Biomarkers for the Diagnosis of Neonatal Sepsis

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KEYWORDS

• Biomarker • C-reactive protein • Complete blood count • Neonate • Procalcitonin • Sepsis

KEY POINTS

- Early, accurate diagnosis of neonatal sepsis improves time to effective therapy for infants with sepsis while minimizing antibiotic exposure in uninfected infants.
- An ideal biomarker for neonatal sepsis should become abnormal before clinical signs develop and have near-perfect sensitivity and a rapid turnaround time.
- At present, no biomarker (including complete blood count with differential, C-reactive protein, and procalcitonin) has sufficient sensitivity to preclude the need for empiric antibiotic treatment of infants with suspected sepsis.
- Existing biomarkers have mediocre specificity, which has contributed to unnecessary antibiotic therapy for infants with culture-negative sepsis.
- Research efforts in partnership with biomedical engineers may identify novel biomarkers, including ones that can be detected via noninvasive sensors.

INTRODUCTION

Neonatal sepsis remains a substantial cause of morbidity and mortality in the nursery setting.[1] Sepsis in neonates and young infants is challenging to diagnose, because infants manifest nonspecific clinical signs in response to sepsis (eg, respiratory distress, hypotension, apnea) that could indicate noninfectious conditions. Furthermore, time to antibiotics affects neonatal sepsis outcome; therefore, there are both clinical and compliance motivations for identifying and treating neonates with sepsis expeditiously.[2,3] As a result, clinicians commonly use serum biomarkers to measure inflammation and infection and assess the infant's risk of sepsis. This article reviews the current state of neonatal sepsis diagnostics, highlights the uses and limitations of

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current biomarkers, and discusses the characteristics and development pathway of a theoretic ideal biomarker for neonatal sepsis.

DEFINITIONS

Neonatal sepsis has been loosely defined as an infection of a sterile site (eg, blood, urine, cerebrospinal fluid) and clinical signs of illness. Infection in the first 72 hours of life is defined as early-onset sepsis (EOS) and is generally associated with perinatal risk factors such as intrauterine infection and inflammation (ie, chorioamnionitis), prolonged rupture of membranes, and maternal group B *Streptococcus* colonization.[4,5] For infants cared for in the nursery or neonatal intensive care unit (NICU) setting, infection beyond age 72 hours is defined as late-onset sepsis (LOS) and is associated with health care–associated transmission.[6] Infections among infants aged more than 72 hours who have been discharged is generally associated with community-acquired pathogens and is referred to by a variety of names, including invasive bacterial infection, fever without a source, and serious bacterial infection.[7,8] However, a discussion of sepsis diagnostics for young infants presenting to care from the community with suspected sepsis is beyond the scope of this article.

Sepsis in adults is defined as life-threatening organ dysfunction as a result of a dysregulated response to infection.[9] Since the early 1990s, the diagnosis of sepsis has been based on a group of clinical findings designed to measure organ dysfunction systemic inflammatory response syndrome (SIRS). SIRS criteria have poor sensitivity and specificity for neonatal sepsis. Coggins and colleagues[10] evaluated SIRS criteria in a case-control study of infants with LOS in their NICU; the sensitivity and specificity of SIRS criteria were 42% and 74%, respectively. Concerningly, most septic infants who developed organ dysfunction did not meet SIRS criteria at the time cultures were obtained. The current diagnostic definition, the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), mark an intentional shift away from SIRS.[9] The Sepsis-3 task force created the Sequential Organ Failure Assessment (SOFA). SOFA uses a variety of objective clinical components, including fraction of inspired oxygen; mean arterial pressure, including need for vasopressors; Glasgow coma scale score; need for mechanical ventilation; and bilirubin, platelet, and creatinine concentrations. The neonatal-specific SOFA score can be used to improve prediction of mortality in neonatal sepsis but is not intended as a diagnostic tool.[11,12] However, experts have called for specific consensus definitions of sepsis for preterm and term infants.[13]

CHALLENGES IN SEPSIS DIAGNOSTICS

The diagnosis of neonatal sepsis is difficult (**Fig. 1**). Distinguishing the individual components of sepsis from a dysregulated response to the infection is challenging. The 3 primary issues in neonatal sepsis diagnosis are (1) the myriad of clinical findings that mimic sepsis rather than represent it, and, as a direct result, (2) concern for falsely negative bacterial cultures, also known as culture-negative sepsis, and (3) the need to treat empirically for a minimum of 24 to 48 hours while cultures incubate. A further complication is that the initiation of antimicrobial therapy before cultures are obtained can sterilize subsequent cultures and decrease the opportunity for accurate diagnosis of sterile site infections.

Neonatal sepsis is rare; conditions that mimic sepsis are collectively common. For example, apnea, respiratory distress, and hypotension are all 10 to 100 times more common than EOS in very-low-birthweight (<1500 g) infants.[14] Transient tachypnea of the newborn, which also mimics EOS, is approximately 200 times more common

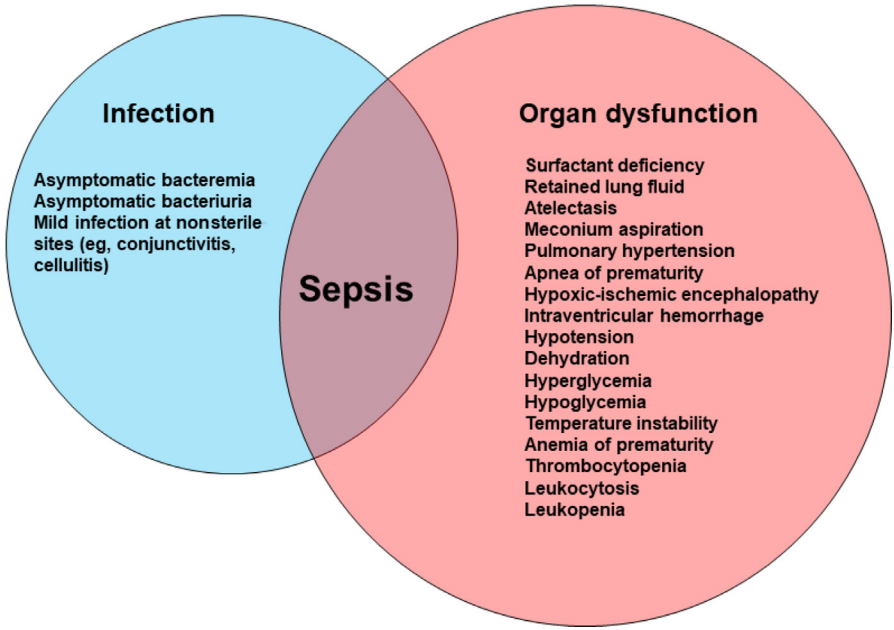

Fig. 1. Although a formal consensus definition of neonatal sepsis has not been developed, it is generally defined as an infection causing organ dysfunction because of a dysregulated response. As shown, there are many causes of organ dysfunction in infants, particularly those who are premature. Therefore, a careful evaluation for infection and organ dysfunction is indicated before assigning sepsis as the cause of these clinical signs.

than EOS (~60 per 1000 live births vs 0.3 per 1000 live births) and results in unnecessary evaluation and treatment of sepsis.[15,16] Respiratory distress syndrome affects most preterm infants, including virtually all infants less than 30 weeks' gestation, and is clinically and radiographically indistinguishable from pulmonary manifestations of EOS.[17] Clinicians appreciate the urgency of a neonatal sepsis diagnosis and institution of therapy, but identifying the 1 infant who is septic out of the many who have noninfectious presentations is challenging. Schulman and colleagues[18] evaluated antibiotic use in 116 California nurseries and found that the median number of infants treated for EOS for each proven case was 95 (range, 11–336). Antibiotic use for EOS did not correlate with the incidence of EOS at that center.[18] Similar studies in both well-baby nurseries and in NICUs have shown that antibiotic use does not correlate with infant or maternal risk factors.[19,20] Diagnostic subjectivity and inefficiency are major contributors to unnecessary antibiotic use in the nursery setting and have spurred efforts to develop objective sepsis biomarkers.[21]

Appropriately obtained bacterial cultures (usually blood culture alone for EOS and blood, urine, and cerebrospinal fluid for LOS) are the gold standard for the diagnosis of neonatal sepsis.[22] Cultures should be obtained before antibiotic therapy is initiated. Blood culture sensitivity is directly linked to volume; a weight-based approach is recommended by the American Academy of Pediatrics.[23,24] For most term neonates, this equates to 1 mL. The sensitivity of blood cultures approaches 100% if 1 mL of blood is obtained and the culture is processed correctly.[25–27] However, many clinicians caring for neonates with suspected sepsis incorrectly view the sensitivity of blood cultures as poor. In many situations, preanalytical issues such as inadequate blood volume or

contamination are responsible for the absence of detection of a pathogen or detection of an organism that is not considered to be a pathogen. This problem results in the far-too-common practices of either providing prolonged antibiotic therapy to infants with sterile cultures for culture-negative sepsis or treating a contaminant organism as a true pathogen.[28–30] These practices are unnecessary at best and harmful at worst, leading to increased dysbiosis, adverse short-term and long-term outcomes, and antimicrobial resistance.[31] A full discussion of culture-negative sepsis is beyond the scope of this article, but it is an important contributor to the need for better sepsis biomarkers.

Although the sensitivity of a properly obtained blood culture is excellent to detect bacterial causes of neonatal sepsis, these cultures require incubation for hours to days to detect growth.[32] Most clinical microbiology laboratories incubate blood cultures for 3 to 7 days.[33] Identification of the causative bacteria for neonatal sepsis can be achieved in more than 99% of patients by 36 hours in LOS, and recent data suggest that as little as 24 hours may be sufficient to detect pathogens associated with EOS.[34–38] However, even if empiric antibiotics are discontinued after 24 hours of administration, infants are still exposed to at least 1 dose of aminoglycosides and/or multiple doses of β-lactams or vancomycin. Even a single dose of antibiotic is capable of causing significant dysbiosis that can persist for months and cause increased susceptibility to infection and autoimmune disease.[39–41]

These real and perceived limitations to culture-based approaches have fueled interest in developing rapid, accurate biomarkers for neonatal sepsis. Jörn-Hendrik Weitkamp[42] has described the ideal neonatal sepsis biomarker as needing near-perfect sensitivity and a rapid turnaround time. This combination would allow clinicians to delay the initiation of antibiotics for infants with a negative biomarker test. However, at present there is no test for neonatal sepsis that meets the criteria of an ideal biomarker.

CURRENT BIOMARKERS

The most frequently used laboratory parameters as neonatal sepsis biomarkers include complete blood counts (CBCs) with differential, C-reactive protein (CRP), and procalcitonin. However, numerous other assays have been investigated and are discussed briefly later. In general, the laboratory tests currently in use as sepsis biomarkers for neonates and young infants share common characteristics. Most have reasonably good sensitivity and therefore reasonable negative predictive value (NPV). However, specificity and positive predictive value (PPV) are generally poor. These characteristics mean that normal results can be reassuring, but abnormal results are less meaningful because many inflammatory conditions can affect these values in the absence of neonatal sepsis, including maternal preeclampsia, chorioamnionitis, hypoxic-ischemic injury, and in utero growth restriction.[43–47] The negative sequelae of relying on biomarkers with poor PPV to direct therapy may mean that uninfected neonates with sterile cultures are subject to prolonged antibiotic exposure, for culture-negative sepsis.[48,49] In addition, the excellent NPV must be interpreted within the context of the relative rarity of EOS and LOS, and therefore a low pretest probability. Schulman and colleagues[18] showed an incidence of 1.1% for EOS and ~5% for LOS in infants evaluated for sepsis. Using these low pretest probabilities, even a coin flip has excellent NPV (exceeding 95%).[50] The authors are reminded of the classic *Simpsons* episode[51] in which Lisa Simpson teaches her father about such specious reasoning. Lisa picks up a rock and facetiously claims that it keeps tigers away. Homer asks how, and a frustrated Lisa explains that it does not actually work, being just a rock, but there are no tigers in the neighborhood! Homer considers this for a moment, and then tries to buy the rock from his exasperated daughter.

In order to safely delay the initiation of antibiotic therapy from an infant with suspected sepsis, clinicians need a fast, accurate test with a negligible number of false-negatives, not a meaningful number of false-negatives buried in an avalanche of true-negatives. However, many current biomarkers perform well because of the low pretest probability of sepsis and are, in fact, little better than Lisa Simpson's rock.

COMPLETE BLOOD COUNT

White blood cells and their differential count is the oldest biomarker for neonatal sepsis, predating the widespread use of automated blood culture systems. The original description of vacuolization of the neutrophil as a specific finding in neonatal sepsis was published in 1966.[52] The use of the CBC as an adjunctive test for neonatal sepsis has become fairly widespread; a national survey published in 2017 found that 95% of nurseries use a CBC as part of their sepsis evaluations.[53] The exact use of CBCs varies widely; approaches include obtaining CBC at a single time point or serially, evaluating the total white blood cell count, neutrophil count, immature-to-total ratio, temporal trends, and evaluation of red blood cell and platelet size and morphology in addition to leukocytes.[54–56] Receiver-operator curves can be generated for these different values. Specific findings of sepsis (>75%) include low absolute leukocyte counts, severe neutropenia, and increased immature-to-total ratio ($\geq$25%). However, specificity comes at the cost of poor sensitivity for EOS and LOS, where the CBC has less than 50% sensitivity.[57] In a large retrospective cohort using the Pediatrix administrative database, the highest area under the curve (AUC) Hornik and colleagues[58] could generate using different combinations of CBC values was 0.686, and most infants with EOS had normal CBCs. The investigators concluded that the poor sensitivity of CBCs makes them poor diagnostic markers, and that the practice of obtaining a CBC as part of a sepsis evaluation is not supported.[58]

C-REACTIVE PROTEIN

CRP, an acute phase reactant made in the liver in response to inflammatory cytokines, has attracted widespread and prolonged interest as a neonatal sepsis biomarker. Studies evaluating the CRP for the diagnosis of EOS have consistently reported sensitivities of 50% to 70% with unacceptably high false-positives.[59,60] CRP levels increase naturally over the first 1 to 2 days of life to levels that approach abnormal.[61] A meta-analysis by Brown and colleagues[62] of 22 studies evaluating the accuracy of CRP to detect LOS showed that, at median specificity (74%), the sensitivity of CRP was 62%. Together, these data show that CRP is not a useful tool in the diagnosis of neonatal sepsis.

PROCALCITONIN

Current evidence does not support the use of procalcitonin rather than CRP, because both have significant limitations of sensitivity and specificity. Retrospective studies suggest a range of sensitivity and specificity for procalcitonin that is on par with or slightly superior to CBC and CRP, in the 65% to 85% range.[60,63–67] Meta-analysis of 39 studies comparing procalcitonin with CRP for EOS and LOS found a slightly superior sensitivity (77% vs 66%) and no difference in specificity ($\sim$80%–82%) for procalcitonin compared with CRP. In the neonatal procalcitonin intervention study (NeoPInS), which investigated antibiotic use, Stocker and colleagues[68] showed an AUC of 0.921 for procalcitonin at age 36 hours, which was slightly inferior to the AUC of CRP in the same study. Meta-analyses of 17 studies and 1086 neonates

showed that, at the median sensitivity of 85%, the specificity of procalcitonin was 54%.[69] Despite initial excitement, the data do not support the use of procalcitonin as an ideal sepsis biomarker.

OTHERS

Numerous other serum biomarkers have been considered for the identification of neonatal sepsis.[70] These include, but are not limited to, interleukin-6,[71] presepsin,[72] cluster of differentiation (CD) 64,[73] CD11b,[74] serum amyloid A,[75] S100 protein A12,[76] lipopolysaccharide binding protein,[77] volatile organic compounds,[78] and soluble triggering receptor expressed on myeloid cell-1.[79] In addition, microbiome monitoring[80] and the application of mass spectroscopy to serum samples during sepsis evaluations[81] are novel approaches for biomarkers. Noninvasive biomarkers, such as heart rate characteristic (HRC) monitoring in preterm infants, have also been studied. In a randomized controlled trial of HRC monitoring for very-low-birthweight infants, Moorman and colleagues[82] saw a reduction in mortality following sepsis for infants receiving HRC monitoring compared with controls (10% vs 16.1%, absolute risk reduction of 6.1%, $P = .01$). A larger follow-up study showed a similar reduction in mortality among extremely low birthweight infants (<1000 g) receiving HRC monitoring.[83] This effect, presumably, is caused by HRC alerting clinicians about impending deterioration and improving time to sepsis evaluation and initiation of effective antimicrobial therapy.

Biosensing, in which a detector is used to directly sense the presence of 1 or more circulating biomolecules, is an exciting emerging field in sepsis diagnostics.[84] A variety of techniques, including electrochemical, optical, bioluminescent, and thermal, can be used to amplify and identify different circulating factors. Previous work has used biosensors to measure the biomarkers discussed earlier, such as CRP and interleukin-6.[85] However, more recent studies have turned toward direct identification of bacterial components such as lipopolysaccharide or bacterial ribosomal RNA.[86] Optical biosensors can measure a variety of physiologic changes via transcutaneous capture, including creatinine, bilirubin, or nitric oxide concentration.[87] If these technologies can be combined and optimized, it is possible that a wrist probe might be able to accurately detect an increased concentration in biomolecules caused by sepsis before clinical signs develop.

OPTIMAL CURRENT PRACTICE

In the absence of a fast, accurate sepsis biomarker with excellent sensitivity, how should clinicians approach infants with suspected sepsis? As endorsed by the American Academy of Pediatrics, the most effective current strategy for neonatal sepsis is the use of objective clinical risk factors to determine the pretest probability of sepsis, in combination with serial observation for equivocal or low-risk infants.[23] Several studies investigating the impact of observation-based instead of laboratory-based approaches have shown similar safety outcomes with reduced need for sepsis evaluations and antibiotic exposure. Cantoni and colleagues[88] performed a 2-year study in northeastern Italy that included 15,239 infants. In the first year, infants with 1 or more risk factors for EOS were evaluated with blood culture and CBC with differential. In the second year, infants were evaluated with serial physical examination alone and cultured only if clinical signs of illness developed. The investigators saw no difference in EOS incidence, time to antibiotic initiation, or mortality. However, they did see a 58% reduction in antibiotic use in the cohort evaluated during the second study year. A similar 4-year study from Norway evaluated a change in practice in which clinicians relied on serial physical examination and limited laboratory diagnostics and

found a 60% reduction in antibiotic use with no change in EOS incidence.[89] The most common tool to help guide objective risk assessment in term and late-preterm infants is the Neonatal Sepsis Calculator,[90] which has been validated in numerous studies and has been shown to reduce sepsis evaluations and unnecessary antibiotic use.[91–93] In addition, instead of using 0.3 to 0.5 mL of blood on imperfect and generally unhelpful biomarkers, providers should add that blood to the culture bottle in order to optimize volume. This method improves the sensitivity of the gold standard test and minimizes confusing or inaccurate biomarker results.

FUTURE RESEARCH

Imagine for a moment a future in which the ideal biomarker has been identified. This mythological test, the SeptiCheck (Cantey Fantasy Industries, San Antonio, TX) is a rapid point-of-care test that requires 0.3 mL of blood and results in a qualitative yes/no result within 10 minutes. It has 99.3% sensitivity and 99% specificity for neonatal sepsis. Infants with suspected sepsis who have a negative SeptiCheck can be observed closely; infants whose SeptiCheck is positive, which occurs infrequently because sepsis is rare and the excellent specificity of the test minimizes false-positives, cultures are obtained and the infant is started on empiric antibiotic therapy pending cultures. Sepsis evaluations and unnecessary antibiotic exposure plummet, and morbidity and mortality from proven sepsis decrease with faster time to initiation of antibiotic therapy.

How do clinicians get there from here? First and foremost, clinicians and researchers must not be discouraged by the middling utility of current sepsis biomarkers, but instead should continue the search for biomarkers that are informative. Translational studies that enroll neonates before they develop clinical illness will be needed to identify biomarkers with early, predictive kinetics: a test that becomes abnormal before clinical illness, peaks concomitantly with sepsis, and decreases with disease resolution. Novel approaches such as microbiome monitoring, mass spectroscopy, and others should be applied to well and sick neonates to generate novel biomarker targets for formal hypothesis testing. In addition, continued partnerships with engineers will be critical. The ideal biomarker, if it exists, cannot be found without collaboration with biomedical, electrical, chemical, and computer science engineers. The ultimate goal for clinicians is not to shorten antibiotic duration for infants with suspected sepsis but to have a rapid, sensitive test that supports initiation of antibiotics only when needed. Until that day comes, clinicians should reconsider how laboratory parameters can be optimally used in combination with the prenatal history and physical examination applying diagnostic and antimicrobial stewardship.

CLINICS CARE POINTS

- Early diagnosis of neonatal sepsis improves time to effective therapy, but current biomarkers are insufficiently sensitive or specific to be clinically useful.
- There is currently no consensus definition of neonatal sepsis, and extrapolation of sepsis criteria from older children or adults is highly inaccurate.
- The most evidence-based clinical role for current biomarkers (complete blood count, C-reactive protein, or procalcitonin) is to help providers discontinue empiric antibiotics at 24-48 hours when cultures are sterile.
- Abnormal biomarkers are not an indication to continue antibiotic therapy in a well-appearing infant whose cultures are sterile.

- Management guided by serial examination and objective risk factors is non-inferior to biomarker-based management and can reduce unnecessary blood draws and antibiotic exposure.

Best practices

What is the current practice for diagnosing neonatal sepsis?

- Neonatal sepsis is frequently suspected when infants have clinical signs consistent with sepsis, or for well-appearing infants with risk factors for sepsis.
- Bacterial cultures of blood (and urine, cerebrospinal fluid, or other sterile sites for late-onset sepsis) are the reference standard for neonatal sepsis.
- Serum biomarkers (e.g., complete blood count with differential, C-reactive protein, procalcitonin) are frequently obtained to help make the diagnosis of neonatal sepsis.
- Current biomarkers are insufficiently sensitive or specific to be consistently useful in the diagnosis of neonatal sepsis.
- The ideal biomarker would have near-perfect sensitivity, rapid turnaround time, and the ability to identify sepsis before clinical signs develop.
- Continued collaboration between clinicians, informaticists, and biomedical engineers is essential to develop novel biomarkers that meet these criteria.

What changes in current practice are likely to improve outcomes?

- Providing education for clinicians regarding the limitations of current sepsis biomarkers
- Re-emphasizing the importance of culture- or molecular-based technologies in the diagnosis of neonatal sepsis

Major recommendations

- The systematic use of current sepsis biomarkers should be discouraged; instead, that blood volume should be added to the blood culture to optimize sensitivity of the reference standard.
- Nurseries should have process improvement methods in place to ensure that adequate ($\geq$1 mL) blood volume is inoculated into culture media.
- Perform collaborative, exploratory studies that incorporate novel approaches such as mass spectrometry and other biomedical engineering approaches, aimed at identifying novel biomarkers that can be adapted for clinical use.

Rating for strength of the evidence: Quality of evidence moderate, strength of recommendation moderate.

DISCLOSURE

The authors have nothing to disclose.

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