

Recent Developments and Current Issues in the Epidemiology, Diagnosis, and Management of Bacterial and Fungal Neonatal Sepsis

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Abstract

Identifying neonates with sepsis is complicated by variability in clinical presentation. The incidence of early onset sepsis (EOS) resulting from invasive group B streptococcal (GBS) infections has been notably reduced by the widespread delivery of intrapartum antibiotic prophylaxis. Rates of EOS attributable to non-GBS etiologies have remained constant, and ampicillin-resistant *Escherichia coli* has become more prevalent. Late-onset sepsis (LOS) attributable to gram-positive organisms including coagulase-negative *Staphylococci* and *Staphylococcus aureus* is associated with increased morbidity and mortality among premature infants. Invasive candidiasis is an emerging cause of LOS, especially among infants who receive broad-spectrum antimicrobial agents. Prophylactic fluconazole administration to very low-birth-weight (VLBW) neonates during the first 6 weeks of life prevents invasive candidiasis in neonatal intensive care units (NICU) with high rates of fungal infections. Targeted fluconazole prophylaxis may be beneficial in VLBW neonates who receive care in NICUs with lower rates of invasive fungal infections. Assessment of immune function, neutrophil markers, acute phase reactants, and utilization of sepsis screening scores may contribute to the management of sepsis. Maternal decolonization, antimicrobial stewardship, early enteral feeding, and optimal infection control practices are potential practical strategies for reducing the burden of neonatal sepsis.

Keywords

- ▶ neonatal sepsis
- ▶ invasive candidiasis
- ▶ epidemiology
- ▶ management

The definition of neonatal sepsis is complicated by the frequent presence of noninfectious conditions that resemble those of sepsis, especially in very low-birth-weight (VLBW) preterm infants and by the absence of optimal diagnostic tests. Although growth of an organism from a sterile site is the “gold standard” for definitive diagnosis, it is not always possible to isolate a causative pathogen. Invasive infections can occur in seemingly asymptomatic neonates. Therefore, assessment of history and risk factors in combination with diagnostic tests are used to identify neonates who are more likely to be infected.

The definitions of early onset sepsis (EOS) and late onset sepsis (LOS) are subject to subspecialist variation. Many investigators, including those who participate in the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Neonatal Network and the Vermont Oxford Network, define EOS by the onset of signs/symptoms and an associated positive culture at or before 72 hours of life. LOS is characterized by the onset of symptoms consistent with sepsis at greater than 72 hours of life. These classifications of EOS and LOS reflect the differing etiologies and proposed pathophysiology of pathogens commonly associated with the timing of

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onset of these conditions. The impaired innate immune function of premature infants predisposes them to invasive infections. As the fetal immune response begins at 24 weeks of age and development occurs until term, premature neonates do not benefit from complete immune system development, making them more susceptible to infection with organisms that term infants may be able to suppress.¹

Early Onset Sepsis

Risk factors for EOS include prematurity and associated immunologic immaturity,² maternal group B streptococcal (GBS) colonization, rupture of membranes greater than 18 hours, and maternal intra-amniotic infection.^{3,4} The maternal vaginal flora is the most common source of organisms associated with EOS. Maternal pathogens are introduced during passage through the birth canal or with ascending maternal infection in a fetus that inhales or digests infected amniotic fluid. Chorioamnionitis is defined as a maternal fever $> 38^{\circ}\text{C}$ and two or more of the following: maternal leukocytosis $> 15,000$ cells/mm³, maternal tachycardia > 100 beats per minute, fetal tachycardia > 160 beats per minute, uterine tenderness, or a foul odor to the amniotic fluid.⁵ Although maternal fever occurs in one in four or five laboring women who receive epidural anesthesia, histological or microbiologic chorioamnionitis rarely occurs in women with an isolated fever.⁶ Surveillance of high-risk preterm infants at 20 academically affiliated neonatal intensive care units (NICUs) of the NICHD Neonatal Research Network (NRN) noted that between 14% and 28% of women with preterm deliveries at 22 to 28 weeks' gestation had clinical presentations consistent with chorioamnionitis.⁷ In contrast, in an earlier study under 1% of women with term gestations had recovery of organisms by culture from their amniotic fluid.⁸ Among women who experienced preterm labor with intact membranes, 32% had one or more organisms isolated from their amniotic fluid, and the amniotic fluid of almost three-quarters of women with preterm premature rupture of membranes (PPROM) yielded one or more organisms.⁹ In another study of women with preterm deliveries at 23 to 32 weeks' gestation, 23% infants were found to have cord blood cultures positive for *Ureaplasma* spp. or *Mycoplasma* spp., suggesting that these intrauterine infections may be more frequent than previously appreciated.¹⁰

Bacterial Pathogens

GBS and *Escherichia coli* are the most common pathogens associated with EOS in the United States.^{11,12} Population-based surveillance from 2005 to 2008 conducted through the Active Bacterial Core sites identified 658 cases of EOS from 2005 to 2008; 72 (11%) were fatal. Overall incidence remained stable during the 3 years at 0.76 to 0.77 cases/1,000 live births. GBS (38%) was the most commonly reported pathogen followed by *E. coli* (24%). African-American preterm infants had the highest incidence (5.14 cases/1,000 live births) and case fatality (24.4%). The estimated national annual burden of EOS was ~3,320 cases (95% confidence interval [CI]: 3,060 to 3,580), including 390 deaths (95% CI: 300 to 490). The burden

of EOS among preterm infants was slightly less than half of the total number of EOS cases.¹¹ A study from the NICHD NRN of almost 400,000 infants born at NRN hospitals reported an overall rate of EOS of 0.98 cases per 1,000 live births, with rates for GBS and *E. coli* of 0.41/1,000 live births and 0.28/1,000 live births, respectively. Most infants with GBS were term (73%); most with *E. coli* were preterm (81%).¹² Between 1997 and 2010, 1,032 cases of EOS were identified among 108,000 VLBW admissions to 313 community based NICUs. Gram-negative organisms were isolated most frequently in this VLBW cohort with an increased risk of death among infants with EOS (odds ratio [OR] 1.45; 95% CI: 1.21 to 1.73).¹³

Group B Streptococci

Prior to universal recommendations for intrapartum antibiotic prophylaxis (IAP) in at-risk women, almost half of infants born to women colonized with GBS developed invasive early onset GBS disease.^{14,15} Recommendations for IAP in women who are colonized with GBS or have other risk factors for EO GBS have been instrumental in reducing the rates of EO GBS disease.¹⁶ Despite the benefit of IAP, rates of uptake are limited by inadequate screening practices and missed opportunities to administer IAP.¹² Additionally, the efficacy of IAP has only been demonstrated for EOS attributable to GBS; IAP does not prevent LOS. With increasing use of IAP, concerns have been raised about an increase in non-GBS EOS and about increasing risk of antimicrobial resistance. Most population-based studies report stable rates of EOS caused by non-GBS pathogens.¹⁷ However, several studies have suggested that ampicillin-resistant *E. coli* may be increasing, especially among VLBW infants.^{18,19}

Late-Onset Sepsis

LOS is a frequent complication of extreme prematurity. Several studies from the NICHD NRN have documented rates of infection among VLBW ($< 1,500$ g) infants. In a recent study, 36% of 9,575 extremely low-gestational-age infants (22 to 28 weeks) developed LOS.⁷ An earlier NRN study reported that 21% of 6,215 VLBW infants who survived more than 3 days developed LOS. Gram-positive organisms are most commonly associated with LOS among VLBW infants. In the NRN cohort, 70% of infections were associated with gram-positive organisms; coagulase-negative staphylococci (CoNS) contributed 48%, gram-negative 18%, and fungal 12%. In some evaluations, more than one organism was isolated.¹⁸ The predominance of gram-positive organisms among VLBW infants was also seen in a large study of community-based NICUs over a 14-year period. Among 12,204 cases of LOS, gram-positive organisms were most frequent.¹³ Several studies have noted an increased risk of mortality among infants with LOS compared with those who are uninfected.^{13,18,20} Understanding the epidemiology of LOS helps to identify infants with possible infection and also to select appropriate empiric antimicrobial therapy while awaiting culture results.

Coagulase-Negative Staphylococci

In a large retrospective cohort study conducted in 248 NICUs from 1997 to 2009, 17,624 episodes of CoNS sepsis were

identified among 16,629 VLBW infants; most episodes were classified as possible infections. Infants with lower birth weights and gestational age were more likely to have a CoNS isolated during an episode of sepsis. A CoNS infection was characterized as isolation of the organism from two or more blood cultures, one blood culture and one other sterile site, or one blood culture with a significant infection. The number of central lines, clinical presentation of lethargy, and gastric acid residuals but not central line duration were risk factors for a CoNS infection.²¹ As CoNS are frequently isolated from neonates with clinical sepsis, it is important to understand risk factors for their recovery and strategies to decrease their prevalence to optimize management.

Staphylococcus aureus

A retrospective evaluation of NICHD NRN sites from 2007 to 2009 identified 8,444 VLBW neonates who survived greater than 3 days. Among these infants, 316 (3.7%) had *Staphylococcus aureus* bacteremia or meningitis; 88 infants had methicillin-resistant *Staphylococcus aureus* (MRSA) infections, 228 infants had methicillin-susceptible *Staphylococcus aureus* (MSSA) infections, and there were no coinfections. Almost all of the infants who had MRSA infections had manifestations after 7 days of age. Nine of 20 participating centers had no cases of MRSA. Morbidities did not differ between neonates with MRSA and MSSA infections. Although *S. aureus* infections comprised only 1% of all-cause bacteremia and meningitis, mortality rates of neonates with both MRSA and MSSA infections were high (26% and 24%, respectively) and comparable.²²

Candidiasis

Although less frequent than gram-positive or gram-negative infections, invasive infections with fungal organisms, primarily *Candida* spp., result in substantial morbidity and mortality.^{23,24} Approximately 2.5% of all bloodstream infections in VLBW neonates are estimated to be due to fungal etiologies.²⁵ The risk for fungal sepsis is increased by colonization acquired vertically from maternal sources as well as horizontally from the NICU environment. A positive correlation exists between multiple sites of colonization and risk for invasive candidiasis.²³ Risk factors supporting the use of empiric antifungal therapy in a neonate exhibiting signs and symptoms of sepsis include gestational age, exposure to third-generation cephalosporin antibiotics in the 7 days prior to symptom onset, and thrombocytopenia.²⁶ Invasive candidiasis occurred in 137 of 1,515 (9%) of neonates weighing < 1,000 g at birth in a prospective observational cohort study conducted in 19 NICHD NRN sites. Incidence of invasive infection varied from 2% to 28% among the sites that enrolled more than 50 neonates.²⁷ Overall mortality in a cohort of 730 infants with invasive candidiasis from 192 NICUs enrolled between 1997 and 2003 was 19%.²⁸ Among infants weighing between 401 and 1,000 g at birth born between 1993 and 2001 who had an invasive fungal infection, 31 (30%) had a head circumference less than the 10th percentile at 18 to 22 months of corrected gestational age, a statistically significant ($p < 0.05$) difference compared with uninfected neonates and comparable to neonates with gram-negative invasive infections.²⁹

Special Populations

As surgical techniques to correct congenital cardiac anomalies have become more successful, the survival of infants with these conditions has improved. Extended hospitalizations for surgical recovery and rehabilitation place these neonates at risk for sepsis. Among a cohort of 11,638 infants with congenital heart disease (CHD) receiving care in 250 U.S. NICUs from 1996 to 2007, 656 (6%) neonates had 821 episodes of sepsis, resulting in a cumulative incidence of 71/1,000 admissions. Gram-positive organisms, most commonly CoNS and *S. aureus*, were isolated in (64%) of episodes. Similar to infants without CHD, infants with CHD and culture-proven sepsis were more likely to die than uninfected infants with CHD. Mortality rates were highest for infants with candidemia followed by those with gram-negative bacteremia.³⁰ Most studies of neonates with CHD include both term and preterm neonates, accounting for the lower overall rates of sepsis and the comparatively lower rates of gram-negative bacteremia and candidemia than are noted when analysis is limited to preterm infants.

Diagnostics

One of the most challenging aspects in the management of neonates with sepsis is making the diagnosis. Many of the complications of prematurity, including respiratory distress syndrome and CHD, have similar manifestations to those associated with sepsis. Several physiological and laboratory parameters have been assessed to diagnose sepsis in neonates (► Table 1).

Culture-Based Diagnostics

Blood Culture

Blood culture is the gold standard diagnostic test for neonatal sepsis. At least 1 mL of whole blood inoculated into each blood culture bottle provides an optimal specimen. Up to 25% of neonates with bacterial sepsis have a low organism burden that would not be detected with culture of smaller blood volumes.^{31,32} Blood cultures of adequate volume are twice as likely to yield an organism than blood cultures containing a lesser blood volume.³³

Cerebrospinal Fluid Culture

Evaluation of the cerebrospinal fluid (CSF) is needed to diagnose meningitis. A lumbar puncture is recommended for all infants with suspected sepsis or with blood culture-proven bacteremia, if they have stable cardiorespiratory and coagulation status. Meningitis occurs in up to 23% of bacteremic infants.^{34,35} Organisms are not isolated from blood in up to 38% of infants who have a pathogen isolated from the CSF, underscoring the importance of examining the CSF.^{36,37}

Urine Culture

Most clinicians do not obtain a urine culture as part of an EOS evaluation as it is unusual for a neonate to develop a urinary tract infection in the first 72 hours, without associated bacteremia. A urine culture may be useful as part of an evaluation for

Table 1 Culture-based and non-culture-based diagnostics for neonatal sepsis

Category	Parameter	Optimal timing, volume of specimen, routine/investigational ^a	Applicability for neonatal sepsis
Culture-based			
Blood	Culture	> 1 mL of whole blood Routine	Gold standard for bacteremia
CSF	Culture	When clinically feasible Routine	Optimize antimicrobial therapy
Urine	Culture	> 72 h of life Routine	Not useful for EOS; potential benefits for LOS
Tracheal aspirate	Culture	Routine	Usually reflects colonization
Non-culture-based			
Immune function	MHC II TNF- α	Investigational Investigational	Both decreased in chorioamnionitis and sepsis
Neutrophil indices	Neutropenia Absolute neutrophil count Absolute immature neutrophil count	After 12 h of life Consider GA, delivery mode, altitude, arterial versus venous sampling, time since birth Routine	Neutropenia better predictor for sepsis than leukocytosis
Neutrophil markers	CD64	Elevated for 24 h after infection Requires 50 μ L blood Results within hours Investigational	Cut points between 2.38 and 3.62 optimal sensitivity, specificity and NPV for EOS
Platelet count	Thrombocytopenia and thrombocytosis	Late findings; slow to respond Routine	Thrombocytopenia associated with fungal infection
CSF cell count	CSF WBC	Uninfected neonates: mean 10 cells/mm ³ ; range up to 20 cells/mm ³ Routine	Does not predict culture-proven meningitis
CSF chemistries	CSF protein CSF glucose	Term < 100 mg/dL Preterm higher; 70–80% of serum glucose Routine	Elevated in fungal meningitis Low glucose specific for bacterial meningitis
Acute phase reactants	CRP Procalcitonin	8–24 h after infection 2–12 h after infection Routine/investigational	Good NPV Better sensitivity but less specificity than CRP
Sepsis panels/ scores		After 24 h of life Investigational	Most useful for NPV and discontinuation of antimicrobial therapy

Abbreviations: CRP, C-reactive protein; CSF, cerebrospinal fluid; EOS, early onset sepsis; GA, gestational age; LOS, late onset sepsis; MHC II, major histocompatibility complex class II; NPV, negative predictive value; TNF, tumor necrosis factor; WBC, white blood cell count.

^aRoutine refers to an assay or parameter that is routinely available and widely used; *investigational* refers to an assay or parameter that is undergoing evaluation for clinical use and applicability.

LOS, especially when colonization with *Candida* is suspected. A secondary analysis of the NRN Early Diagnosis of Nosocomial Candidiasis study demonstrated that extremely low-birth-weight (ELBW) infants with candiduria, both with and without isolation of the organism from additional sterile sites, had a greater risk of neurodevelopmental impairment or death than those ELBW infants with suspected but unproven infection. The authors conclude that the isolation of *Candida* spp. from the urine of an ELBW neonate should result in an investigation for evidence of invasive infection with prompt initiation of treatment.³⁸ Both bacterial organisms and yeast will grow in urine cultures undergoing routine bacterial culture; thus a

request for a fungal urine culture is not needed for diagnosis of candiduria.

Tracheal Aspirate Cultures

Collection of tracheal aspirates after placement of an endotracheal tube is unlikely to be of diagnostic value and will likely reflect colonization rather than infection.

Non-Culture-Based Diagnostics

In addition to blood, CSF, and urine cultures, non-culture-based laboratory tests may be beneficial in identifying infected neonates and in deciding on duration of antimicrobial

therapy (–Table 1). Non-culture-based tests may be more helpful in identifying neonates who are unlikely to have infection, when culture-based methods are inconclusive.³⁹

Immune Function

Immune response in the premature neonate and the relationship to infection is an active area of investigation. Reduced expression of major histocompatibility complex class II in neonates has been associated with a history of maternal preterm labor and PPROM, chorioamnionitis, and neonatal sepsis. Whole blood lipopolysaccharide stimulation assays were notable for decreased tumor necrosis factor- α production in preterm infants who subsequently developed sepsis. Other mechanisms of impaired immune response include in utero exposure to inflammation prior to preterm delivery leading to “endotoxin hyporesponsiveness,” thus increasing the risk for sepsis and end-organ sequelae.⁴⁰

Neutrophil Number and Function

Term and late preterm neonates have impaired neutrophil function compared with that of older infants. Early onset neutropenia might predispose neonates to colonization with pathogens and subsequent EOS or LOS. Neutropenia appears to be a better predictor for neonatal sepsis than leukocytosis, although neutropenic ranges differ by gestational age, mode of delivery, altitude of location of birth, arterial versus venous sampling, and time since birth. A white blood cell count (WBC) obtained within the first 6 to 12 hours of life may be too early to reflect an occult inflammatory response and could be repeated if sepsis is suspected.^{41,42} Two very large retrospective multicenter administrative database studies demonstrated that low WBC and high percentage of immature to total white blood cell (I/T ratios) were associated with increasing odds of infection in EOS.⁴³ High and low WBC, high Absolute Neutrophil count (ANC), high I/T ratios, and low platelet counts were associated with LOS.⁴⁴ However, a single blood cell count-derived index did not have proven sensitivity to reliably exclude EOS or LOS in neonates.^{43,44} The absolute immature neutrophil count and absolute neutrophil count have suboptimal sensitivity and decreased predictive accuracy for EOS as elevation does not consistently distinguish an inflammatory response from a noninfectious etiology.⁴⁵ The I/T ratio is a more sensitive indicator of sepsis; however, single assessments have a better negative predictive value (99%) than positive predictive value (25%).⁴⁶ The I/T ratio is elevated in a quarter to half of presumptively uninfected neonates.⁴⁷ Overall, neutrophil indices seem to be more helpful for excluding infants without infection than for including infants with infection.³⁹

Neutrophil Markers

The CD64 neutrophil marker has been noted to be elevated for 24 hours following infection in neonates with culture-confirmed sepsis. A prospective single-center study demonstrated that a CD64 index with a cut point of 2.38 had 100% sensitivity and specificity and 68% negative predictive value for EOS. A cut point of 3.62 had a sensitivity of 75%, specificity of 77% and a negative predictive value of 96%.⁴⁸ Index values below the cut points could be used to support the discontin-

uation of antimicrobials. Advantages of this assay include the requirement for only 50 μ L of blood and availability of results within hours of the specimen being obtained.⁴⁹ However, determination of the surface expression of CD64 on neutrophils requires the use of flow cytometry that has limited its utility, except at specialized centers with 24-hour flow cytometry access and support.

Platelet Count

Thrombocytopenia and thrombocytosis are later findings in bacterial and fungal infections. Although thrombocytopenia is a risk factor for invasive candidiasis, low platelet counts may persist despite adequate treatment and clinical improvement.^{50,51} Therefore, thrombocytopenia may not be an optimal indicator of onset or resolution of sepsis.

CSF Cell Count and Chemistries

Uninfected preterm or term infants have a CSF mean white blood cell count of less than 10 cells/mm³, with an upper limit of 2 standard deviations equivalent to less than 20 cells/mm³.^{3,37,52,53} CSF white blood cell band number does not predict culture-proven meningitis.⁵⁴ Infants with meningitis attributable to gram-negative organisms have increased CSF parameters compared with infants with meningitis attributable to gram-positive organisms.⁵⁵ The CSF protein concentration in the term uninfected infant is usually less than 100 mg/dL; the CSF protein concentration of the uninfected preterm infant is higher than the term infant and is correlated with prematurity.⁵⁶ The CSF glucose concentration in the term infant is usually between 70% and 80% of a simultaneously measured serum glucose; a low CSF glucose has the greatest specificity for bacterial meningitis.^{37,57} Bacterial meningitis may occur in infants with normal CSF parameters who concomitantly have a high concentration of bacterial organism. This observation accentuates the need to utilize culture-based and non-culture-based assessments in combination with clinical evaluation for optimal diagnosis and management.

Acute Phase Reactants: C-Reactive Protein and Procalcitonin

C-reactive protein (CRP) is associated with a humoral response to bacterial infection and increases within 6 to 8 hours of infection, with a peak noted 24 hours following infection.^{58,59} Low sensitivity within the first few hours of life may be due to delayed synthesis of interleukin-6 during the early immune response. Low CRP values should be considered in the context of infection timing. Two, within range, CRP assessments between 8 to 24 hours after birth and 24 hours later have a negative predictive value of 99.7% for culture-confirmed neonatal sepsis.⁶⁰ Host characteristics, including gestational age, may need to be considered with interpretation of CRP values.⁶¹ CRP assessment may provide guidance for discontinuation of antimicrobial therapy after 48 hours of empiric treatment, but measurement may not be beneficial to determine total duration of antimicrobial therapy.

Procalcitonin measurements appear to have better sensitivity but less specificity than CRP for identifying infants with neonatal sepsis.⁶² Values appear to increase 2 hours following

pathogen exposure, peak within 12 hours, and return to baseline levels within 48 to 72 hours in healthy adults.⁶³ An increase in procalcitonin levels can also occur after parturition and in conditions without a known infectious etiology, such as respiratory distress syndrome.⁶⁴ As procalcitonin reference levels have become available for preterm and term infants and the assay has become available in clinical laboratories, this test is increasingly being used in assessing response to antimicrobial therapy and determining duration.⁶⁵ Both CRP and procalcitonin measurements have been utilized in some centers to guide management of infants with suspected sepsis. The improved negative predictive value of serial CRP assessments compared with a single CRP measurement should be considered when comparing studies of acute phase reactants.

Sepsis Screening Panels/Scores

A variety of sepsis panels and scoring systems have been devised to increase the positive predictive value of individual parameters for neonatal sepsis. Overall, positive predictive value is increased when assessments are obtained after 24 hours of life. A cytokine scoring system combining measurements of granulocyte colony-stimulating factor (G-CSF), interleukin-6, interleukin-8, and tumor necrosis factor- α from 226 neonates > 3 days old with clinical sepsis was noted to have 100% sensitivity and a 69% positive predictive value for gram-negative bacteremia.⁶⁶ However, most scoring systems are more applicable for their negative predictive value and consequently for discontinuation of antimicrobial therapy.^{45,67,68}

Management

Empiric treatment is often initiated in infants thought to be at risk for sepsis, either because of clinical signs and symptoms or pregnancy-related risk factors (►Table 2).

Ampicillin and an aminoglycoside are recommended as empiric therapy for EOS, unless there is an epidemiological or clinical indication for broader-spectrum therapy with a cephalosporin or carbapenem. If an organism is not isolated and there is no clinical evidence of infection, most clinicians discontinue antimicrobial therapy after 48 hours.³⁹ The empiric use of cephalosporins is discouraged due to an increased risk for the development of resistance and candidiasis; however, cephalosporins may be indicated if gram-negative meningitis is suspected.^{69–72} For empiric third-generation cephalosporin therapy in neonates, cefotaxime is preferred over ceftriaxone due to the potential displacement of bilirubin and potential for kernicterus observed with ceftriaxone.

Duration of Treatment

Bacteremia without a focus is treated for 7 to 10 days. When uncomplicated meningitis attributable to GBS is diagnosed, therapy is extended to 14 days. Complications including cerebritis, osteomyelitis, and endocarditis require extended courses of treatment. For gram-negative meningitis, some experts recommend the longer of 14 days after the first negative culture or a total course of 21 days with cefotaxime and an aminoglycoside until culture results are known.⁷³

The optimal empiric therapy and duration for culture-negative clinical sepsis is unknown. Antibiotic treatment of mothers during labor for GBS prophylaxis, suspected chorioamnionitis, or PPROM may reduce the opportunity to isolate a pathogen from the newborn. In many clinical situations, it may be impractical to perform a lumbar puncture to assess for meningitis, and empiric treatment for duration to treat potential meningitis may be indicated. Several studies have associated antimicrobial treatment for greater than 5 days in newborns with culture-negative clinical sepsis with increased risk of death and NEC.^{74–77} This observation may be due to the presence of an unrecognized noninfectious etiology mimicking the presentation of clinical sepsis or to complications from extended courses of empiric antimicrobial therapy.

Management of Candidiasis

The polyenes including amphotericin B deoxycholate and lipid amphotericin preparations are traditional choices for invasive candidiasis. Amphotericin B deoxycholate is well tolerated by neonates who do not exhibit many of the toxicities seen in older children. Fluconazole, with the option of intravenous and oral administration, has activity against approximately 95% of common neonatal candida isolates. However, if invasive candidiasis occurs in a neonate who has received fluconazole prophylaxis, an antifungal from a different class is recommended for treatment. Fluconazole pharmacokinetics demonstrates that doses between 6 and 12 mg/kg per day achieve serum concentrations adequate for treatment of neonatal candidiasis.^{25,78,79} The echinocandins, semisynthetic lipopeptides produced via chemical modification of natural products of fungi, have excellent efficacy against most species of *Candida* and have a comparatively benign safety profile.^{80,81} They have excellent soft tissue and bone penetration, with application in hepatosplenic candidiasis and osteomyelitis.

Although there have been case reports of hepatic insufficiency in some pediatric recipients of the echinocandins, as a class, these agents are well tolerated in the neonate.^{82–85} Two of the most commonly used echinocandins, caspofungin and micafungin, have comparative susceptibility and safety profiles. There are ongoing pharmacokinetic trials of anidulafungin in neonates. Disadvantages of echinocandin use include the higher minimal inhibitory concentration for *Candida parapsilosis*. Additionally, the central nervous system (CNS) penetration of the echinocandins is not optimal. If the possibility of CNS infection exists, the use of an agent with established CNS penetration, such as amphotericin or fluconazole, should be considered. In situations where CNS involvement is unknown or cannot be assessed, an echinocandin could be used in combination with amphotericin or fluconazole. There is no best empiric antifungal for suspected or recognized neonatal candidiasis; rather, the selection of agent(s) should be guided by the clinical presentation and history of antifungal agents received.

Assuming that an antifungal with activity against the organism(s) isolated is administered at the appropriate dose, blood-stream clearance should occur within 3 to 5 days following

Table 2 Management and prevention of neonatal sepsis

Condition	Therapy	Additional considerations
Empiric management		
Early onset sepsis	Ampicillin + aminoglycoside: 10 d for bacteremia; 14 d for GBS and uncomplicated meningitis; extend to 21 to 28 d for complicated infections	Consider a third-generation cephalosporin (cefotaxime preferred) or carbapenem for meningitis. Tailor therapy to pathogen. Consider discontinuation of therapy if pathogen not isolated.
Late-onset sepsis	Vancomycin + aminoglycoside: duration dependent on pathogen and site	Alternatives to vancomycin may be considered based on local epidemiology and clinical presentation. Aminoglycoside-based regimen preferred to cephalosporin given reduced risk of resistance. Consider cephalosporin if meningitis suspected. Consider a carbapenem if third-generation cephalosporin recently received. Consider amphotericin for fungal etiologies. Tailor therapy to pathogen. Consider discontinuation of therapy if pathogen not isolated.
Non-antimicrobial treatment strategies		
Recombinant G-CSF Recombinant G-MSF	Enhance neutrophil number and function, but no reduction in infection when administered as prophylaxis or improvement in survival when administered as therapy	Insufficient evidence to support the clinical use of G-CSF or GM-CSF either as treatment or prophylaxis to prevent systemic infections. ¹⁰⁴
IVIG	Augments antibody-dependent cytotoxicity and improves neutrophilic function, <i>but</i> no evidence that IVIG in suspected or proven sepsis reduces death	Insufficient evidence from 10 RCTs or quasi-RCTs to support use in neonates with confirmed or suspected sepsis. ¹⁰⁷
Prevention strategies		
IAP	Administration of penicillin or ampicillin 4 h prior to parturition	Successfully reduces rates of EOS due to GBS. No effect on LOS GBS.
Fluconazole prophylaxis	Administration of weight-based dosing to neonates < 1,500 g	Most beneficial in NICUs with high baseline rates of invasive candidiasis.
BLF supplementation with a probiotic, LGG	BLF is a human milk glycoprotein with a role in innate immune response; LGG enhances the activity of lactoferrin	BLF supplementation with and without LGG reduced the incidence of first LOS in 472 VLBW neonates in large randomized, double-blind RCT. Additional confirmatory studies warranted.

Abbreviations: BLF, bovine lactoferrin; EOS, early onset sepsis; GBS, group B streptococcus; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage-stimulating factor; IAP, intrapartum antimicrobial prophylaxis; IVIG, intravenous immunoglobulin; LGG, *Lactobacillus rhamnosus* GG; LOS, late-onset sepsis; NICU, neonatal intensive care unit; RCTs, randomized, controlled trials; VLBW, very low birth weight.

initiation of therapy. Rescreening after 5 days of therapy to document clearance of organisms is recommended. Consideration of a second antifungal agent may be warranted if there is evidence of an abscess, persistent candiduria, or 10 days of persistent organism isolation.^{24,86} Improvement in rates of fungal clearance from 67 to 96% with the addition of a second antifungal agent has been noted.⁸⁷

There is debate regarding the need to remove central venous catheters (CVCs) in patients with invasive candidiasis. Improved outcomes have been noted in neonates with CVC removal.^{24,86,88} Replacement of the CVC at a different site, when continued central access is required, has been proposed as an alternative to discontinuation of the CVC. Because a majority of neonates are CVC-dependent for parenteral nutrition and antifungal therapy, the maintenance of venous

access is obligatory.²⁴ A recent evaluation of CVC retention in nonneutropenic adults suggested that CVC removal within 48 hours of organism isolation may not affect outcome.⁸⁹ A Cochrane Review in 2011 assessing early removal versus expectant management of CVCs in neonates with bloodstream infection did not identify any randomized controlled trials using standard search methods of the Cochrane Neonatal Review Group. The authors concluded that observational data suggest that early removal is associated with a decreased incidence of complications and prolonged detection of pathogens. However, the reviewers recommended caution in the application of these findings due to inherent study design biases.⁹⁰ The American Academy of Pediatrics Committee on Infectious Diseases recommends removal of any infected vascular catheter and consideration of replacement when

infection is controlled.⁷³ Additional studies in preterm neonates are needed before definitive recommendations regarding CVC retention can be made in this population.

Prevention of Candidiasis

Fluconazole Prophylaxis

Prophylactic administration of fluconazole during the first 6 weeks of life reduces fungal colonization and invasive fungal infection in infants with birth weights < 1,000 g.⁹¹ In addition to the individual benefit afforded by prophylaxis for VLBW neonates, fluconazole prophylaxis may have a community benefit by decreasing the overall fungal burden of a NICU.²⁵ A single center was able to decrease invasive candidiasis mortality with fluconazole prophylaxis administered to high-risk neonates.⁹² Results from over 14 trials at multiple institutions with 3,100 neonates suggest that fluconazole prophylaxis decreases colonization of the urine, gastrointestinal tract, and integument, without promoting the development of resistance and without adverse effects.^{25,91–97} Targeted therapy of 3 mg/kg of intravenous fluconazole initiated before 48 hours of life to neonates < 1,000 g and continued for the duration of intravenous access is a regimen that has been adopted by some NICUs with high baseline rates of candidiasis.⁹⁵ Of note, enterally administered fluconazole does not appear to provide protection against CVC-associated candidiasis.²⁵ Both the Infectious Diseases Society of America and the American Academy of Pediatrics support the administration of prophylactic fluconazole to preterm infants.^{73,88} Based on an annual U.S. preterm birth cohort of approximately 30,000 VLBW, it has been estimated that fluconazole prophylaxis could prevent about 2,000 to 3,000 cases of invasive candidiasis, about 200 to 300 deaths, and the adverse neurodevelopmental outcomes of invasive candidiasis in approximately 400 to 500 infants per year.²⁵ Differing baseline rates of fungal infections, practices related to CVC removal, severity of illness, and practices related to the use of broad-spectrum antimicrobials make universal recommendations regarding prophylaxis challenging.

Other Preventive Strategies

Additional strategies that have been proposed to reduce the risk of invasive candidiasis include maternal decolonization, through targeted therapy of women with symptomatic vaginal candidiasis, or empiric therapy for all antepartum women.⁹⁸ Neonatal practices that may reduce the risks of invasive candidiasis include limited use of broad-spectrum antimicrobials in hosts with documented resistant pathogens, use of an aminoglycoside instead of a cephalosporin for empiric therapy when meningitis or antimicrobial resistance is not suspected, limitation of postnatal steroid use in VLBW infants, early enteral feeding, and the establishment of the neonatal gut microbiome with human milk feeding.^{24,99} Infection prevention practices in the NICU, including the utilization of trained teams with standardized practices for the insertion and care of CVCs, have been shown to reduce the incidence of catheter-related infections.^{100,101} Active surveillance of organisms associated with infection, antifungal resistance, and outcomes is important to optimize management.

Other Management Strategies

Recombinant G-CSF and recombinant granulocyte-macrophage-stimulating factor (GM-CSF) have both been shown to enhance the neutrophil function of leukocytes removed from premature infants and evaluated in the laboratory setting.¹⁰² A prospective, randomized case-control study conducted over a 13-month period between 2009 and 2010 demonstrated that G-CSF administered to 60 neonates with clinical sepsis resulted in decreased antibiotic utilization and lengths of stay compared with 30 infants who did not receive G-CSF.¹⁰³ Treatment with colony-stimulating factor therapy (G-CSF and GM-CSF) of 97 neonates who had documented culture-positive sepsis (largely due to gram-negative infection and GBS) and neutropenia (absolute neutrophil count < 1,700/mL) significantly reduced the risk of death (relative risk 0.34; 95% CI: 0.12 to 0.92).¹⁰⁴

These studies suggest the potential for enhancing immune response by supplementing conventional therapies with G-CSF or GM-CSF. However, large-scale randomized, placebo-controlled trials will be necessary to support the clinical use of G-CSF and GM-CSF.^{104,105}

An interest in the potential benefits of intravenous immunoglobulin in the management of neonatal sepsis stems from the potential of supplemental immunoglobulin G to activate the immune response by augmenting antibody-dependent cytotoxicity and improving neutrophil function. A Cochrane review of 10 randomized or quasi-randomized controlled trials of intravenous immunoglobulin for treatment of suspected bacterial or fungal infection in newborn infants < 28 days of age concluded that there is still insufficient evidence to support the administration of intravenous immunoglobulin to prevent mortality in infants with clinical or culture-proven sepsis.¹⁰⁶ Mortality or major morbidity at 2 years of age was compared in 3,493 infants from 113 hospitals in nine countries who received directed or empiric antimicrobial therapy and polyvalent immunoglobulin G immune globulin or a placebo. There were no significant differences in death, major or minor disability at age 2 years, incidence of subsequent sepsis episodes, and adverse events. These results did not support a benefit of immune globulin therapy to neonates with confirmed or suspected sepsis.¹⁰⁷

Prevention

In the United States, IAP for the prevention of invasive GBS disease has been highly successful. Current recommendations call for universal screening of pregnant women at 35 to 37 weeks' gestation to identify those who are colonized with GBS and for the provision of IAP with penicillin or ampicillin to colonized women for at least 4 hours prior to delivery. In the rare situation of penicillin allergy, clindamycin is an alternative agent, if local epidemiology or isolate testing demonstrates susceptibility.¹⁶ IAP does not prevent late-onset GBS. The source of pathogens associated with LOS is from the environment, most often from providers who interact with the infant in the postnatal environment. Infection control strategies targeted toward the health care worker, including hand hygiene, are effective in reducing the risk of LOS.¹⁰⁸

Assessing the risk of colonization from the health care environment requires reliable and rapid methods for assessing whether an organism represents endemic flora or a new outbreak strain. A focus on MRSA in the NICU setting demonstrated that high-throughput sequencing technology can distinguish between outbreak and endemic isolates and may assist with infection control in a clinically relevant time frame.¹⁰⁹ Although the presence of resistant organisms is a focus of prevention efforts in many NICU settings, the comparable morbidity and mortality of infections with antimicrobial susceptible strains such as MSSA should be noted. Prevention efforts and resources should be devoted equally to reducing the prevalence of both resistant and susceptible organisms.²²

The benefits of fluconazole prophylaxis in neonates weighing < 1,500 g for the prevention of candidiasis have been demonstrated in multiple studies and are most beneficial in NICUs with high baseline rates of invasive candidiasis.²³

The ability of bovine lactoferrin (BLF) supplementation alone and in combination with the probiotic *Lactobacillus rhamnosus* GG (LGG) to prevent neonatal sepsis was evaluated in a prospective, multicenter, double-blind, randomized placebo-controlled trial in 11 tertiary care NICUs. Over a 9-month period during 2007 to 2008, 472 VLBW neonates received placebo, LGG, and BLF or BLF only daily from birth through 30 days of life or 45 days for neonates with birth weights of < 1,000 g. Compared with placebo, BLF supplementation with and without LGG reduced the incidence of the first LOS episode in VLBW neonates. Further studies of lactoferrin, with and without probiotics, to reduce risk of neonatal sepsis are indicated.^{110,111}

Conclusion

The epidemiology of neonatal sepsis is a changing landscape. Early and late-onset neonatal sepsis continues to be associated with significant morbidity and mortality, including long-term morbidity. Surveillance for rates of infection, pathogens associated with infection and their antimicrobial susceptibility, antimicrobial and adjunctive treatments used, and short- and long-term outcomes is important as optimal prevention and treatment strategies are explored in larger scale clinical trials.

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