



## Neonatal sepsis

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Neonatal sepsis is the cause of substantial morbidity and mortality. Precise estimates of neonatal sepsis burden vary by setting. Differing estimates of disease burden have been reported from high-income countries compared with reports from low-income and middle-income countries. The clinical manifestations range from subclinical infection to severe manifestations of focal or systemic disease. The source of the pathogen might be attributed to an in-utero infection, acquisition from maternal flora, or postnatal acquisition from the hospital or community. The timing of exposure, inoculum size, immune status of the infant, and virulence of the causative agent influence the clinical expression of neonatal sepsis. Immunological immaturity of the neonate might result in an impaired response to infectious agents. This is especially evident in premature infants whose prolonged stays in hospital and need for invasive procedures place them at increased risk for hospital-acquired infections. Clinically, there is often little difference between sepsis that is caused by an identified pathogen and sepsis that is caused by an unknown pathogen. Culture-independent diagnostics, the use of sepsis prediction scores, judicious antimicrobial use, and the development of preventive measures including maternal vaccines are ongoing efforts designed to reduce the burden of neonatal sepsis.

### Epidemiology and definition of neonatal sepsis

#### Definition of neonatal sepsis

The term neonatal sepsis is used to designate a systemic condition of bacterial, viral, or fungal (yeast) origin that is associated with haemodynamic changes and other clinical manifestations and results in substantial morbidity and mortality. Despite years of clinical experience with the care of neonates with confirmed or suspected sepsis, challenges remain including the absence of a consensus definition of neonatal sepsis.<sup>1</sup> Traditionally, the definition of sepsis has included isolation of a pathogen from a normally sterile body fluid such as blood or cerebrospinal fluid (CSF). However, as the clinical features of sepsis can be induced by potent pro-inflammatory cytokines, the term systemic inflammatory response syndrome (SIRS) has also been used when describing neonatal sepsis.

Neonatal sepsis has been classified as either early-onset or late-onset depending on the age of onset and timing of the sepsis episode. Clinical manifestations of early-onset infections usually appear within the first 72 h of life;<sup>1</sup> some clinicians define early-onset infections, especially those due to group B *Streptococcus* (GBS), as infections occurring at less than 7 days of age. Early-onset infections are acquired before or during delivery and usually represent

vertical mother-to-infant transmission. Late-onset infections present after delivery, or beyond 3 to 7 days of age, and are attributed to organisms acquired from interaction with the hospital environment or the community. In some situations, organisms attributed to late-onset sepsis might be acquired at parturition, but with clinical manifestation of infection after 72 h of life. In extremely low gestational age and high-risk term infants, many of whom have prolonged hospital stays, the designation of late-onset sepsis might apply to any episode of sepsis from birth to hospital discharge regardless of age at the time of the episode. For GBS infections, late onset often refers to disease that occurs from 1 week to 3 months of age, with infections that develop after 3 months of age designated as very-late-onset infection.<sup>2</sup>

#### Burden of neonatal sepsis

Precise estimates of neonatal sepsis burden vary by setting, with differing estimates of burden between countries of different income levels. Defining the rate of neonatal sepsis is important and has been complicated by variation in the denominators used. When comparing rates of neonatal sepsis, it is important to note whether the denominator is comprised of the total number of livebirths or another measure, such as the number of hospital admissions. As noted, it is important to consider if population-based or hospital-based rates of neonatal sepsis are reported. In the USA, the incidence of neonatal bacterial sepsis varies from one to four infections per 1000 livebirths, with geographical location and temporal changes over time accounting for variance. Full-term male infants have a higher incidence of sepsis than full-term female infants, although this association has not been seen in preterm infants. A study from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Neonatal Research Network documented rates of culture-confirmed early-onset sepsis among almost 400 000 livebirths at network centres.<sup>3</sup> The overall rate of early-onset sepsis, defined as a positive blood or CSF bacterial culture at less than

#### Search strategy and selection criteria

We searched the Cochrane Library and PubMed for publications in English from Jan 1, 2010, to Jan 1, 2017, but also included commonly referenced and highly regarded papers with publication before these dates. We used the search term "neonatal sepsis". We also searched the reference lists of publications identified by the search strategy and selected those that we judged relevant. Review articles and book chapters are cited to provide readers with more details and more references than this Seminar was able to accommodate. Our reference list was modified on the basis of comments from peer reviewers.

72 hours of age, was 0·98 infections per 1000 livebirths, with rates inversely related to birthweight (10·96 per 1000 livebirths for 401–1500 g birthweight, 1·38 for 1501–2500 g birthweight, 0·57 for >2500 g birthweight).<sup>3</sup>

2·5 million sepsis-related hospital admissions (30·8 per 1000 livebirths) were noted from 1988 to 2006 in infants less than 3 months of age in a cross-sectional study of records contained in the US National Hospital Discharge Survey.<sup>4</sup> The authors noted that episodes of clinical neonatal sepsis declined following the widespread implementation of intrapartum antimicrobial prophylaxis (IAP) that paralleled declines in GBS early-onset neonatal sepsis. A modest but steady decline occurred in hospital admission rates for full-term infants, and less so for preterm infants during the surveillance period.<sup>4</sup> By comparison, a retrospective study from the Canadian Neonatal Network of early-onset sepsis,<sup>5</sup> defined as a bacterial isolate from culture of blood or CSF obtained from infants in the first 72 h of life who were admitted to neonatal intensive care units, revealed an early-onset neonatal sepsis rate of 6·8 per 1000 admissions from 2003 to 2005 and 6·2 per 1000 admissions from 2006 to 2008. Similar to observations in the USA, the authors noted a significant reduction in GBS and an increased isolation rate of non-GBS organisms as possible causes of early-onset neonatal sepsis.<sup>5</sup>

## Pathophysiology and causative agents of neonatal sepsis

### Early-onset sepsis

Early-onset neonatal sepsis occurs in utero from either a transplacental or, more commonly, ascending bacteria entering the uterus from the vaginal environment following membrane rupture. Additionally, the newborn child might become infected when exposed to potentially pathogenic bacteria, viruses, or fungi during passage through the birth canal. The human birth canal is colonised with aerobic and anaerobic bacterial organisms that can be vertically transmitted from an ascending infection of the amniotic fluid or natal infection of the neonate<sup>6</sup> during labour or delivery.<sup>7</sup>

Chorioamnionitis, often referred to as intra-amniotic infection, is an acute inflammation of the fetal membranes, presumably due to bacterial infection. Chorioamnionitis results from microbial invasion of amniotic fluid, often as a result of prolonged rupture of the chorioamniotic membrane. The clinical syndrome of chorioamnionitis might include maternal signs and symptoms (fever, leucocytosis, cloudy or odorous discharge, and lower abdominal tenderness) and fetal signs (tachycardia is most common). Chorioamnionitis might also present asymptotically with laboratory or pathological abnormalities supporting the syndrome. The rate of histological chorioamnionitis is inversely related to gestational age at birth and directly related to duration of membrane rupture.<sup>8–11</sup> *Ureaplasma parvum* and *Ureaplasma urealyticum*, both genital mycoplasmas, are

the most common bacteria isolated from placentas with histological chorioamnionitis and from amniotic fluid. *Ureaplasma* spp colonisation of the respiratory tract of preterm infants has been associated with bronchopulmonary dysplasia. The understanding of the association between maternal chorioamnionitis and neonatal outcomes is an area of active investigation by maternal and neonatal research teams.<sup>12</sup>

### Late-onset or acquired sepsis

During the first 3 months of life, the innate immune system, including phagocytes, natural-killer cells, antigen presenting cells, and the complement system, provide a defence against pathogens. Decreased function of neutrophils and low concentrations of immunoglobulins increase the susceptibility of preterm infants to invasive infection. As infants age, they are exposed to environmental organisms that might be pathogenic to those with an immature immune system. Contact with hospital personnel, family members, nutritional sources, and contaminated equipment all represent opportunities for pathogen exposure. Hand contamination is the most common source of postnatal infections in infants admitted to hospital, underscoring the importance of hand hygiene.

Late-onset bloodstream infections occur more frequently in neonates with central venous access than in infants without central venous access who are usually older, and these infections are more likely to be attributed to Gram-positive organisms, including coagulase-negative staphylococci and streptococci. Most cases of meningitis are late-onset infections resulting from haematogenous spread via the choroid plexus into the CNS; less often, late-onset meningitis results from contiguous spread as a result of contamination of open neural tube defects, congenital sinus tracts, ventricular devices, or penetrating wounds from fetal scalp monitors. Abscess formation, ventriculitis, septic infarcts, hydrocephalus, and subdural effusions are complications of meningitis that occur more often in neonates.<sup>13</sup>

### Causes of neonatal sepsis

Neonatal sepsis can be the result of infections with bacterial, viral, or fungal (mostly yeast) microorganisms. The most common organisms associated with early-onset neonatal sepsis are *Streptococcus agalactiae* (GBS) and *Escherichia coli*. In almost 400 000 livebirths from 2006 to 2009 at academic-based neonatal centres in the USA, 389 newborn infants had early-onset infection (0·98 cases per 1000 livebirths) with 43% due to GBS (0·41 per 1000 livebirths) and 29% to *E coli* (0·28 per 1000 livebirths). Most infants with GBS infections were full term (73%) although 81% of those with *E coli* infections were preterm; infection rates increased with decreasing birthweight. Case fatality rate overall was 16%, but it was inversely related to gestational age: 54% at 22–24 weeks, 30% at 25–28 weeks, 12% at 29–33 weeks, and 3% at more than

37 weeks' gestation. Although 9% of infants with a GBS sepsis and 33% of infants with *E coli* sepsis died, the risk of death was not significantly higher for infants with sepsis associated with *E coli* infection compared with sepsis associated with GBS infection after adjustment for gestational age. This prospective study showed that although GBS remains the most frequent pathogen of early-onset infection, there has been a shift from GBS to *E coli* as the most important pathogen associated with early-onset infection in preterm and very low birthweight infants. Despite national guidelines for use of intrapartum antibiotics to reduce vertical transmission of GBS infection, there were notable missed opportunities for GBS intrapartum chemoprophylaxis.<sup>3,14</sup> Although less frequent than GBS and *E coli*, *Listeria monocytogenes* (usually acquired transplacentally), non-typeable *Haemophilus influenzae*, and Gram-negative enteric bacilli other than *E coli* also have been implicated in early-onset neonatal sepsis, as have *Candida* spp, which are often associated with a cutaneous erythematous rash.<sup>15,16</sup>

Late-onset neonatal sepsis might also be associated with GBS, *E coli*, other Gram-negative aerobes, or *L monocytogenes* infection. The incidence of neonatal listeriosis has decreased substantially in recent years.<sup>17</sup> However, in the neonatal intensive care unit, coagulase-negative staphylococci are the most commonly isolated pathogens in neonates with late-onset sepsis.<sup>18–21</sup> *Staphylococcus aureus* is also associated with late-onset sepsis, most commonly in neonates with vascular-access catheters. For example, in 117 episodes of *S aureus* sepsis in infants in 13 neonatal units in the UK, eight (7%) episodes were attributed to methicillin-resistant *S aureus* (MRSA). The mean gestational age of infants was 27 weeks and the mean birthweight was 850 g. The overall *S aureus* incidence was 0.6 per 1000 livebirths and 23 per 1000 livebirths in infants less than 1500 g. 94% of the episodes were classified as late-onset, occurring more than 48 h of life; all of the seven episodes categorised as early-onset were attributed to MRSA. Half of the infants exhibited non-localising signs of sepsis, and half of the infants had central venous access at the time of the *S aureus* infection.<sup>22</sup> Other infrequent causes of both early and late sepsis are *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *Enterococcus faecalis*, and in neonates in the community, *Streptococcus pneumoniae*. Additionally, *Neisseria meningitidis*, *Ureaplasma* spp, and *Mycoplasma hominis* have been associated with early-onset sepsis, meningitis, pneumonia, osteomyelitis, and cerebral abscesses. The prevalence of pathogens varies considerably by international setting, with a notable burden contributed by Gram-negative organisms in resource-poor areas.<sup>23</sup>

The most common viral causes of sepsis are herpes simplex virus (HSV) and enterovirus infections, both of which are more frequently associated with late-onset presentations. Neonatal HSV infections are associated with substantial morbidity and mortality. Manifestations of infections can result in presentations similar to sepsis and might be localised to the skin, eyes, and mouth,

involve the CNS, or might be disseminated involving liver, lungs, adrenal glands, with onset between days 5–9 of life. Neonatal HSV can result from infection with either HSV-1 or HSV-2, with HSV-1 becoming more prevalent with increases in genital infections due to HSV-1.<sup>24–26</sup>

Neonates with enterovirus infections might develop meningoencephalitis, myocarditis, and hepatitis, following poor feeding, lethargy, fever, irritability, hypoperfusion, and jaundice. Infants younger than 10 days of age who are exposed to echoviruses, parechoviruses, and coxsackie group B viruses through maternal shedding are unable to mount a substantial immune response and because of the timing of recent maternal infection, usually do not benefit from the transplacental transfer of maternal antibodies.<sup>27–29</sup>

Fungi, notably yeast, have been implicated in an increasing number of systemic infections, usually acquired during prolonged hospital stay of preterm neonates. *Candida* spp are the third most common cause of late-onset neonatal sepsis in low birthweight infants (<1500 g), with the emergence of *Candida parapsilosis* as a major pathogen in neonates with central venous access. Geographical variation has been reported, with relatively lower incidence of *C parapsilosis* infection in Europe compared with North America and Australia.<sup>30</sup> As with other neonatal infections, risk factors included prematurity, gastrointestinal colonisation, and vascular catheterisation, suggesting that control of transmission in the hospital environment could prevent colonisation and infection.<sup>31</sup> In a prospective observational cohort of 1515 infants with 1000 g birthweight or less who received care at one of 19 academic medical centres in the USA, invasive candidiasis occurred in 137 (9%) infants with marked centre-to-centre variability. Potentially modifiable risk factors included central venous catheters, receipt of broad-spectrum and antenatal antibiotics including third-generation cephalosporins, receipt of intravenous lipid emulsion, postnatal corticosteroids, antacid medications, and the presence of an endotracheal tube.<sup>32</sup>

## Risk factors

### Infant risk factors

The most important neonatal factor predisposing to infection that could result in sepsis is prematurity or low birthweight. Preterm low birthweight infants have a 3–10 times higher incidence of infection than full-term normal birthweight infants. Immune dysfunction and an absence of transplacentally acquired maternal IgG antibodies in premature infants might increase risk of infection. Additionally, preterm infants often require prolonged intravenous access, endotracheal intubation, or other invasive procedures that provide a portal of entry or impair barrier and clearance mechanisms, placing them at increased risk for hospital-acquired infections. Furthermore, lower neonatal 25-hydroxyvitamin D concentrations have been associated with early-onset sepsis.<sup>33</sup>

Symptoms	
General	Fever, temperature instability; "not doing well", poor feeding, or oedema
Gastrointestinal system	Abdominal distention, vomiting, diarrhoea, or hepatomegaly
Respiratory system	Apnoea, dyspnoea, tachypnoea, retractions, flaring, grunting, or cyanosis
Renal system	Oliguria
Cardiovascular system	Pallor, mottling, cold, clammy skin, tachycardia, hypotension, or bradycardia
CNS	Irritability, lethargy, tremors, seizures, hyporeflexia, hypotonia, abnormal Moro reflex, irregular respirations, full fontanel, or high-pitched cry
Haematological system	Jaundice, splenomegaly, pallor, petechiae, purpura, or bleeding

Adapted from Nelson Textbook of Pediatrics<sup>34</sup> with permission from Elsevier.

**Table 1: Initial signs and symptoms of infection in newborn infants**

### Maternal risk factors

Maternal history provides important information about exposure to infectious diseases, bacterial colonisation, immunity (natural and acquired), and obstetric risk factors (prematurity, prolonged rupture of membranes of 18 h or greater, chorioamnionitis, and urinary tract infections). Attack rates of neonatal sepsis increase substantially in low birthweight infants in the presence of maternal chorioamnionitis. Factors influencing how and whether infant colonisation results in disease including prematurity, underlying illness, invasive procedures, inoculum size, virulence of the infecting organism, genetic predisposition, the innate immune system, host response, and the acquisition of transplacental maternal IgG antibodies, are not completely understood. Aspiration or ingestion of bacteria in amniotic fluid might lead to congenital pneumonia or systemic infection, with manifestations frequently apparent before delivery (fetal distress and tachycardia), at delivery (apnoea, respiratory distress, and shock), or after a latent period of a few hours to 1–2 days (respiratory distress, haemodynamic instability, or shock). Additionally, maternal GBS bacteriuria, indicative of a heavy burden of GBS colonisation, represents a notable risk for acquisition of neonatal GBS infection.

Resuscitation at birth, including emergent endotracheal intubation or insertion of an umbilical vascular catheter, is associated with an increased risk of bacterial infection. This infection might be due to exposure to organisms associated with maternal colonisation at the time of birth or acquisition of translocated pathogens during the procedures associated with resuscitation.

### Diagnosis

#### Clinical signs and symptoms of neonatal sepsis

Neonates with bacterial sepsis might show non-specific signs and symptoms or focal signs of infection, including temperature instability, hypotension, poor perfusion

#### Panel 1: Integrated management of childhood illness (IMCI) and WHO criteria for severe infections in children

- Neurological: convulsions, drowsy, or unconscious, decreased activity, or bulging fontanelle
- Respiratory: respiratory rate more than 60 breaths per minute, grunting, severe chest indrawing on intake of breath, or central cyanosis
- Cardiac: poor perfusion, or rapid and weak pulse
- Gastrointestinal: jaundice, poor feeding, abdominal distension, or emesis
- Dermatological: skin pustules, periumbilical erythema, or purulence
- Musculoskeletal: oedema or erythema overlying bones or joints
- Other: temperature greater than 37.7°C (or feels hot) or less than 35.5°C (or feels cold)

Adapted from WHO: Pocket book of hospital care for children: Guidelines for the management of common childhood illnesses.

For the **Guidelines for the management of common childhood illnesses** see [http://www.who.int/maternal\\_child\\_adolescent/documents/child\\_hospital\\_care/en/](http://www.who.int/maternal_child_adolescent/documents/child_hospital_care/en/)

with pallor and mottled skin, metabolic acidosis, tachycardia or bradycardia, apnoea, respiratory distress, grunting, cyanosis, irritability, lethargy, seizures, feeding intolerance, abdominal distention, jaundice, petechiae, purpura, and bleeding (table 1). Initial symptoms might be few and could include apnoea alone or tachypnoea with retractions, nasal flaring, grunting, or tachycardia. Later complications of sepsis might include respiratory failure, pulmonary hypertension, cardiac failure, shock, renal failure, liver dysfunction, cerebral oedema or thrombosis, adrenal haemorrhage or insufficiency, bone marrow dysfunction (neutropenia, thrombocytopenia, anaemia), and disseminated intravascular coagulation (panels 1 and 2, appendix).

Non-infectious presentations of organ failure might mimic the clinical presentation of neonatal sepsis. Additionally, infectious and non-infectious causes might coexist in the same host. For example, clinical observations have shown that respiratory distress syndrome secondary to surfactant deficiency might be present with bacterial pneumonia.

#### Conventional diagnostics

Traditionally, laboratory-confirmed neonatal sepsis is diagnosed by isolating the causative agent from a normally sterile body site (blood, CSF, urine, and pleural, joint, and peritoneal fluids; table 2). To optimise diagnosis, specimens of adequate volume obtained aseptically are essential. For blood cultures, a minimum of 0.5–1 mL of blood should be obtained, preferably from two different venipunctures from two separate sites. True pathogens are more likely to be present in both culture specimens. In the presence of a central venous catheter, blood cultures ideally would be obtained simultaneously, with one from a peripheral and one from a vascular catheter so that differential time to positivity can be assessed.<sup>35</sup> This

See Online for appendix

**Panel 2: Assessment of a neonate for sepsis****History (specific risk factors)**

*Maternal infection during gestation or at parturition (type and duration of antimicrobial therapy):*

- Urinary tract infection
- Chorioamnionitis—clinical or histological diagnosis
- Maternal colonisation with group B streptococci, *Neisseria gonorrhoeae*, or herpes simplex virus
- Low gestational age or birthweight
- Multiple infants from a single gestation
- Duration of membrane rupture
- Complicated delivery
- Fetal tachycardia (distress)
- Alterations in heart rate variability
- Age at infection onset (in utero, birth, early postnatal, late)
- Location at infection onset (hospital, community)

*Medical intervention:*

- Vascular access
- Endotracheal intubation
- Parenteral nutrition
- Surgery

**Evidence of other disease processes\***

- Congenital malformations (heart disease, neural tube defect)
- Respiratory tract disease (respiratory distress syndrome, aspiration)
- Necrotising enterocolitis
- Metabolic disease—eg, galactosaemia

**Evidence of focal or systemic disease**

- General appearance and neurological status
- Abnormal vital signs

- Organ system disease
- Reduced feeding, stools, urine output, and extremity movement

**Laboratory studies**

*Evidence of infection*

- Culture from a normally sterile site (blood or cerebrospinal fluid [CSF])
- Confirmed presence of a microorganism in tissue or fluid
- Molecular detection (blood, urine, or CSF)
- Maternal or neonatal serology (syphilis, toxoplasmosis)
- Autopsy

*Evidence of inflammation*

- Leucocytosis: increased immature:total neutrophil count ratio
- Acute-phase reactants: C-reactive protein, erythrocyte sedimentation rate
- Cytokines: interleukin 6, interleukin B, tumour necrosis factor
- Pleocytosis in CSF or synovial or pleural fluid
- Disseminated intravascular coagulation: fibrin degradation products, D-dimer

*Evidence of multiorgan system disease*

- Metabolic acidosis
- Decreased pulmonary function
- Decreased renal function
- Decreased hepatic function or injury
- Bone marrow function: neutropenia, anaemia, thrombocytopenia

Adapted from Nelson Textbook of Pediatrics<sup>34</sup> with permission from Elsevier.

\*Disease processes or conditions that increase the risk of infection or might overlap with signs of sepsis.

facilitates identification of peripheral bacteraemia versus catheter-related bloodstream infections and has implications for clinical management. Because some organisms might be detected only in CSF and not in the blood at the time of a sepsis assessment, in symptomatic neonates the sepsis assessment should also include a lumbar puncture procedure.<sup>36</sup> Automated blood culture systems continuously monitor specimens and alert when positive signalling is detected, facilitating further processing for pathogen identification. Matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry can assist with early identification of organisms from blood cultures, allowing for directed antibiotic therapy in the setting of bloodstream infections.<sup>37</sup> More recently, the use of multiplex PCR on positive blood culture specimens can identify common bacterial and fungal organisms as well as antimicrobial resistance genes within hours of organism growth.<sup>38</sup> Similar technology has been used on CSF samples to improve time to identification of bacterial organisms.<sup>39</sup>

Urinary tract infections do not occur in the first 72 h of age, and therefore, suprapubic bladder aspiration or urinary catheterisation is not done as part of the

assessment for early-onset neonatal sepsis. However, urinary tract infections are common in older term and preterm infants and a urinary source should be considered with late-onset presentations of sepsis.<sup>40</sup>

Examination of the placenta with attention to pathology might suggest both chronic and acute intrauterine inflammation. Although placental cultures could reveal potentially pathogenic bacteria, such a finding is likely to represent fetal exposure rather than true infection, and should not be the reason for prolonged antibiotic therapy of the infant.

**Culture-independent diagnostics**

Because PCR is a highly sensitive and rapid technique, it is increasingly being applied to bodily fluids directly without the need to first culture causative agents (table 2). Quantitative real-time amplification systems (qPCR) based on bacterial 16S ribosomal DNA have a very high negative predictive value and results are usually available in a timely manner. Additionally, a small volume sample is frequently sufficient, and the test can be done on surgical tissues and body fluids such as pleural effusions and ascites. Disadvantages of qPCR include the inability

	Parameter	Optimal conditions for specimen collection	Applicability for neonatal sepsis
<b>Culture-based</b>			
Blood	Culture	0.5–1 mL of whole blood from two sites at time of symptom onset	Gold standard for bacteraemia
CSF	Culture	When clinically feasible, >1 mL CSF	Optimise antimicrobial therapy
Urine	Culture	At >72 h of life, >1 mL urine	Not useful for EOS; potential benefits for LOS
Tracheal aspirate	Culture	Obtained with concern for new onset of lower respiratory tract infection	Usually reflects colonisation
<b>Culture-independent</b>			
Immune function	MHC II and TNF $\alpha$	Investigational	Both decreased in chorioamnionitis and sepsis
Neutrophil indices	Neutropenia Absolute neutrophil count Absolute immature neutrophil count	After 12 h of life, with consideration of gestational age, delivery method, altitude, arterial versus venous blood sampling, and time since birth	Neutropenia better predictor for sepsis than leucocytosis
Neutrophil markers	CD64	Increased for 24 h after infection, requires 50 $\mu$ L of blood, investigational	Cutoff points between 2.38–3.62 optimal sensitivity, specificity, and NPV for EOS
Platelet count	Thrombocytopenia and thrombocytosis	Late findings occurring after clinical manifestations have occurred, usually >72 h after infection onset	Thrombocytopenia associated with fungal infection
CSF cell count	CSF WBC	Uninfected neonates mean 10 cells per $\text{mm}^3$ , range up to 20 cells per $\text{mm}^3$	Does not predict culture-proven meningitis
CSF chemistries	CSF protein and glucose concentrations	Fullterm <0.1 g/dL, with preterm neonates with higher concentrations (70–80% of serum glucose)	Increased in fungal meningitis; low glucose specific for bacterial meningitis
Acute phase reactant–CRP	CRP	CRP assessed 8–24 h after infection	Good NPV
Acute phase reactant–procalcitonin	Procalcitonin	Procalcitonin assessed 2–12 h after infection, investigational	Better sensitivity but less specificity than CRP
Sepsis panels scores	Multiple laboratory tests	After 24 h of life, investigational	Most useful for NPV and discontinuation of antimicrobial therapy

Adapted from Nelson Textbook of Pediatrics<sup>34</sup> with permission from Elsevier. Routine refers to an assay or parameter that is usually available and widely used. Investigational refers to an assay or parameter that is undergoing assessment for clinical use and applicability. \*CSF=cerebrospinal fluid. EOS=early-onset sepsis. LOS=late-onset sepsis. MHC II=major histocompatibility complex class II. TNF  $\alpha$ =tumour necrosis factor  $\alpha$ . NPV=negative predictive value. WBC=white blood cell count. CRP=C-reactive protein.

**Table 2: Culture-based and culture-independent diagnostics for neonatal sepsis**

to do susceptibility testing and a high sensitivity that does not differentiate between active infection and recent infections that have resolved.<sup>41</sup> The possibility of detecting contaminants is also high, and therefore clinical correlation with results is mandatory.

Other commonly used non-culture based diagnostic tests include the total and differential white blood cell (WBC) count, absolute and immature neutrophil counts, and the ratio of immature to total neutrophils (I/T<sup>2</sup>). Although the WBC count has limitations in terms of sensitivity, an immature-to-total neutrophil ratio of 0.2 or greater has been suggestive of a bacterial infection.<sup>42</sup> The I/T<sup>2</sup> score was found to be predictive when used in combination with complete blood cell counts obtained at more than 4 h of age.<sup>43</sup> However, abnormal WBC counts could also result from fetal exposure to in-utero inflammation and not sepsis as frequently seen following maternal chorioamnionitis.<sup>44</sup> It seems that the main benefit of the WBC count is its negative predictive value since normal serial values make it unlikely that a blood or CSF culture will be positive. It is also worth noting that WBC values are dynamic during the first 12 h of life, so serial measurements over 24 h might be more informative than a single assessment.<sup>45</sup>

Other diagnostic tests that measure an inflammatory response include C-reactive protein (CRP), procalcitonin (PCT), haptoglobin, fibrinogen, proteomic markers in amniotic fluid, inflammatory cytokines (including interleukin 6, interleukin 8, and tumour necrosis factor  $\alpha$ ), and cell surface markers (including soluble CD14 subtype [presepsin], and neutrophil CD64).<sup>46,47</sup> CRP has been used as a marker of humoral response to bacterial infection. Because of the requirement for hepatic synthesis of CRP before appreciable concentrations are noted, decreased sensitivity has been reported when the CRP is measured at the onset of an infectious process, which occurs before adequate time for hepatic metabolism might have occurred. Serial measurements of CRP in combination with other acute phase reactants and markers, such as procalcitonin and interleukin levels (interleukin 6 and interleukin 8), might improve the accuracy of detection of an infectious process.<sup>48,49</sup> Similar to the WBC count, the absence of serial abnormalities has a high negative predictive value, supporting discontinuation of antibiotic therapy.

#### Diagnostic algorithms

Investigators have attempted to develop and validate so-called sepsis scores by incorporating different

	Therapy	Additional considerations
<b>Empirical management</b>		
EOS	Ampicillin plus aminoglycoside; 10 days for bacteraemia; 14 days for GBS bacteraemia and uncomplicated meningitis; extend to 21–28 days 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
LOS	Vancomycin plus aminoglycoside; duration of treatment dependent on pathogen and site	Alternatives to vancomycin can be considered on the basis of local epidemiology and clinical presentation; an aminoglycoside-based regimen is preferred to cephalosporin given the reduced risk of resistance; consider cephalosporin if meningitis suspected, a carbapenem if the patient has recently been given a third-generation cephalosporin; and amphotericin for fungal causes; and tailor therapy to pathogen and consider discontinuation of therapy if pathogen is not isolated
<b>Non-antimicrobial treatment strategies</b>		
Recombinant G-CSF <sup>54,55</sup> and recombinant GM-CSF	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.
IVIg <sup>56</sup>	Augments antibody dependent cytotoxicity and improves neutrophilic function but no evidence that IVIG in suspected or proven sepsis reduces death	Insufficient evidence from ten RCTs or quasi-RCTs to support use in neonates with confirmed or suspected sepsis
<b>Prevention Strategies</b>		
IAP <sup>14</sup>	Administration of penicillin or ampicillin 4 h before parturition	Successfully reduces rates of EOS due to GBS; and no effect on LOS GBS
Fluconazole prophylaxis	Administration of weight-based dosing to neonates less than 1500 g	Most beneficial in NICUs with high baseline rates of invasive candidiasis
BLF supplementation with a probiotic, LGG <sup>57</sup>	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 randomised, double-blind RCT. Additional confirmatory studies warranted.
No recommended durations are provided for non-antimicrobial therapies since there is insufficient evidence for their use. Adapted from Nelson Textbook of Pediatrics <sup>34</sup> with permission from Elsevier. EOS=early onset sepsis. GBS=group B streptococcus. LOS=late-onset sepsis. IVIG=intravenous immunoglobulin. RCT=randomised controlled trials. IAP=intrapartum antimicrobial prophylaxis. NICU=neonatal intensive care unit. G-CSF=granulocyte colony stimulating factor. GM-CSF=granulocyte macrophage stimulating factor. BLF=bovine lactoferrin supplementation. LGG=Lactobacillus rhamnosus GG. VLBW=very low birthweight.		
<b>Table 3: Management and prevention of neonatal sepsis</b>		

combinations of inflammatory response parameters, laboratory assessments, and physical examination findings, but a single score has not proven to be consistently reliable. In a prospective observational study<sup>50</sup> that enrolled 113 infants with median age 14 days, birthweight 1500 g or greater, from five European countries, the predictive value of the criteria developed by an expert panel to identify culture-proven late-onset sepsis was 61% (95% CI 52–70). 69 infants had an organism isolated (28 coagulase-negative staphylococci, 24 enterobacteriaceae, 11 other Gram-positive organisms, and six Gram-negative non-fermentative organisms). There was notable variation in empirical treatment with 43 different antibiotic regimens noted.<sup>50</sup> A quantitative stratification algorithm for the risk of early-onset sepsis in newborn babies at 34 weeks' gestation or greater was developed using a Bayesian approach.<sup>51</sup> Maternal and infant data collected from over 600 000 livebirths occurring at 14 hospitals between 1993–2007 identified 350 neonates with early-onset neonatal sepsis that were frequency-matched to 1063 control neonates without early-onset neonatal sepsis. The neonatal population was divided into three groups by a risk-stratification scheme: administer empirical antibiotics (4.1% of all livebirths, 60.8% of all early-onset neonatal sepsis, and sepsis incidence of 8.4 per 1000 livebirths); observe and assess (11.1% of births, 23.4% of all early-onset neonatal sepsis, and 1.2 per 1000 livebirths); and continued observation (84.8% of

births, 15.7% of all early-onset neonatal sepsis, and 0.11 per 1000 livebirths). The application of this schema was estimated to reduce antibiotic treatment of between 80 000–240 000 newborns in the USA annually.<sup>51</sup> An online early-onset newborn sepsis calculator is available to predict the probability of early-onset infection and guide decisions with respect to initiation of antibiotic therapy.<sup>51,52</sup>

## Management

### Empirical therapy

Treatment of neonatal infections can be divided into antimicrobial therapy for the suspected (empirical) or known (definitive) pathogens. Consideration of early-onset or late-onset presentation and exposures (community versus hospitalised status at the time of symptom onset) affects antimicrobial choice. The most important components are a thorough and complete history and physical examination as well as cultures of clinical specimens. Although it is preferable to obtain cultures before the initiation of antimicrobial therapy to optimise recovery of organisms, antimicrobial therapy administration should not be unduly delayed for specimen collection in severely ill neonates in septic shock. In general, empirical therapy should be guided by the antimicrobial resistance patterns of bacterial isolates commonly detected in the neonatal intensive care unit or in community settings. Initial empirical treatment of early-onset bacterial infections should consist of ampicillin

For the newborn sepsis calculator see <https://neonatalesepsiscalculator.kaiserpermanente.org>

and an aminoglycoside (usually gentamicin), with third-generation or fourth-generation cephalosporin drugs reserved for suspected Gram-negative meningitis. Infections due to extended-spectrum  $\beta$ -lactamase-producing Gram-negative bacilli require treatment with carbapenems, such as meropenem. Treatment with piperacillin–tazobactam and ampicillin–sulbactam is being used increasingly among infants admitted to hospital in the neonatal intensive care unit; however, the penetration of tazobactam into the CNS is unreliable and should not be used for treatment of meningitis. However, the  $\beta$ -lactamase inhibitor sulbactam, when combined with ampicillin, does seem to achieve high concentrations in the CSF (table 3).<sup>53</sup>

Health-care-associated infections acquired in a neonatal intensive care unit are more likely to be caused by coagulase-negative staphylococci, and less often due to *S aureus* and Gram-negative bacteria. Although bloodstream infections due to coagulase-negative staphylococci in preterm infants are associated with substantial short-term morbidity as well as long-term neurodevelopmental impairment, they are not associated with increased mortality. With improvement in blood culture techniques that provide real-time culture results, narrow empirical therapy with a  $\beta$ -lactam anti-staphylococcal antibiotic such as nafcillin combined with an aminoglycoside, could be initiated in infants not colonised with MRSA and altered if pathogen recovery suggests alternative antimicrobial coverage. Such a strategy has been shown to reduce vancomycin use in the neonatal intensive care unit.<sup>58,59</sup>

Fungal infections including candidiasis, aspergillosis, and zygomycoses, should be aggressively managed when they are suspected and diagnosed. Empirical antifungal therapy with amphotericin deoxycholate can be considered in high-risk infants with risk factors for invasive candidiasis. Involvement of a paediatric infectious disease physician, a pharmacist with expertise in neonatal infections, and use of a guide containing neonatal dosing by weight and gestational age optimises antimicrobial use. Peak and trough measurements of antimicrobials might be useful to minimise toxicity if the antimicrobial will be administered for more than 2–3 days and in the treatment of particular infections such as meningitis where CSF penetration is needed. Trough measurements might be indicated in infants with compromised kidney or liver function.

### Directed therapy

Once the pathogens have been identified and their susceptibilities known, and the site or sites of infection identified, the most appropriate antimicrobial or antimicrobials should be administered. Penicillin or ampicillin are effective against GBS, with gentamicin providing synergy until the blood and CSF cultures are sterile, at which time it can be discontinued. Ampicillin alone is adequate for *L monocytogenes*, although the aminoglycoside

also provides synergy at treatment onset. Enterococci should be treated with a penicillin-containing antibiotic, with the addition of an aminoglycoside if synergy is documented to provide bactericidal and post-antibiotic effects. The aminoglycoside can be discontinued when cultures are sterile or there is improvement in clinical status. Infections due to ampicillin-resistant enterococci are treated with vancomycin without the addition of an aminoglycoside.

Since the majority, if not all, of coagulase-negative staphylococci isolates are resistant to  $\beta$ -lactam drugs including the penicillinase-resistant penicillins, vancomycin remains the drug of choice for proven infections. Instances with persistent coagulase-negative staphylococcal bacteraemia without a source, might benefit from the addition of rifampin. Linezolid and daptomycin are alternative therapies that should be reserved for treatment failure or resistance to first-line drugs.

For Gram-negative enteric bacteria, ampicillin (if susceptible) or an aminoglycoside is sufficient for treatment. However, if meningitis is suspected or confirmed, a third-generation or fourth-generation cephalosporin (eg, cefotaxime, ceftazidime, or cefepime if *Pseudomonas* spp coverage is needed) or carbapenem agent (eg, meropenem) should be used. Invasive infections due to extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Enterobacteriaceae* spp are best treated with a carbapenem treatment, although the use of cefepime could be considered. Treatment of infections caused by carbapenemase-producing *Enterobacteriaceae* spp requires infectious disease consultation; a carbapenem-containing regimen with colistin or high-dose tigecycline or an aminoglycoside may be needed.

Clindamycin, ampicillin–sulbactam, or metronidazole are appropriate for anaerobic infections; metronidazole is preferred for anaerobic infections that involve the CNS. The exact duration of antimicrobial therapy has insufficient supportive evidence; however, at a minimum, antibiotics should be continued until cultures are sterile and there is clinical recovery. This usually translates to a minimum of 7 days for bloodstream infections, 14 days for Gram-positive meningitis, and 21 days for Gram-negative meningitis. Vancomycin-resistant enterococci and vancomycin-insensitive *S aureus* are emerging pathogens resulting from the widespread use of vancomycin. Although vancomycin is frequently used by neonatal units where MRSA is endemic, its use can be reduced by limiting empirical therapy to neonates with severe infection possibly due to coagulase-negative staphylococci or MRSA, and by discontinuing therapy after 48 h when blood culture results are sterile. When susceptibility results are available and there is no evidence of CNS or endovascular involvement, clindamycin might be a suitable alternative for therapy of uncomplicated bacteraemia and skin and soft tissue infections attributed to MRSA in a neonate. Infants who have been exposed to antibiotics have been shown to have higher rates of necrotising enterocolitis, sepsis, and

morbidity than infants who have not been exposed to antibiotics, presumably due to intestinal dysbiosis induced by antibiotic exposure.<sup>60</sup>

Amphotericin deoxycholate remains the treatment of choice for invasive candidiasis when meningitis is a consideration; liposomal amphotericin or an echinocandin (caspofungin or micafungin) are options for hepatic or splenic candidiasis. Fluconazole might be an effective therapy for susceptible organisms. Successful treatment outcomes are dependent on the underlying condition of the host infant, duration of positive cultures, extent of disease, and ability to remove the source, if the infection is associated with central venous catheter access.

### Adjunctive therapies

Neutrophil storage pool depletion has been associated with poor prognosis and mortality in neonatal sepsis. Therapies that increase the number or improve the function of neutrophils have been studied, including granulocyte transfusions, granulocyte macrophage colony-stimulating factor (GM-CSF), G-CSF, and intravenous immune globulin (IVIG). Paradoxically, neonates with sepsis actually have high concentrations of circulating G-CSF and GM-CSF despite low neutrophil counts. Several studies have been unable to show a consistent beneficial effect of GM-CSF or G-CSF on mortality.<sup>54,55,61,62</sup> Moreover, timely administration of granulocyte transfusions is problematic, and there is insufficient time to screen potential donors. IVIG has been shown in a small case series to increase blood immature neutrophil counts, presumably from improved egress of neutrophils from the bone marrow. However, a study by the International Neonatal Immunology Study Group (INIS Collaborative Group)<sup>63</sup> and a Cochrane Review<sup>56</sup> involving over 7000 infants in total showed that IG-IV infusions do not have an effect on immediate morbidity or long-term mortality.

Pentoxifylline is an agent that improves microcirculation and decreases tumour necrosis factor  $\alpha$  concentrations associated with sepsis. Two randomised controlled trials in 140 neonates suggested that there were improved survival rates in infants who had culture-confirmed sepsis and received pentoxifylline.<sup>64</sup> Additional large-scale trials will be needed to reproduce this potential benefit. Several clinical trials relating to neonatal sepsis are ongoing.

### Conclusions and outstanding research questions

Although the burden of early-onset sepsis attributed to GBS has been reduced because of the widespread implementation of prenatal screening and administration of intrapartum antibiotics, missed opportunities for diagnosis and intervention still exist. The widespread use of antibiotic prophylaxis raises questions about the emergence of resistance among co-colonising organisms and continued active

surveillance will be important to monitor this concern. The significance of coagulase-negative staphylococci as colonising organisms versus pathogens in the neonate remains an important area of investigation, especially with concern for emergence of vancomycin resistance. The use of non-culture based diagnostics and sepsis scores to predict and diagnose septic neonates are areas of active investigation. The next frontier for antibiotic stewardship in the neonatal intensive care unit must be development of strategies to decrease antibiotic use and minimise adverse effects by a thorough study of duration of therapy. As knowledge of the neonatal microbiome emerges, the importance of minimising antibiotic exposure to decrease necrotising enterocolitis, as well as other sequelae such as asthma, obesity, inflammatory bowel disease, and neurological disorders is paramount. Although prevention of neonatal infections is the ultimate goal, maintenance of a pathogen-limited neonatal environment for premature infants remains a challenge as long-term vascular access and respiratory support is needed. Monitoring and assessing long-term outcomes of neonatal sepsis as neonates age remains a notable health-care challenge.

### Contributors

All authors contributed to the preparation of the manuscript, have reviewed and edited the content, and concur with submission and publication. ALS had full access to all of the information presented and had final responsibility for the decision to submit for publication.

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