Published in final edited form as:

Pediatr Crit Care Med. 2014 July ; 15(6): 523–528. doi:10.1097/PCC.0000000000000157.

Time for a neonatal-specific consensus definition for sepsis

James L. Wynn, MD^a, Hector R. Wong, MD^b, Thomas P. Shanley, MD^c, Matthew J. Bizzarro, MD^d, Lisa Saiman, MD^e, and Richard A. Polin, MD^f

^aVanderbilt University, Department of Pediatrics, Division of Neonatal-Perinatal Medicine

^bCincinnati Children's Medical Center, Division of Pediatric Critical Care Medicine; University of Cincinnati College of Medicine, Department of Pediatrics

^cUniversity of Michigan, Department of Pediatrics, Division of Pediatric Critical Care Medicine

^dYale University School of Medicine, Department of Pediatrics, Division of Neonatal-Perinatal Medicine

^eColumbia University Medical Center, Department of Pediatrics, Division of Infectious Diseases

^fColumbia University Medical Center, Department of Pediatrics, Division of Neonatal-Perinatal Medicine

Abstract

Objective—To review the accuracy of the pediatric consensus definition of sepsis in term neonates and to determine the definition of neonatal sepsis used.

Study selection—The review focused primarily on pediatric literature relevant to the topic of interest.

Conclusions—Neonatal sepsis is variably defined based on a number of clinical and laboratory criteria that make the study of this common and devastating condition very difficult. Diagnostic challenges and uncertain disease epidemiology necessarily result from a variable definition of disease. In 2005, intensivists caring for children recognized that as new drugs became available, children would be increasingly studied and thus, pediatric-specific consensus definitions were needed. Pediatric sepsis criteria are not accurate for term neonates and have not been examined in preterm neonates for whom the developmental stage influences aberrations associated with host immune response. Thus, specific consensus definitions for both term and preterm neonates are needed. Such definitions are critical for the interpretation of observational studies, future training of scientists and practitioners, and implementation of clinical trials in neonates.

Correspondence: James L. Wynn, MD, Vanderbilt University Medical Center, Pediatrics/Neonatology, 2215 B Garland Avenue, 1125 MRB4/Light Hall, Nashville, TN 37232-0656, Phone: 615.343.0206, james.wynn@vanderbilt.edu.

Financial Disclosure Statement for all authors: No author has any financial relationships that could be broadly relevant to the work.

Contributor's statements:

James L. Wynn: Dr. Wynn conceived the special article topic, wrote the first draft, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Hector R. Wong: Dr. Wong reviewed and revised the manuscript, and approved the final manuscript as submitted.

Thomas P. Shanley: Dr. Shanley reviewed and revised the manuscript, and approved the final manuscript as submitted.

Matthew J. Bizzarro: Dr. Bizzarro reviewed and revised the manuscript, and approved the final manuscript as submitted.

Lisa Saiman: Dr. Saiman reviewed and revised the manuscript, and approved the final manuscript as submitted.

Richard A. Polin: Dr. Polin reviewed and revised the manuscript, and approved the final manuscript as submitted.

Keywords

Newborn; neonate; term; preterm; sepsis; infection; SIRS; septic shock; definition; consensus

Statement of the problem

Neonatal sepsis results in death or major disability for 39% of those affected even with timely antimicrobial treatment¹. The incidence of severe sepsis in newborns *doubled* (from 4.5 to 9.7 cases per 1,000 births) between 1995 and 2005². The frequency of sepsis during the birth hospitalization varies inversely with gestational age at birth and may reach 60% in the most immature infants³. The short-term economic burden of caring for and hospitalizing these infected infants is staggering and is estimated at approximately \$700 million in the US⁴. Neither the treatment of neonatal sepsis, nor the neurodevelopmental outcomes in surviving infants has changed significantly over the last thirty years despite multiple failed attempts to reduce the burden of infection^{5,6}. These disappointments have occurred in the context of tremendous advances in other areas of newborn care including nutrition, management of respiratory distress and pulmonary hypertension, and therapeutic cooling following hypoxic-ischemic encephalopathy.

Adult and pediatric intensivists currently use consensus definitions for sepsis for goal-based therapeutic interventions^{7–10}. These definitions are critical to facilitate epidemiologic studies, to accurately determine disease incidence, to select patients for clinical trials, to improve training, and ultimately, to improve the delivery of care. The pediatric consensus definition for sepsis, established in 2005, was intended for all children (<18 years old) and including term (>37 weeks completed gestation) neonates (Supplemental Table 1)⁷. Preterm neonates (<37 weeks completed gestation) were specifically excluded from the pediatric consensus definitions and neonatal-perinatal subspecialists were not represented among the pediatric consensus experts. To investigate whether the pediatric consensus definitions for systemic inflammatory response syndrome (SIRS) and sepsis applied to term infants, Hofer et al retrospectively examined 476 term neonates and found that the consensus definitions applied to only 53% of cases of culture-positive early-onset sepsis (EOS)¹¹. The authors determined that the sensitivity of more comprehensive clinical and laboratory criteria to define SIRS in the setting of culture-proven EOS (n = 30) was 20% for hypothermia or fever, 43% for abnormal WBC and neutrophil indices, 87% for respiratory signs, and 33% for cardiovascular signs. To date, the accuracy of the pediatric consensus definitions has not been assessed in preterm infants, nor have consensus definitions been developed or tested in this unique developmentally immature population.

How has sepsis been defined in neonates?

There is remarkable heterogeneity among studies regarding the definition of neonatal sepsis (Supplemental Table 1). For example, in 12/42 (29%) selected studies/guidelines single or combinations of laboratory tests were incorporated into the definition of sepsis and included C-reactive protein (CRP) (n=5)^{12–16}, total WBC (n=4)^{7,12,17,18}, metabolic acidosis (n=3)^{12,18,19}, unspecified lab studies (n=2)^{20,21}, I/T ratio (n=3)^{7,13,18}, neutropenia (n=1)¹³, abnormal fibrinogen (n=1)¹², thrombocytopenia (n=1)¹², hyperglycemia (n=1)¹⁹ and

hypoglycemia (n=1)²². In many cases, there was additional variability among lab results defined as abnormal. Clinical findings were integrated in 26/42 (62%) of the selected studies/guidelines and included unspecified signs of sepsis (n=16)^{14,15,20,21,23–34}, cardiovascular signs [tachycardia/bradycardia, hypotension, poor perfusion (n=12)]^{7,12,13,17–19,22,30,32,35,36}, respiratory signs [apnea, cyanosis, tachypnea, need for ventilator, increased oxygen requirement (n=9)]^{1,7,12,13,17–19,22,35}, abnormal temperature [fever or hypothermia (n=7)]^{7,12,17–19,22,35}, central nervous system signs [lethargy, hypotonia, seizure (n=2)]^{12,22}, and feeding problems (n=1)¹². In some reports, neonatal sepsis was defined by the duration of antimicrobial treatment (at least 5 or more days)^{6,37–39}. Thus, there is an unmet need for the development of a consensus definition for sepsis in both preterm and term neonates to be used for future clinical studies.

The pediatric consensus definition of sepsis is SIRS in the presence of or as a result of suspected or proven infection. SIRS requires either 1) abnormal white count [total white blood cell (WBC) increased or decreased for age -or- >10% immature neutrophils] or 2) abnormal core temperature (>38.5° or <36°C)⁷. Because abnormal WBC indices or an abnormal core temperature are required for the definition of SIRS, we will focus on the predictive accuracy of these tests in term and preterm neonates.

Total leukocyte counts and neutrophil indices are inaccurate to define sepsis in term or preterm neonates

The complete blood count (CBC) with differential is commonly ordered with a sepsis evaluation in the NICU. Many factors can affect the WBC and differential counts including maternal hypertension, the method of delivery, the infant's sex, age in hours, and the method of blood sampling⁴⁰. The impact of gestational age on the range of normal WBC indices must also be taken into account. Following specific exclusion of infants with sepsis or suspected sepsis, Schmutz et al studied the range of ANC in >30,000 CBCs obtained within 72 hours of birth from infants of 23 to 42 weeks completed gestation including 852 infants with <28 weeks gestation⁴¹. The upper and lower limits for the ANC from birth to the 3rd day of life were 1500 to 41000/μL; from the 3rd day until the 10th day of life counts ranged from 1100 to 15300/μL.

Hornik et al retrospectively examined the utility of the CBC and WBC indices (total leukocyte counts, immature to total neutrophil (I/T) ratio and absolute neutrophil counts [ANC]) from >200,000 infants (including both preterm and term infants), and concluded no CBC parameter had sufficient utility for identifying infants with early-onset or late-onset sepsis^{42,43}. The area under receiver operating characteristics curves (AUC) was 0.668 for all individual indices including WBC, I/T, ANC, and platelet counts highlighting the limited utility of the CBC and in particular the WBC to identify infants with early-onset sepsis. A significant proportion of infants (14.4%) with LOS had normal CBC indices (WBC count 5000/mm³–19,000/mm³, ANC 1500/mm³, I/T ratio <0.2)⁴². In a study investigating the diagnostic value of waiting a period of time to obtain a CBC after birth to identify near-term (34–36 weeks completed gestation) and term infants with blood culture-positive sepsis Newman concluded, “...even when the CBC is optimally interpreted, decisions about

antibiotic treatment should remain highly dependent on maternal risk factors and newborn symptoms of infection”⁴⁰.

While pediatricians/neonatologists often reply upon a “shift to the left” to identify infected infants, the identification of band forms is problematic. Van der Meer et al examined the range of variance for band form neutrophils by surveying >750 technician responses from >100 laboratories in Europe⁴⁴. All observers received *identical* slides with 100 Giemsa-stained cells from a patient with sepsis. They reported wide intra- and inter-laboratory variation in interpretations for neutrophils (15–72%, SD 11%) and for band forms (4–64%, SD 11%). This study suggests the enormous variability of interpretation of the band count and thus the I/T ratio may limit use of the I/T ratio as a definition criterion.

Core temperature is inaccurate to define sepsis in term or preterm neonates

The use of an abnormal core temperature as a criterion for sepsis has significant limitations in preterm infants. Preterm neonates manifest a baseline lack of temperature maintenance due to physiologic immaturity that requires servo-controlled incubators. Thus, hypothermia may be secondary to inadequate provision of heat rather than sepsis. Among 395 symptomatic preterm infants with blood culture-positive late-onset sepsis (LOS), only 10.8% had temperature instability that occurred 24 hours before or after the first positive blood culture (positive predictive value 10.2%)³⁴. Neonates, particularly preterm neonates, rarely manifest fever greater than 38°C even with sepsis and septic shock, and are more likely to manifest temperature instability or hypothermia. Thus, an abnormal core temperature is a difficult criterion to interpret in the setting of neonatal SIRS/sepsis. Voora et al showed that among 10,092 term newborns, 1% (n=100) developed an axillary or rectal temperature 37.8°C within 4 days of birth and only 10% of those with a temperature 37.8°C (n=10) had a positive blood culture⁴⁵.

Interpretation of blood culture results in defining sepsis in term or preterm neonates (Type I and Type II errors)

When a potential pathogen grows from a blood culture, it is important to distinguish true infection from a potential contaminant (type I error). As many as 62% of extremely low birth weight infants who survive >12 hours after birth have a positive blood culture during their hospitalization³. Coagulase-negative *Staphylococci* (CoNS) represents greater than 50% of isolated bacteria from blood cultures of preterm infants in the United States and many centers worldwide^{16,46,47}. Management of a single blood culture positive for CoNS varies widely, from careful observation to aggressive treatment, which implies some clinicians may view it as a contaminant⁴⁸. The rate of false positive blood cultures (of which >67% are due to CoNS) is directly proportional to the age of the child and is as high as 17% in infants <12 weeks of age⁴⁹.

Although a positive culture is often considered the “gold standard” definition of infection, culture-negative, clinical sepsis is considered a real entity in all age groups. Furthermore, a pathogen may be identified in as little as 36–51% of cases of sepsis in adults^{50,51} even

though sepsis is defined as SIRS in response to an inciting infection. A similar rate of culture-negative sepsis is seen in pediatric patients even in the setting of shock¹⁸. In a study of newborns with unequivocal infection documented at autopsy, premortem blood cultures were negative in 14% of cases⁵². In another study of 92 neonates 34 weeks with culture-positive bacterial meningitis, 35 (38%) had negative blood cultures⁵³. When blood and other sterile site cultures are negative, but the infant manifests signs concerning for infection they are considered to have clinical sepsis. In neonates, this clinical scenario is far more common than blood-culture positive sepsis⁵⁴, and represented nearly 60% of the subjects enrolled in the recent International Neonatal Immunotherapy Study (INIS) that examined treatment of neonatal sepsis with intravenous immune globulin¹. Furthermore, neonates often have low colony count bacteremia and when an insufficient volume of blood is obtained for culture, the blood culture may be falsely negative (type II error)⁵⁵. Finally, the possibility of a non-bacterial cause of infection must also be considered. Fungal and viral infections may also generate a systemic inflammatory response and sepsis. There is increasing evidence for novel viral pathogens associated with sepsis-like syndrome in preterm infants (e.g., echovirus, enterovirus, parechovirus, coxsackie, adenovirus, parainfluenza, rhinovirus, coronavirus)^{56–59}.

How should we move forward?

We must first establish a consensus definition of neonatal sepsis to refine future observational studies and clinical trial designs. The limited diagnostic accuracy of common laboratory tests (including the WBC indices and acute phase reactants) for diagnosing neonatal sepsis is well described (Supplemental table 2) and has been reviewed in detail elsewhere^{60,61}. Improved diagnostic accuracy may come from novel microscale-based molecular methods including 16sRNA DNA PCR, genome-wide expression profiling, flow cytometry-based blood analysis, and proteomic approaches. For example, by first examining patients that met the consensus definition for pediatric septic shock, Wong et al used genome-wide expression profiling on whole blood to demonstrate multiple subclinical molecular endotypes⁶². Utilization of these robust methods within a definitive disease framework is highly likely to reveal markers that facilitate early identification of patients at high risk of poor outcomes and thus permit stratification risk for future clinical trials. However, in the absence of a consensus definition for sepsis, sensitive molecular diagnostic tests derived from subjects in observational studies where multiple sepsis definitions are used (Supplemental table 1) will only further amplify the heterogeneity of the populations and would thus be at risk of demonstrating poor diagnostic accuracy between clinical cohorts.

To complement classic and modern molecular laboratory testing, defining neonatal sepsis should include a severity of illness description. The PIRO model (predisposition, insult/infection, response, organ dysfunction⁶³) is one example of a severity of illness model that has been used to study sepsis in adults and identify patients at high risk of mortality^{64,65}. Examples of predisposition factors for sepsis in neonates would include maternal risk factors (e.g., preterm premature rupture of membranes or vaginal colonization with group B streptococcus⁶⁶) and neonatal risk factors (prematurity or need for invasive monitoring). Infection factors might include the type and antimicrobial resistance pattern of the pathogen

(bacteria, virus, or fungus-if identified) and the site of infection (e.g. blood, urine, CSF, trachea, multi-site or disseminated). Host response factors might encompass the inflammatory response (longitudinal plasma cytokines and acute phase reactants), severity and duration of clinical signs (e.g., tachypnea, increased oxygen requirement, apnea, tachycardia) and laboratory data such as neutropenia. Host response assessments may permit identification of temporal stages of sepsis pathology amenable to different treatment approaches. Organ dysfunction factors might include the requirement for inotropes, intubation for respiratory failure, oliguria, or coagulopathy. Importantly, no matter what criteria constitute the initial consensus definition for sepsis, revision and refinement of the definition should be expected to occur as new data is acquired.

Potential limitations of a consensus definition of sepsis

The present consensus definitions for sepsis in children and adults are threshold-based and thus static. A static definition of sepsis has potential advantages, but also has inherent limitations including the inability to incorporate change in status over time. For example, a patient may meet criteria (laboratory or clinical) for the definition of sepsis at one point in their disease process but not at another. Thus, a static definition of sepsis will likely be associated with limitations in diagnostic accuracy because sepsis is a dynamic, complex and heterogeneous condition⁶⁷. This challenging feature of sepsis, in addition to the biology/cutoff value used for biomarkers and variability of sepsis definitions between studies, likely contributes to the frequently limited positive predictive value but high negative predictive value seen for clinical signs and common laboratory tests used in neonates (Supplemental table 2). Although important for diagnostic accuracy, time is a difficult variable to integrate into the static decision to enroll a patient in a prospective interventional trial. Therefore, the variable of time (e.g. time of clinical presentation/biomarker measurement, duration of clinical and laboratory signs) should be collected in diagnostic studies and RCTs. Another potential limitation of a consensus definition is the background of the consensus members. A homogeneous consensus (e.g. all newborn critical care providers) is at risk of reflecting the bias of common training. Therefore, any expert consensus should have diversity among its members. Lastly, the consensus should only defer to expert opinion when data is not available. This last point illustrates the need to collect data for diagnostic and interventional studies in order to refine the criteria that constitute the definition of neonatal sepsis. Any useful definition would need to address these points and would need validation with outcomes especially in preterm neonates. A consensus definition is not meant to be the last word on how to define sepsis. Rather, the establishment of an accepted definition to be used by clinical researchers is but the first of many steps towards improving outcomes for neonates with sepsis. We have shown the present definition of neonatal sepsis is both *static and variable*; the combination of which we believe is entirely inadequate to allow progress.

Summary

Sepsis is a common and devastating problem in the NICU. Diagnostic and clinical interventional trials focused on neonatal sepsis are significantly hampered by use of a widely diverse definition for this disease. The first step toward addressing the poor outcomes is to reduce the variability in our definition of sepsis through the establishment of a consensus

definition. Though intended to facilitate study of this important disease in all children, the pediatric consensus definitions for sepsis are not accurate for term neonates and were not created for preterm neonates. Thus, consensus definitions for sepsis in term and preterm infants are needed so that future epidemiologic studies and trials of diagnostic and therapeutic interventions can be interpreted and implemented. Definitions are also needed to help clinicians in determining true sepsis and providing appropriate clinical care. It is therefore critical that a consensus conference be held to establish evidence-based neonate-specific sepsis definitions and to develop a research agenda to validate these definitions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding source: This project was done with no specific support.

The authors wish to thank Dr. Bill Walsh and Dr. Jeff Reese for their intellectual input.

Abbreviations used

NICU	Neonatal intensive care unit
SIRS	systemic inflammatory response syndrome
ACCP	American College of Chest Physicians
SCCM	Society of Critical Care Medicine
MODS	multiple organ dysfunction syndrome
WBC	white blood cell
EOS	early-onset sepsis
LOS	late-onset sepsis
ANC	absolute neutrophil count
I/T ratio	immature to total neutrophil ratio
CBC	complete blood count
VLBW	very low birth weight
CRP	C-reactive protein
APR	acute phase reactant
CoNS	coagulase-negative <i>Staphylococci</i>
SD	standard deviation
AUC	area under curve

References

1. Brocklehurst P, Farrell B, King A, et al. Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med*. 2011; 365(13):1201–1211. [PubMed: 21962214]
2. Hartman ME, Linde-Zwirble WT, Angus DC, Watson RS. Trends in the epidemiology of pediatric severe sepsis*. *Pediatr Crit Care Med*. 2013; 14(7):686–693. [PubMed: 23897242]
3. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics*. 2010; 126(3):443–456. [PubMed: 20732945]
4. Prabhudas M, Adkins B, Gans H, et al. Challenges in infant immunity: implications for responses to infection and vaccines. *Nat Immunol*. 2011; 12(3):189–194. [PubMed: 21321588]
5. Wynn JL, Neu J, Moldawer LL, Levy O. Potential of immunomodulatory agents for prevention and treatment of neonatal sepsis. *J Perinatol*. 2009; 29(2):79–88. [PubMed: 18769381]
6. Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *JAMA*. 2004; 292(19):2357–2365. [PubMed: 15547163]
7. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med*. 2005; 6(1):2–8. [PubMed: 15636651]
8. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest*. 1992; 101(6):1644–1655. [PubMed: 1303622]
9. Brierley J, Carcillo JA, Choong K, et al. Clinical practice parameters for hemodynamic support of pediatric and neonatal septic shock: 2007 update from the American College of Critical Care Medicine. *Crit Care Med*. 2009; 37(2):666–688. [PubMed: 19325359]
10. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Intensive Care Med*. 2008; 34(1):17–60. [PubMed: 18058085]
11. Hofer N, Zacharias E, Muller W, Resch B. Performance of the definitions of the systemic inflammatory response syndrome and sepsis in neonates. *J Perinat Med*. 2012; 40(5):587–590. [PubMed: 23120762]
12. Auriti C, Fiscarelli E, Ronchetti MP, et al. Procalcitonin in detecting neonatal nosocomial sepsis. *Arch Dis Child Fetal Neonatal Ed*. 2012; 97(5):F368–F370. [PubMed: 22933097]
13. Wu TW, Tabangin M, Kusano R, Ma Y, Ridsdale R, Akinbi H. The utility of serum hepcidin as a biomarker for late-onset neonatal sepsis. *J Pediatr*. 2013; 162(1):67–71. [PubMed: 22796049]
14. Kuhn P, Messer J, Paupe A, et al. A multicenter, randomized, placebo-controlled trial of prophylactic recombinant granulocyte-colony stimulating factor in preterm neonates with neutropenia. *J Pediatr*. 2009; 155(3):324–330. e321. [PubMed: 19467544]
15. Been JV, Rours IG, Kornelisse RF, et al. Histologic chorioamnionitis, fetal involvement, and antenatal steroids: effects on neonatal outcome in preterm infants. *Am J Obstet Gynecol*. 2009; 201587(6):e581–e588.
16. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics*. 2002; 110(2 Pt 1):285–291. [PubMed: 12165580]
17. Madan JC, Salari RC, Saxena D, et al. Gut microbial colonisation in premature neonates predicts neonatal sepsis. *Arch Dis Child Fetal Neonatal Ed*. 2012; 97(6):F456–F462. [PubMed: 22562869]
18. Wynn JL, Cvijanovich NZ, Allen GL, et al. The Influence of Developmental Age on the Early Transcriptomic Response of Children with Septic Shock. *Molecular Medicine*. 2011; 17(11–12):1146–1156. [PubMed: 21738952]
19. Ng PC, Ang IL, Chiu RW, et al. Host-response biomarkers for diagnosis of late-onset septicemia and necrotizing enterocolitis in preterm infants. *J Clin Invest*. 2010; 120(8):2989–3000. [PubMed: 20592468]

20. Manzoni P, Rinaldi M, Cattani S, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. *JAMA*. 2009; 302(13):1421–1428. [PubMed: 19809023]
21. Benitz WE, Han MY, Madan A, Ramachandra P. Serial serum C-reactive protein levels in the diagnosis of neonatal infection. *Pediatrics*. 1998; 102(4):E41. [PubMed: 9755278]
22. Ottolini MC, Lundgren K, Mirkinson LJ, Cason S, Ottolini MG. Utility of complete blood count and blood culture screening to diagnose neonatal sepsis in the asymptomatic at risk newborn. *Pediatr Infect Dis J*. 2003; 22(5):430–434. [PubMed: 12792384]
23. Dutta S, Reddy R, Sheikh S, Kalra J, Ray P, Narang A. Intrapartum antibiotics and risk factors for early onset sepsis. *Arch Dis Child Fetal Neonatal Ed*. 2010; 95(2):F99–F103. [PubMed: 19996327]
24. Chaaban H, Singh K, Huang J, Siryaporn E, Lim YP, Padbury JF. The role of inter-alpha inhibitor proteins in the diagnosis of neonatal sepsis. *J Pediatr*. 2009; 154(4):620–622. e621. [PubMed: 19324226]
25. Poindexter BB, Ehrenkranz RA, Stoll BJ, et al. Parenteral glutamine supplementation does not reduce the risk of mortality or late-onset sepsis in extremely low birth weight infants. *Pediatrics*. 2004; 113(5):1209–1215. [PubMed: 15121931]
26. Lin HC, Hsu CH, Chen HL, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics*. 2008; 122(4):693–700. [PubMed: 18829790]
27. Weisman LE, Stoll BJ, Kueser TJ, et al. Intravenous immune globulin therapy for early-onset sepsis in premature neonates. *J Pediatr*. 1992; 121(3):434–443. [PubMed: 1517923]
28. Fanaroff AA, Korones SB, Wright LL, et al. A controlled trial of intravenous immune globulin to reduce nosocomial infections in very-low-birth-weight infants. National Institute of Child Health and Human Development Neonatal Research Network. *N Engl J Med*. 1994; 330(16):1107–1113. [PubMed: 8133853]
29. Klinger G, Levy I, Sirota L, Boyko V, Lerner-Geva L, Reichman B. Outcome of early-onset sepsis in a national cohort of very low birth weight infants. *Pediatrics*. 2010; 125(4):e736–e740. [PubMed: 20231184]
30. Tita AT, Landon MB, Spong CY, et al. Timing of elective repeat cesarean delivery at term and neonatal outcomes. *N Engl J Med*. 2009; 360(2):111–120. [PubMed: 19129525]
31. Strunk T, Doherty D, Jacques A, et al. Histologic chorioamnionitis is associated with reduced risk of late-onset sepsis in preterm infants. *Pediatrics*. 2012; 129(1):e134–e141. [PubMed: 22157134]
32. Mercer BM, Crouse DT, Goldenberg RL, et al. The antibiotic treatment of PPRM study: systemic maternal and fetal markers and perinatal outcomes. *Am J Obstet Gynecol*. 2012; 206(2):145, e141–e149. [PubMed: 22000668]
33. Kingsmore SF, Kennedy N, Halliday HL, et al. Identification of diagnostic biomarkers for infection in premature neonates. *Mol Cell Proteomics*. 2008; 7(10):1863–1875. [PubMed: 18622029]
34. Fanaroff AA, Korones SB, Wright LL, et al. Incidence, presenting features, risk factors and significance of late onset septicemia in very low birth weight infants. The National Institute of Child Health and Human Development Neonatal Research Network. *Pediatr Infect Dis J*. 1998; 17(7):593–598. [PubMed: 9686724]
35. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control*. 2008; 36(5):309–332. [PubMed: 18538699]
36. Kermorvant-Duchemin E, Laborie S, Rabilloud M, Lapillonne A, Claris O. Outcome and prognostic factors in neonates with septic shock. *Pediatr Crit Care Med*. 2008; 9(2):186–191. [PubMed: 18477932]
37. Griffin MP, Lake DE, Moorman JR. Heart rate characteristics and laboratory tests in neonatal sepsis. *Pediatrics*. 2005; 115(4):937–941. [PubMed: 15805367]
38. Wynn JL, Hansen NI, Das A, et al. Early sepsis does not increase the risk of late sepsis in very low birth weight neonates. *J Pediatr*. 2013; 162(5):942–948. e943. [PubMed: 23295144]

39. Mitha A, Foix-L'Hélias L, Arnaud C, et al. Neonatal infection and 5-year neurodevelopmental outcome of very preterm infants. *Pediatrics*. 2013; 132(2):e372–e380. [PubMed: 23878051]
40. Newman TB, Puopolo KM, Wi S, Draper D, Escobar GJ. Interpreting complete blood counts soon after birth in newborns at risk for sepsis. *Pediatrics*. 2010; 126(5):903–909. [PubMed: 20974782]
41. Schmutz N, Henry E, Jopling J, Christensen RD. Expected ranges for blood neutrophil concentrations of neonates: the Manroe and Mouzinho charts revisited. *J Perinatol*. 2008; 28(4): 275–281. [PubMed: 18200025]
42. Hornik CP, Benjamin DK, Becker KC, et al. Use of the complete blood cell count in late-onset neonatal sepsis. *Pediatr Infect Dis J*. 2012; 31(8):803–807. [PubMed: 22531232]
43. Hornik CP, Benjamin DK, Becker KC, et al. Use of the complete blood cell count in early-onset neonatal sepsis. *Pediatr Infect Dis J*. 2012; 31(8):799–802. [PubMed: 22531231]
44. van der Meer W, van Gelder W, de Keijzer R, Willems H. Does the band cell survive the 21st century? *Eur J Haematol*. 2006; 76(3):251–254. [PubMed: 16412143]
45. Voora S, Srinivasan G, Lilien LD, Yeh TF, Pildes RS. Fever in full-term newborns in the first four days of life. *Pediatrics*. 1982; 69(1):40–44. [PubMed: 7033912]
46. Hornik CP, Fort P, Clark RH, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. *Early Hum Dev*. 2012; 88(Suppl 2):S69–S74. [PubMed: 22633519]
47. Cohen-Wolkowicz M, Moran C, Benjamin DK, et al. Early and late onset sepsis in late preterm infants. *Pediatr Infect Dis J*. 2009; 28(12):1052–1056. [PubMed: 19953725]
48. Rubin LG, Sanchez PJ, Siegel J, Levine G, Saiman L, Jarvis WR. Evaluation and treatment of neonates with suspected late-onset sepsis: a survey of neonatologists' practices. *Pediatrics*. 2002; 110(4):e42. [PubMed: 12359815]
49. Norberg A, Christopher NC, Ramundo ML, Bower JR, Berman SA. Contamination rates of blood cultures obtained by dedicated phlebotomy vs intravenous catheter. *JAMA*. 2003; 289(6):726–729. [PubMed: 12585951]
50. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med*. 2003; 348(16):1546–1554. [PubMed: 12700374]
51. Seymour CW, Iwashyna TJ, Cooke CR, Hough CL, Martin GS. Marital status and the epidemiology and outcomes of sepsis. *Chest*. 2010; 137(6):1289–1296. [PubMed: 20173054]
52. Squire E, Favara B, Todd J. Diagnosis of neonatal bacterial infection: hematologic and pathologic findings in fatal and nonfatal cases. *Pediatrics*. 1979; 64(1):60–64. [PubMed: 450562]
53. Garges HP, Moody MA, Cotten CM, et al. Neonatal meningitis: what is the correlation among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters? *Pediatrics*. 2006; 117(4):1094–1100. [PubMed: 16585303]
54. Lukacs SL, Schrag SJ. Clinical sepsis in neonates and young infants, United States, 1988–2006. *J Pediatr*. 2012; 160(6):960–965. e961. [PubMed: 22261508]
55. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP. Volume of blood required to detect common neonatal pathogens. *J Pediatr*. 1996; 129(2):275–278. [PubMed: 8765627]
56. Smit PM, Pronk SM, Kaandorp JC, et al. RT-PCR detection of respiratory pathogens in newborn children admitted to a neonatal medium care unit. *Pediatr Res*. 2013; 73(3):355–361. [PubMed: 23202720]
57. Kusuvara K, Saito M, Sasaki Y, et al. An echovirus type 18 outbreak in a neonatal intensive care unit. *Eur J Pediatr*. 2008; 167(5):587–589. [PubMed: 17593390]
58. Verboon-Maciolet MA, Krediet TG, Gerards LJ, Fleer A, van Loon TM. Clinical and epidemiologic characteristics of viral infections in a neonatal intensive care unit during a 12-year period. *Pediatr Infect Dis J*. 2005; 24(10):901–904. [PubMed: 16220089]
59. Civardi E, Tzialla C, Baldanti F, Strocchio L, Manzoni P, Stronati M. Viral outbreaks in neonatal intensive care units: What we do not know. *Am J Infect Control*. 2013; 41(10):854–856. [PubMed: 23623159]
60. Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. *Clin Perinatol*. 2010; 37(2):421–438. [PubMed: 20569816]

61. Srinivasan L, Harris MC. New technologies for the rapid diagnosis of neonatal sepsis. *Current opinion in pediatrics*. 2012; 24(2):165–171. [PubMed: 22273634]
62. Wong HR, Cvijanovich NZ, Allen GL, et al. Validation of a gene expression-based subclassification strategy for pediatric septic shock. *Critical care medicine*. 2011; 39(11):2511–2517. [PubMed: 21705885]
63. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Critical care medicine*. 2003; 31(4):1250–1256. [PubMed: 12682500]
64. Granja C, Pova P, Lobo C, Teixeira-Pinto A, Carneiro A, Costa-Pereira A. The predisposition, infection, response and organ failure (Piro) sepsis classification system: results of hospital mortality using a novel concept and methodological approach. *PLoS One*. 2013; 8(1):e53885. [PubMed: 23349756]
65. Howell MD, Talmor D, Schuetz P, Hunziker S, Jones AE, Shapiro NI. Proof of principle: the predisposition, infection, response, organ failure sepsis staging system. *Critical care medicine*. 2011; 39(2):322–327. [PubMed: 21099424]
66. Escobar GJ, Puopolo KM, Wi S, et al. Stratification of risk of early-onset sepsis in newborns ≥ 34 weeks' gestation. *Pediatrics*. 2014; 133(1):30–36. [PubMed: 24366992]
67. Lynn LA. The diagnosis of sepsis revisited - a challenge for young medical scientists in the 21st century. *Patient Saf Surg*. 2014; 8(1):1. [PubMed: 24383420]
68. Bassler D, Stoll BJ, Schmidt B, et al. Using a count of neonatal morbidities to predict poor outcome in extremely low birth weight infants: added role of neonatal infection. *Pediatrics*. 2009; 123(1):313–318. [PubMed: 19117897]
69. Weston EJ, Pondo T, Lewis MM, et al. The burden of invasive early-onset neonatal sepsis in the United States, 2005–2008. *Pediatr Infect Dis J*. 2011; 30(11):937–941. [PubMed: 21654548]
70. Bizzarro MJ, Raskind C, Baltimore RS, Gallagher PG. Seventy-five years of neonatal sepsis at Yale: 1928–2003. *Pediatrics*. 2005; 116(3):595–602. [PubMed: 16140698]
71. Escobar GJ, Li DK, Armstrong MA, et al. Neonatal sepsis workups in infants ≥ 2000 grams at birth: A population-based study. *Pediatrics*. 2000; 106(2 Pt 1):256–263. [PubMed: 10920148]
72. Vergnano S, Menson E, Kennea N, et al. Neonatal infections in England: the NeonIN surveillance network. *Arch Dis Child Fetal Neonatal Ed*. 2011; 96(1):F9–F14. [PubMed: 20876594]
73. Puopolo KM, Draper D, Wi S, et al. Estimating the probability of neonatal early-onset infection on the basis of maternal risk factors. *Pediatrics*. 2011; 128(5):e1155–e1163. [PubMed: 22025590]
74. Kudawla M, Dutta S, Narang A. Validation of a clinical score for the diagnosis of late onset neonatal septicemia in babies weighing 1000–2500 g. *J Trop Pediatr*. 2008; 54(1):66–69. [PubMed: 17698886]
75. Streimish I, Bizzarro M, Northrup V, et al. Neutrophil CD64 as a diagnostic marker in neonatal sepsis. *Pediatr Infect Dis J*. 2012; 31(7):777–781. [PubMed: 22481422]
76. Weirich E, Rabin RL, Maldonado Y, et al. Neutrophil CD11b expression as a diagnostic marker for early-onset neonatal infection. *J Pediatr*. 1998; 132(3 Pt 1):445–451. [PubMed: 9544899]
77. Lacaze-Masmonteil T, Rosychuk RJ, Robinson JL. Value of a single C-reactive protein measurement at 18 h of age. *Arch Dis Child Fetal Neonatal Ed*. 2014; 99(1):F76–F79. [PubMed: 24008814]
78. Franz AR, Bauer K, Schalk A, et al. Measurement of interleukin 8 in combination with C-reactive protein reduced unnecessary antibiotic therapy in newborn infants: a multicenter, randomized, controlled trial. *Pediatrics*. 2004; 114(1):1–8. [PubMed: 15231900]
79. Laborada G, Rego M, Jain A, et al. Diagnostic value of cytokines and C-reactive protein in the first 24 hours of neonatal sepsis. *Am J Perinatol*. 2003; 20(8):491–501. [PubMed: 14703598]