capable of being activated, there may be variations in their activation level. In addition, profound C9 deficiency has been observed in neonates, reducing the ability to form bacteriolytic C5b-9 (m), which will increase the risk of acquiring severe invasive bacterial infections.[27,28]

ETIOLOGIC AGENTS IN NEONATAL SEPSIS

The etiologic agents associated with neonatal sepsis in the United States have changed over time.[5] In this section, we review current data on organisms associated with early-onset and late-onset neonatal sepsis (**Table 2**).

EARLY-ONSET SEPSIS

Group B-streptococcus

Despite widespread use of IAP to prevent vertical transmission of invasive GBS disease, missed opportunities for prevention exist and GBS remains the most common

Table 2
Organisms associated with early-onset and late-onset neonatal sepsis

Early-Onset Sepsis	Late-Onset Sepsis
Group B *Streptococcus*	Coagulase-negative *Staphylococcus*
Escherichia coli	*Staphylococcus aureus*
Listeria monocytogenes	Enterococci
Other streptococci: *Streptococcus pyogenes,* viridans group streptococci, *Streptococcus pneumoniae*	Multidrug-resistant gram-negative rods (*E coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Citrobacter*, *Serratia*)
Enterococci	*Candida*
Nontypable *Haemophilus influenzae*	

organism associated with EOS in the United States. According to the Centers for Disease Control and Prevention (CDC), rates of early-onset invasive GBS disease have declined by 80% since the CDC prevention guidelines were first published.[9] GBS are gram-positive encapsulated bacteria for which 10 different serotypes have been identified; serotype III strains are responsible for most of disease (54%).[29] GBS commonly colonize the gastrointestinal (GI) and genital tracts, with rates up to 20% in the adult population.[30] Transmission occurs late in pregnancy or during labor and delivery, and the likelihood of disease, as well as the severity, has been associated with the density of recto-vaginal carriage.[31,32] GBS possess different virulence factors that determine its ability to cause invasive disease: (1) capsular polysaccharide, which helps evade phagocytosis; (2) pili, which allows adherence of GBS to the host's epithelial cells as well as transepithelial migration; and (3) C5a peptidase, which inhibits human C5a, a neutrophil chemoattractant produced during complement activation. Among infected newborns, clinical manifestations develop very early after delivery and most infants will have signs of respiratory distress and cardiovascular instability. Infants with early-onset GBS are at increased risk for meningitis. Rapid deterioration of the clinical status is expected unless prompt antibiotic management is started. Risk of death is inversely related to gestational age, with mortality of 20% to 30% among infected infants of less than 33 weeks' gestation, compared with a mortality of 2% to 3% in full-term infants.[1,33]

Escherichia coli

E coli, a gram-negative rod that commonly colonizes the maternal urogenital and GI tracts, is considered the second most common cause of neonatal sepsis in term infants and the most common cause in VLBW neonates with rates of 5.09 per 1000 live births.[3,34,35] The antigenic structure of *E coli* is represented by multiple antigens (O), (K), and (H), which in combination account for the genetic diversity of the bacteria. Strains with the K1 antigen have been associated with the development of neonatal sepsis and meningitis, as well as with increased risk of mortality when compared with K1-negative strains.[36] Some studies suggest a more aggressive presentation for infants infected with *E coli*, with a higher risk of thrombocytopenia and death in the first days of life.[3] Several US studies have shown high rates of ampicillin resistance in *E coli* strains that infect newborns. Although some studies have shown an association between intrapartum antibiotic exposure and ampicillin-resistant *E coli*, ampicillin resistance has increased throughout the community and a direct link between intrapartum use of ampicillin and the higher likelihood of resistance has not been established.[3,37–39]

Listeria monocytogenes

Listeria is a facultative anaerobic, gram-positive bacterium found in soil, decaying vegetation, fecal flora, and raw unprocessed food.[40] Multiple virulence factors allow *Listeria* to escape the immune system, including listeriolysin, which helps the organism avoid the oxidative stress of phagolysosomes, allowing intracellular replication. *Listeria* proteins ActA, phospholipase C, and lecithinase allow polymerization of actin and lysis of phagosomal membranes, enabling cell-to-cell transmission.[41,42] Pregnant women have 17% higher risk of *Listeria* infection than nonpregnant women, and infection has been associated with spontaneous abortions and stillbirths. Early neonatal infections have a similar clinical presentation, as EO GBS infections, with respiratory distress, sepsis, and meningitis. In severe cases, patients may present with a granulomatous rash (small patches with erythematous base), known as granulomatosis infantisepticum. Most cases of neonatal *Listeria* are caused by serotypes 1, 2, and 4, with

the latter serotype responsible for almost all cases of meningitis.[43] Suspicion for *Listeria* sepsis should be increased in ill infants of mothers who have consumed raw milk, unpasteurized cheeses, or other unprocessed food products that have been contaminated with the organism.[44,45]

Other Bacterial Etiologic Agents Seen in EOS

Other less common but important pathogens associated with EOS include other streptococci (*Streptococcus pyogenes*, viridans group streptococci, *S pneumoniae*), enterococci, staphylococci, and nontypable *H influenzae*. *S pyogenes* (group A Streptococcus [GAS]) was once the predominant organism responsible for neonatal sepsis. Although overall incidence has decreased significantly, severe cases of EO GAS continue to be reported. A recent literature review identified 38 cases of neonatal GAS sepsis (24 with EOS). Patients were most likely to present with pneumonia and empyema (42%) or toxic shock syndrome (17%); 70% of the isolates were M1 serotype and they were all susceptible to penicillin. Mortality was estimated to be 38% among patients with EOS.[46] The presentation of pneumococcus, groups C and G streptococci, and viridans streptococci neonatal sepsis is very similar to GBS infection, and transmission seems to be secondary to bacterial colonization of the maternal genital tract.[47–51] Enterococcal EOS is usually mild compared with LOS and is characterized by either a mild respiratory illness or diarrhea without a focal infection. *Enterococcus faecalis* is more frequently isolated than *Enterococcus faecium,* and most of the isolates remain ampicillin susceptible.[52] Although nontypable *H influenzae* frequently colonizes the maternal genital tract, neonatal infection is relatively rare, but with high mortality rates, especially in preterm neonates.[3,53] Hershckowitz and colleagues[54] reported a cluster of 9 cases with 3 deaths; similar high mortality rates were reported in a series by Takala and colleagues.[55]

LOS

The increased survival of preterm low birth weight infants, particularly those who are VLBW, with need for prolonged hospitalization and use of invasive procedures and devices, especially long-term intravascular catheters, results in ongoing risk of infection. LOS is largely caused by organisms acquired from the environment after birth. The following section reviews the most common organisms associated with LOS (see **Table 2**).

CoNS and Staphylococcus aureus

CoNS has emerged as the single most commonly isolated pathogen among VLBW infants with LOS and is associated with 22% to 55% of LOS infections among VLBW infants.[56,57] *S aureus* is associated with 4% to 8%.[7,58] *Staphylococcus* commonly colonizes the human skin and mucous membranes and is capable of adhering to plastic surfaces with the subsequent formation of biofilms. These biofilms protect the bacteria from antibiotic penetration and can produce substances that will help them evade the immune system. Although CoNS infections are usually secondary to *Staphylococcus epidermidis,* other strains such as *Staphylococcus capitis*, *Staphylococcus haemolyticus,* and *Staphylococcus hominis* have also been reported.[59] Methicillin-resistant *S aureus (*MRSA) has been isolated in 28% of staphylococcal infections in preterm neonates with no significant differences between MRSA and methicillin-susceptible organisms in terms of morbidity, mortality, and length of hospital stay. Overall, 25% of infants infected with MRSA die, with no significant difference in death rates between infants infected with MRSA or methicillin-susceptible *S aureus*.[58]

Gram-negative Organisms

Gram-negative organisms are associated with about one-third of cases of LOS, but 40% to 69% of deaths due to sepsis in this age group. Transmission occurs from the hands of health care workers, colonization of the GI tract, contamination of total parenteral nutrition or formulas, and bladder catheterization devices.[60,61] The most common gram-negative organisms isolated include *E coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Citrobacter,* and *Serratia*.[62] In some case series, *Klebsiella* is recognized as the most common gram-negative agent associated with LOS, ranging from 20% to 31% of cases.[63,64] Infections caused by *Pseudomonas* have been associated with the highest mortality.[65] *Citrobacter* is uniquely associated with brain abscesses, but dissemination can occur to other organs. Its ability to survive intracellularly has been linked to the capacity of creating chronic central nervous system (CNS) infections and abscesses.[66,67]

Candida Infections

Infections caused by *Candida* species are the third leading cause of LOS in premature infants. Risk factors of infection include low birth weight, use of broad-spectrum antibiotics, male gender, and lack of enteral feedings.[68] *Candida albicans* and *Candida parapsilosis* are the species most commonly associated with disease in neonates.[69,70] Poor outcomes, including higher mortality rates and neurodevelopmental impairment, have been associated with the ability of the organisms to express virulence traits, such as adherence factors and cytotoxic substances.[71] *Candida* easily grows in blood culture media, but its isolation may require larger volumes of blood than normally obtained in neonates and therefore multiple cultures may be necessary to document infection and clearance. Among those with a positive cerebrospinal fluid (CSF) culture, as many as 50% will have a negative blood culture; the discordance of blood and CSF cultures underscores the need for a lumbar puncture (LP).[68] Prompt removal of contaminated catheters is also recommended based on the ability of *Candida* species to create biofilms, as well as better survival rates and neurodevelopmental outcomes in patients who had early removal and clearance of the infection.[68]

HSV

HSV is a potentially devastating cause of late-onset neonatal infection. HSV should be included in the differential diagnosis and treatment strategy of newborns who present with signs and symptoms of sepsis, especially after the first few days of life. For a detailed discussion on neonatal HSV infection, refer to the article by Kimberlin and colleagues, elsewhere in this issue.

CLINICAL MANIFESTATIONS

Both EOS and LOS have nonspecific clinical manifestations (**Table 3**). The importance of a lumbar puncture in neonates with suspected sepsis and without specific CNS clinical manifestations is underscored by studies showing growth of CSF cultures despite negative blood cultures, especially in VLBW infants.[72]

DIAGNOSTIC METHODS

Early diagnosis of neonatal sepsis is challenging because clinical characteristics are nonspecific and difficult to differentiate from those of noninfectious etiologies, and because the repertoire of ancillary laboratory tests is limited and not always reliable. Blood culture remains the gold standard for diagnosis of neonatal sepsis, but the

Table 3
Clinical manifestations in patients with early-onset and late-onset neonatal sepsis

Sepsis/Meningitis	Temperature instability Respiratory distress Apnea Jaundice & feeding intolerance Bulging fontanel Seizures
Other	Skin lesions: may occur in disseminated staphylococcal, *Listeria,* and *Candida* infections Joint and bone: may be the preceding event

rate of positivity is low, influenced by factors such as intrapartum antimicrobial administration and limitations in blood volume per culture that can be obtained in neonates.[73,74] Here we review the standard evaluation of neonatal sepsis, followed by a discussion of recent data on inflammatory markers and diagnostic methods in neonatal sepsis.

General Evaluation of Neonatal Sepsis

A neonate with signs and symptoms of sepsis (see **Table 3**) requires prompt evaluation and initiation of antibiotic therapy. Blood, CSF (as clinical condition allows), and urine cultures (useful only after the third day of life) should be obtained.[72,75,76] Chest radiograph is indicated in patients having respiratory symptoms. If disseminated herpes is suspected (herpetic skin lesions, elevated hepatic transaminases, maternal peripartum herpes infection), surface cultures from conjunctiva, mouth, skin, and anus, as well as herpes DNA polymerase chain reaction (PCR) from CSF and blood should also be ordered.[77,78] Ancillary tests, such as complete blood count (CBC) and C-reactive protein (CRP), should not preclude a sepsis evaluation in a neonate, because they can be normal (see the following sections).[79] If positive, however, they can be useful in supporting the diagnosis and determining length of therapy. Careful maternal and exposure history targeted toward identifying potential risk factors (see **Table 1**), as well as a complete physical examination, including skin and catheter insertion sites, should be obtained.

Complete Blood Cell Count

Contrary to older children and adults, the white blood cell (WBC) count does not accurately predict infection in neonates. A recent multicenter review of CBCs and blood cultures in neonates admitted to 293 neonatal intensive care units (NICUs) in the United States, showed that low WBC and absolute neutrophil counts, as well as high immature-to-total neutrophil ratio (I:T ratio) were associated with increasing odds of infection (odds ratios 5.38, 6.84, and 7.90, respectively); however, the test sensitivities for detection of sepsis were low.[80] Studies looking at serial values of WBC counts and I:T ratios have shown better outcomes. Murphy and Weiner,[81] in a single-center historical cohort study, showed that 2 serial normal I:T ratios and a negative blood culture in the first 24 hours of life had a negative predictive value (NPV) of 100% (95% confidence interval [CI]: 99.905%–100%), but the specificity and positive predictive value were 51.0% and 8.8% respectively. CBC values need to be interpreted cautiously and in conjunction with other clinical and laboratory parameters.

CRP

CRP was first described in the 1930s and since then multiple studies have shown elevation of the CRP in several infectious and noninfectious etiologies that share a common background of inflammation or tissue injury.[82] In neonates, serial measurements of the CRP in the first 24 to 48 hours of symptoms increases the sensitivity of the test, with suggestion that normal CRP values during this period have a 99% negative predicted value for determination of infection.[83,84] In contrast, elevated levels of CRP may be more difficult to interpret, especially for diagnosis of EOS, because factors such as premature rupture of membranes (PROM), maternal fever, pregnancy-induced hypertension, prenatal steroid use, and fetal distress may also cause elevation of the CRP.[85,86] Additionally, studies have suggested a physiologic variation of the CRP during the first few days of life limiting the use of single values.[86] Gestational age influences CRP kinetics, with preterm infants having a lower and shorter CRP response compared with healthy term infants.[87,88] Studies suggest that CRP is best used as part of a group of ancillary diagnostic tests to help determine if an infant has infection, rather than as a single test.

Procalcitonin

Tissue release of procalcitonin (PCT) increases with infection, making it a potential marker for early detection of sepsis. PCT differs from CRP, in that PCT levels increase more rapidly and may be more useful for detection of EOS. Auriti and colleagues,[89] in a multicenter, prospective observational study of 762 neonates, showed a significant increase in the median value of PCT level in neonates with sepsis compared with those without sepsis (3.58 vs 0.49 ng/mL; $P<.001$), In addition, a cutoff value of 2.4 ng/mL was suggested as the most accurate level for differentiation of sepsis in neonates regardless of gestational age, with a sensitivity of 62% and a specificity of 84%. A meta-analysis of 16 studies (1959 neonates), showed that PCT had a pooled sensitivity and specificity of 81% and 79% respectively.[90] Although these are promising results, further studies are needed to clarify the use of PCT in clinical practice.

Mannose-Binding Lectin

Mannose-binding lectin (MBL) is a plasma protein, primarily produced by the liver, with an important role in the innate immune defense. MBL activates the lectin pathway of the complement system, increasing opsonization and enhancing phagocytosis.[91] Genetic polymorphisms in the MBL gene have been associated with an increased risk of sepsis. In a recent study in which MBL levels were measured in 93 neonates, development of sepsis was associated with lower levels of MBL and with the presence of BB genotype in exon 1 of the MBL gene. MBL remains a research tool with further studies needed to confirm diagnostic utility.[92]

Cytokine Profile

Multiple cytokines have been studied for diagnosis of neonatal sepsis including IL-6, IL-8, IL-10, and TNF-alpha. IL-6 and IL-8 increase very rapidly with bacterial invasion, but they promptly normalize in serum levels (within the first 24 hours), limiting their ability to be used as clinical markers. TNF-alpha has not shown to have high sensitivity, but the ratio of IL-10 and TNF-alpha has been used for diagnosis of LOS in VLBW neonates with some success.[93,94] Evaluating a combination of cytokine profiles may increase the likelihood of identifying infection more than single measurements.

Neutrophil CD64 and Neutrophil/Monocyte CD11B

The specific markers, neutrophil CD64 and neutrophil/monocyte CD11B, are cell surface antigens whose production increases after activation of leukocytes by bacteria and therefore can potentially be used for diagnosis of neonatal sepsis. Their upregulation precedes that of CRP, suggesting potential use in EOS. A recent study by Genel and colleagues[95] showed that CD64 had a sensitivity and specificity to accurately identify neonatal sepsis of 81% and 77% respectively, with an NPV of 75%. Similarly, CD11b had a sensitivity and specificity of 66% and 71%. Cost and processing time may be barriers to use of these markers in clinical practice.

Molecular Techniques for Early Detection of Neonatal Sepsis

Important advances have been made in molecular diagnostics, and studies of real-time PCR and a broad range of conventional PCR assays suggest improved sensitivity and specificity for sepsis diagnosis. A meta-analysis done by Pammi[96] found that sensitivity and specificity of real-time PCR was 0.96 (95% CI for sensitivity: 0.65–1.00 and 95% CI for specificity: 0.92–0.98). Similarly, broad-range PCR had a sensitivity of 0.95 (95% CI 0.84–0.98) and specificity of 0.98 (0.95–1.0); however, neither test achieved the minimum limits of sensitivity or specificity set up by the study and results were insufficient to replace blood cultures for diagnosis of neonatal sepsis. Currently these techniques should be seen as adjunctive methods in the diagnosis of neonatal sepsis, with limitations that include the inability to provide information about antibiotic susceptibility, as well as significant cost of implementation in clinical practice. An exception to this is the use of HSV PCR for the diagnosis of HSV encephalitis, which is the gold standard test for this condition. The role of HSV PCR from the blood in diagnosing disseminated HSV infection is less clear.

Genomics and Proteomics

Exciting alternatives for detection of neonatal sepsis include the use of genomics and proteomics for identification of host response biomarkers. Genomics targets genes that are upregulated with infection and proteomics analyzes the structure, function, and interactions of proteins produced by a particular gene. Early studies in neonates have suggested potential utility in these techniques for identification of sepsis and necrotizing enterocolitis. A score based on proapolipoprotein CII (Pro-apoC2) and a desarginine variant of serum amyloid, was used to withhold antibiotics in 45% of patients with suspected infection and to discontinue antibiotics in 16%.[93,97] Studies are needed for validation of this score, as well as for detection of other potential biomarkers.

TREATMENT

Prevention and Infection Control Practices

Prevention of neonatal sepsis is the goal, through implementation of what is known and development of new prevention strategies. Maternal prenatal care continues to be important for prevention of EO GBS sepsis with identification of maternal carriage of GBS through universal screening for all pregnant women. Early recognition of chorioamnionitis, with appropriate antimicrobial therapy for the mother, decreases maternal fetal transmission. The recent CDC GBS prevention guidelines emphasize the need for universal maternal GBS screening at 35 to 37 weeks of gestation and includes chromogenic agar and nucleic amplification tests as newer diagnostic techniques that can be used to increase the yield of GBS identification. The guidelines also clarify the definition of adequate intrapartum prophylaxis as the use of penicillin, ampicillin, or cefazolin at least 4 hours before delivery (**Table 4**). Clindamycin and

vancomycin, which can be used in penicillin-allergic patients, are not considered effective prophylaxis therapy.[9] Other potential prevention strategies include rapid GBS testing during labor and a safe and effective GBS vaccine. Diagnostic tests during labor will identify colonized women who either had a negative screen at 35 to 37 weeks or were not screened before labor. Further studies are needed to improve sensitivity and specificity of rapid tests.[98]

Efforts to reduce hospital-acquired late-onset infections require much closer attention to appropriate hand washing, infection control, and proper techniques for placement and management of central catheters. Recently, the American Academy of Pediatrics published guidelines for the prevention of health care–associated infections in the NICU. These guidelines emphasize the need for 100% compliance with appropriate hand-washing techniques and recommend use of alcohol-based products for hand hygiene. Catheter bundles that include guidelines for insertion and management of central lines have been shown to reduce risk of central line–associated bloodstream infection.[99–102] The use of heparin for prevention of line infections has been successful in single-center randomized studies, but has not been confirmed in larger multicenter studies; therefore, its routine use is not recommended.[103,104] Daily check lists that document continued need for central lines to reduce duration of line use and a goal of zero central line–associated infections should be the responsibility of the whole health care team.[99]

Prevention Strategies

A variety of interventions to decrease rates of neonatal sepsis have been studied, including postnatal use of lactoferrin, antistaphylococcal monoclonal antibodies, intravenous immunoglobulin (IVIG), granulocyte-macrophage colony-stimulating factors, probiotics, glutamine, and fluconazole prophylaxis for invasive candida infection (**Table 5**).

IVIG

Delay in the synthesis of immunoglobulin and a decrease in transplacental antibody transfer in neonates suggested that the use of IVIG could be a potential strategy for prevention of neonatal sepsis. In 1994, a randomized controlled trial in 2416 infants failed to show a decreased incidence of nosocomial infections, morbidity/mortality, and duration of hospital stay among VLBW infants.[105] A follow-up meta-analysis of 10 trials showed that mortality was reduced with the use of IVIG in suspected (relative risk [RR] 0.58, 95% CI 0.38–0.89) and proven infection (RR 0.55, 95% CI 0.31–0.98), but the investigators caution about their conclusions based on concerns about individual study quality.[106] Recently, the International Neonatal Immunotherapy Study randomized 3493 infants with suspected or proven sepsis to receive either 2 doses of polyvalent IgG immune globulin or placebo 48 hours apart. Their primary outcome

Table 4
Recommended intrapartum antibiotic prophylaxis for Group B *Streptococcus*–positive women

Antibiotic	Dose
Penicillin G	5 million units IV as an initial dose followed by 2.5 million units every 4 h until delivery
Ampicillin	2 g IV as an initial dose, followed by 1 g IV every 4 h until delivery
Cefazolin	2 g IV as an initial dose, followed by 1 g IV every 8 h until delivery

Abbreviation: IV, intravenous.

Table 5
Evidence of strategies for prevention of neonatal sepsis

Prevention Strategy	Notes
Lactoferrin	Small studies show reduction of both fungal and bacterial infection; large studies needed.
Intravenous immunoglobulin	No proven efficacy for prevention of neonatal sepsis.
Antistaphylococcal monoclonal antibodies	Monoclonal antibodies against capsular polysaccharide and clumping Factor A have shown no effect in prevention of sepsis. Antilipoteichoic acid antibodies may have an effect but further randomized controlled studies are required.
Probiotics	No proven efficacy for neonatal sepsis. Useful as prevention of necrotizing enterocolitis.
GM-CSF/G-CSF	No proven efficacy of neonatal sepsis.
Glutamine	No proven efficacy of neonatal sepsis.
Fluconazole	Efficacious in prevention of *Candida* sepsis in VLBW infants.

Abbreviations: G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; VLBW, very low birth weight.

was rate of death or major disability at the age of 2 years. There was no difference in the primary outcome between the 2 groups (RR 1.00, 95% CI 0.92–1.08).[107]

Antistaphylococcal monoclonal antibodies

Because of the burden of staphylococcal infections in neonatal sepsis, different antistaphylococcal monoclonal antibodies had been developed. These include antibodies against the capsular polysaccharide antigen, antibodies against microbial surface components that recognize adhesive matrix molecules, antibodies to clumping factor A, and anti-lipoteichoic acid (LTA) antibodies.[108–110] Initial animal studies, as well as phase I and II trials in humans, showed that capsular-directed antibodies and anti-clumping factor A were well tolerated and had the potential to reduce staphylococcal sepsis[111,112]; however, a meta-analysis showed that their efficacy in decreasing staphylococcal infections was limited and recommended against their use.[113] Recently, Weisman and colleagues[110] showed safety and tolerance of pagibaximab (anti-LTA antibody) at doses of 60 and 90 mg/kg in VLBW infants, with those receiving 90 mg/kg per dose showing no staphylococcal sepsis. Although these are exciting results, randomized controlled studies are needed to further confirm these findings.

Granulocyte/Granulocyte-macrophage colony-stimulating factors

Granulocyte-macrophage colony-stimulating factor (GM-CSF) increases T-Helper 1 immune responses and enhances bactericidal activity by stimulating neutrophils and monocytes. Animal studies have shown that both GM-CSF and granulocyte colony-stimulating factor (G-CSF), given before bacterial inoculation, decrease mortality. A single blinded multicenter study looking at the use of GM-CSF and the development of sepsis showed that although the absolute neutrophil count on the treatment arm increased significantly faster ($P = .002$), there was no difference in sepsis-free survival to day 14 from study entry (risk difference –8%, 95% CI –18% to 3%).[114] A meta-analysis that included 4 small studies had similar results (RR 0.89, 95% CI 0.43–1.86).[96]

Glutamine

Based on promising adult studies, glutamine supplementation has been suggested as a potential intervention to reduce neonatal sepsis. A randomized double-blinded study from the NICHD NRN among 1433 extremely low birth weight infants showed no difference in mortality and late-onset sepsis outcomes (RR 1.07, 95% CI 0.97–1.17; $P = .18$).[115] Similar results were obtained in a meta-analysis of 5 trials that included 2240 neonates looking at the effects of glutamine in the incidence of culture-proven invasive infection (RR 1.01, 95% CI 0.91–1.13).[116] Glutamine supplementation is not currently recommended as a strategy to decrease or prevent neonatal sepsis.

Probiotics

Evidence continues to grow regarding the use of probiotics to prevent necrotizing enterocolitis,[117,118] but data on use of probiotics for prevention of neonatal sepsis are limited and contradictory. A major problem with the use of probiotics is the lack of standardization and federal regulation. If probiotics are considered food additives, no formal evaluation is required and a wide of variety of products are available. Some investigators have stressed the need for review and approval by the Food and Drug Administration, especially if probiotics are to be used in at-risk VLBW infants. Although small studies have indicated potential benefits of probiotics in decreasing neonatal sepsis, a recent meta-analysis (RR 0.90, 95% CI 0.76–1.07) and a systematic review (RR 0.98, 95% CI 0.81–1.18) found no evidence that probiotics decreased sepsis in preterm VLBW infants.[117,119]

Lactoferrin

Lactoferrin is a glycoprotein present in human milk that contains immunomodulatory properties by increasing cytokine production in the gut and associated lymphoid tissue, as well as by showing antibioticlike activities against gram-negative and gram-positive bacteria. A recent multicenter randomized study in 472 VLBW neonates demonstrated decreased bacterial and fungal LOS among newborns treated with lactoferrin (5.9% in lactoferrin group vs 17.3% in controls, $P = .002$).[120] Further studies are needed to confirm these findings and to evaluate optimal dosage as well as short-term and long-term safety.

Fluconazole prophylaxis

Studies have documented that fluconazole prophylaxis among high-risk VLBW infants is effective in reducing the number of severe infections. Kaufman and colleagues[121] found a 22% decrease in risk of fungal colonization among infants weighing less than 1000 g who were on fluconazole prophylaxis. In addition, they found no fungal invasive infections in those who received fluconazole compared with 20% in the placebo group. Similar results were obtained by the Italian Task Force in 322 neonates who were randomized to receive fluconazole or placebo.[122] The success in decreasing the incidence of fungal infections in the neonatal units has not been translated into decreased mortality; however, all prospective studies of fluconazole prophylaxis have consistently revealed decreasing mortality trends.[121–124] In addition, Healy and colleagues,[125] in a retrospective 4-year study looking at the incidence of invasive candidiasis, showed that there were no attributable deaths among patients who received fluconazole prophylaxis compared with 21% of those who did not. Current recommendations advocate for the use of fluconazole prophylaxis in infants with birth weights of less than 1000 g in neonatal units with high rates of invasive candidiasis.[126] Recently, Kaufman and colleagues[123] reported an 8-year to 10-year follow-up study evaluating the long-term and neurodevelopmental outcomes of fluconazole prophylaxis. They found no significant neurodevelopmental impairment or

difference in head circumference growth and cholestasis in patients on prophylaxis when compared with placebo. A larger study is needed to verify these findings. Another important aspect of fluconazole prophylactic use has been the concern regarding emergence of fluconazole-resistant strains; however, studies have failed to show such an association, and a significant increase in fluconazole-resistant *Candida* species has not yet occurred.[125]

Judicious Use of Antibiotics

Appropriate use of antibiotics is important to save lives and reduce complications. Indiscriminate use increases risk of multidrug-resistant organisms and other complications, including disseminated candida infections and necrotizing enterocolitis.[127,128] Reports of vancomycin-resistant enterococcus, beta lactamase–producing organisms (*E coli, Klebsiella, Enterobacter*), and highly resistant *Acinetobacter*, *Burkholderia*, *Chryseobacterium meningosepticum,* and *Serratia* are increasing in the neonatal population, calling for a judicious use of antibiotics.[129–134] A study looking at antibiotic prescribing practices in 4 NICUs found that approximately 28% of the established antibiotic courses and 24% of antibiotic days were inappropriate for the prescribing indication. The most common cause of inappropriate use was excessive continuation of antibiotics, rather than inappropriate initiation, followed by inability to target the specific pathogen once isolated.[135] General principles for antibiotic use, as well as effective antibiotic stewardship programs, may help decrease misuse of antibiotics in the NICU (**Table 6**). Such programs require developing antibiotic guidelines and education initiatives, accompanied by preprescription approval and postprescription review (ie, modification of empiric antibiotic regimen, dose optimization, therapeutic monitoring, oral antimicrobial conversion, and drug-drug interactions). Close involvement and communication among an infectious disease specialist, neonatologist, clinical pharmacist, and infection control and microbiology personnel are essential for the success of the program.[136] Efforts to document and measure the success of stewardship programs are important as well as generating nonpunitive feedback on antibiotic prescription practices. The NICU has specific characteristics in antibiotic prescription practices, as multiple providers may be involved in the decision of initiating, continuing, or discontinuing antibiotics; adapting programs to such characteristics is important.[137] Recently, the CDC launched the *Get Smart for Health Care* campaign, which focuses on improving antibiotic use in inpatient/outpatient health care facilities and offers tools for implementation and improvement of stewardship efforts (http://www.cdc.gov/getsmart/healthcare).

Ampicillin and gentamicin continue to be the preferred antibiotics for empiric treatment of suspected early-onset neonatal sepsis. Increasing ampicillin-resistant *E coli* in some centers has led to increased use of third-generation cephalosporins as part of the empiric treatment; however, studies have shown a potential association with

Table 6
Principles for antibiotic use

Empiric initiation of antibiotics	Use only when bacterial infections are likely and discontinue empiric treatment when they have not been identified.
Switch antibiotics based on susceptibility patterns	Change the antibiotic agents to those with the narrowest spectrum.
Define duration of antibiotic therapy	Establish a final duration of antibiotic management based on the disease process.

Table 7
Duration of antibiotic therapy in neonatal sepsis based on clinical presentation

Clinical Presentation	Duration of Antibiotic Therapy
Early-onset sepsis without meningitis	10 d
Late-onset sepsis without meningitis	10–14 d
Meningitis: early-onset or late-onset sepsis	14–21 d[a]

[a] Gram-negative rod meningitis: will require at least 21 days of therapy.

increased mortality and the development of multidrug-resistant organisms associated with this practice. In addition, cephalosporins are not active against *Listeria* and enterococcus. In LOS, coverage with vancomycin should be considered, especially in hospitalized VLBW infants at risk for CoNS infection.

Definitive therapy should be chosen based on antibiotic susceptibilities. Combination therapy for gram-negative organisms is not required for patients without meningitis, and for patients with an inducible B-lactamase–producing organism (*Serratia*, indol-positive *Proteus*, *Citrobacter,* and *Enterobacter*), the use of a carbapenem should be considered. The duration of therapy is based on the disease process (**Table 7**).

Amphotericin and fluconazole continue to be the antifungal drugs of choice for treatment of neonatal candidiasis. Local susceptibility patterns should be followed if *Candida glabrata*, *Candida krusei,* or *Candida lusitaniae* are isolated, as susceptibility of these species to both fluconazole and amphotericin may be decreased. *C krusei* is intrinsically fluconazole resistant, and may have reduced susceptibility to amphotericin, whereas *C lusitanaie* is among the few *Candida* species to show full in vitro resistance to amphotericin in some isolates. *Candida tropicalis* and *Candida parapsilosis* are frequently resistant to fluconazole, although resistance rates vary by area, but they are usually susceptible to amphotericin. The experience with the use of echinocandins (micafungin, caspofungin, anidulafungin) in neonates continues to increase, but their use is not yet approved in this population, and their poor CNS penetration is also a concern.[138] Micafungin is the echinocandin that has been studied the most in the neonatal population and these studies show that higher doses may be required, especially for better CNS penetration.[139–141]

SUMMARY

Neonatal sepsis continues to be a significant cause of morbidity and mortality in term and preterm infants. Although GBS and *E coli* are the most common pathogens associated with EOS, and CoNS are the most frequently isolated agents in newborns with LOS, other organisms, as well as multidrug-resistant pathogens, need to be considered. Development of accurate novel early diagnostic markers will allow clinicians to better assess the risk of infection and need for antibiotic therapy. Adherence to infection-control policies, including attention to strict hand hygiene, antibiotic stewardship, and catheter management, are required to decrease the number of infections in hospitalized neonates.

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