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Diagnosis of Neonatal Sepsis: The Past, Present and Future

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Abstract

Sepsis remains a significant cause of neonatal mortality and morbidity, especially in low and middle-income countries. Neonatal sepsis presents with nonspecific signs and symptoms that necessitate tests to confirm the diagnosis. Early and accurate diagnosis of infection will improve clinical outcomes and decrease overuse of antibiotics. Current diagnostic methods rely on conventional culture methods, which is time-consuming and may delay critical therapeutic decisions. Nonculture-based techniques including molecular methods and mass spectrometry may overcome some of the limitations seen with culture-based techniques. Biomarkers including hematological indices, cell adhesion molecules, interleukins and acute phase reactants have been used for diagnosis of neonatal sepsis. In this review, we examine past and current microbiological techniques, hematological indices and inflammatory biomarkers that may aid sepsis diagnosis. The search for an ideal biomarker that has adequate diagnostic accuracy, early in sepsis is still ongoing. We discuss promising strategies for the future that are being developed and tested that may help us diagnose sepsis early and improve clinical outcomes.

Keywords

neonate; sepsis; diagnosis; biomarkers; molecular; culture; future

MESH terms:

Neonatal Sepsis; Anti-Bacterial Agents; Sepsis; Biomarkers; Early Diagnosis; Microbiological Techniques

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INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by non-specific signs and symptoms caused by invasion by pathogens^{1,2}. Sepsis is deemed culture-proven if confirmed by microbial growth on blood cultures or other sterile bodily fluids. Debate exists over the occurrence of culture-negative sepsis and whether antibiotics should be continued in culture-negative cases³. Sepsis is categorized as early-onset if diagnosed within the first 72 hours of life, which is due to perinatal risk factors, or late-onset if diagnosed after 72 hours and secondary to nosocomial risk factors. Neonatal sepsis is still a major cause of morbidity and mortality despite advances in neonatal medicine⁴. Incidence varies from 1–4 cases per 1000 live births in high-income countries but as high as 49–170 cases in low and middle-income countries with case fatality rate up to 24%^{5–8}. Survivors of neonatal sepsis are at increased risk for adverse neurodevelopmental outcomes including cerebral palsy, hearing loss, visual impairment and cognitive delays even in those whose cultures were negative but were treated with antibiotics^{9,10}.

The diagnosis of confirmed sepsis relies on conventional microbiologic culture techniques, which can be time-consuming¹¹. Despite the high sensitivity in detecting low bacterial loads (1–4 CFU/mL), many providers view negative blood cultures with skepticism when presented with a sick infant¹². The diagnosis “culture-negative” sepsis or ‘clinical sepsis’ has led to a 10-fold increase in antibiotic use in neonates with evidence of unintended harm including increased risk for necrotizing enterocolitis, fungal infections, bronchopulmonary dysplasia, and death¹².

Advances in rapid culture techniques, antibiotic stewardship, and bundled approaches to prevent central line associated bloodstream infections (CLABSIs) have reduced morbidity and mortality from neonatal sepsis^{13,14}. Newer molecular approaches and nonculture-based methods to assist in timely detection and accurate diagnosis of sepsis are needed. Current biomarkers and adjunct hematological indices used in routine clinical practice have limited value and are difficult to interpret due to low sensitivity and changing normal ranges during the neonatal period^{15,16}. An ideal marker should have sensitivity and negative predictive value (NPV) approaching 100%; specificity and positive predictive value (PPV) over 85%^{17,18}. None of the biomarkers or combination of biomarkers have adequate diagnostic accuracy to be used reliably in the diagnosis of neonatal sepsis¹⁹. We aim to review the past and current diagnostic modalities and present some insight on future diagnostic strategies in neonatal sepsis (Figure 1).

PATHOPHYSIOLOGY OF NEONATAL SEPSIS

Host immune responses including cytokines and chemokines during neonatal sepsis may aid in the diagnosis and/or assessing the severity of sepsis. A summary of the biomarkers associated with host immune pathways that change during sepsis is depicted in Figure 2. Paneth cells and intestinal lymphoid cells produce interleukin-17 (IL-17), which has a role in local defense and development of systemic inflammatory response syndrome²⁰. Respiratory epithelia secrete antimicrobial proteins and peptides including cathelicidin

and β -defensins²¹. Gram positive microorganisms and their cell wall lipoteichoic acid signal through TLR-2 receptors while gram negative microorganisms and their secreted lipopolysaccharide (LPS) signal through TLR-4 receptors²². These signaling cascades are associated with production of nuclear factor κ B (NF κ B) dependent inflammatory cytokines and chemokines. NOD-like receptors lead to production of IL-1 β and IL-18 by a protein complex called inflammasome²³. Activation of pathogen recognition receptors (PRR) results in generation of inflammatory mediators such as IL-1 β , IL-6, IL-8, IL-12, IL-18, interferon- γ (INF- γ) and tumor necrosis factor- α (TNF- α)²⁴. Proinflammatory cytokines activate endothelial cells leading to increased expression of cell adhesion molecules such as soluble intercellular adhesion molecules, selectins, angiopoietins, CD11b, CD18²⁵. Chemokines including CXCL10, CCL5 (RANTES), CCL3 and complement proteins such as, C3a, C5a cathelicidin and defensins are also stimulated by proinflammatory cytokines²⁶. Damage associated molecular patterns (DAMPs, alarmins) such as high mobility group box 1 (HMGB-1), uric acid are released from damaged cells and induce cytokine production, coagulation cascade and regulate polymorphonuclear cell function²⁷. Anti-inflammatory cytokines such as transforming growth factor- β (TGF- β), IL-4, IL-10, IL-11, IL-13) are expressed to control and balance inflammation²⁸. Acute phase reactants (APRs) such as C-reactive protein (CRP), procalcitonin (PCT), serum amyloid A (SAA) are produced predominantly in the liver in response to complement activation, PAMPs activity and proinflammatory cytokine secretion.

CURRENT METHODS TO DIAGNOSE NEONATAL SEPSIS

1. Microbiological culture methods

Conventional culture techniques remain the “gold standard” to confirm the diagnosis of neonatal sepsis. The introduction of automated systems that detect the presence of growth from bacterial CO₂ production has reduced the time to organism detection to 24–48h^{29,30}. Factors that may influence the recovery of pathogens from the blood include amount of blood volume obtained, timing of collection, and number of samples collected.

In neonates, the presence of low or intermittent bacteremia and maternal intrapartum antimicrobial exposure may decrease sensitivity of blood cultures^{12,31}. The delay in pathogen identification and antibiotic susceptibility testing increases exposure to broad-spectrum antibiotics, which may lead to bacterial antibiotic resistance and delay in targeted antimicrobial therapy^{9,32,33}. The volume of blood sampled for cultures is the single most important factor influencing the recovery of pathogens from blood cultures³⁴. However, collection of optimal blood volume can be difficult in extremely preterm infants and repeated phlebotomy may increase the risk of requiring blood transfusions. Schleonka et al reported that a blood culture volume of 1mL injected into pediatric blood culture bottles had excellent sensitivity even if organisms were present at very low concentrations (< 4 colony forming units (CFU)/mL)³¹.

The need for obtaining anaerobic cultures in neonates before commencing antibiotics is unclear³⁵. The overall incidence of clinically significant anaerobic isolates found in a neonatal population was 0.2% of all blood cultures performed³⁶. Previous studies showed that use of anaerobic blood cultures led to increased identification of both aerobic and

facultative anaerobic bacteria³⁷. Créixems et al reported that among 10,024 paired blood cultures (aerobic and anaerobic), 19% of patients with bacteremia would have been missed if aerobic cultures alone were used, not including the 3 strictly anaerobic infections identified³⁸. In contrast, Dunne et al found increased sensitivity in isolating aerobic and facultative anaerobic isolates from pediatric patients when 2 aerobic blood cultures were performed versus paired aerobic/anaerobic cultures³⁹. It is unclear whether treating anaerobes in routine sepsis management in neonates improves clinical outcomes.

2. Rapid testing methods from positive blood cultures

Several diagnostic systems have been developed for rapid identification of organisms found in positive blood cultures and provide faster turn-around times when compared to conventional methods (Table 2)⁴⁰. These FDA-cleared assays rapidly identify organisms growing in positive blood cultures but do not eliminate the time required for growth from these cultures. Peptide Nucleic Acid Fluorescent In Situ Hybridization Molecular Stains (PNA-FISH)⁴¹ is a well-validated method; the new QuickFISH system has reduced turnaround time to 20 minutes, enabling species identification results to be reported in the same time frame as Gram staining⁴². PCR-based methods, including GeneXpert (1 hour), FilmArray (1 hour), and Verigene (2.5 hours), are somewhat slower than QuickFISH but have little or no sample processing and include selected antibiotic resistance genes⁴⁰. Rapid assays are gradually becoming less labor intensive and has led to improved clinical outcomes, shorter hospital stays, and dramatically lower healthcare costs^{43,44}.

Recent advances in molecular techniques enable amplification of microbial pathogens directly from whole blood samples in under 12 h without relying on initial microbial growth in blood cultures (Table 2)⁴⁰. This provides the advantage of same-day identification and early targeted pathogen-specific antimicrobial therapy, especially in settings where there is pretreatment with antibiotics, low-density bacteremia, or where culture-negative sepsis is common. These molecular techniques predominantly rely on the amplification methods of polymerase chain reaction (PCR) for the bacterial 16S or 23S rRNA genes and the 18S rRNA gene of fungi. Diagnostic accuracy of systems such as SeptiFast, SepsiTst, and, most recently, detection of PCR amplified pathogen DNA from blood that is hybridized to capture probe-decorated nanoparticles detectable by a small portable T2 Magnetic Resonance (MR) platform have been reported^{45,46–48}. The Roche Light Cycler SeptiFast system requires 100 μ L blood and can detect 25 pathogens known to cause >90% of bloodstream infections, with a turnaround time of 6 hours. A competing commercial assay, SepsiTst is able to detect >300 pathogens, however, with a relatively slower turnaround time of 8–12 hours⁴⁶. The T2 MR is an automated nanoparticle-based PCR assay that can detect as few as 1 CFU/mL of *Candida* spp. in the blood in approximately 3 hours⁴⁶.

Some studies report a discordance between conventional culture and PCR methods during validation of molecular pathogen detection methods, which has led to continued uncertainty about the bacterial etiology of sepsis^{49,50}. Furthermore, false positive results were seen with high cycle thresholds thus opening the possibility for nonspecific amplification and raising the questions about whether the bacteria present was the cause for the sepsis syndrome⁵¹. A systematic review concluded that molecular diagnostics had value as adjunctive tests with

an overall sensitivity of 90% and specificity of 96%⁵². Molecular assays are not readily available, may be expensive and have modest diagnostic accuracy. Hence, molecular assays are not ready to replace blood cultures as reference standards but are useful as adjunctive tests in the diagnosis of neonatal sepsis.

3. Hematological Indices

Leukocyte (<5000 or $20000/\text{mm}^3$), absolute neutrophil (<1000 or $5000/\text{mm}^3$) and immature/total neutrophil counts (>0.2), and peripheral blood smear (toxic granulation, vacuolization and Dohle bodies) are traditionally used to aid the diagnosis of neonatal sepsis⁵³.

White blood cell count (WBC)—Leukocyte count starts between 6000 and $30000/\text{mm}^3$ in the first day of life and decreases to 5000 – $20000/\text{mm}^3$ later. Neutrophil count tends to be lower at lower gestational ages (GA) and peaks 6–8 h after birth⁵⁴. Clinical conditions such as maternal fever and hypertension, perinatal asphyxia, meconium aspiration syndrome, delivery route, intraventricular hemorrhage, hemolysis, pneumothorax, convulsion and even crying affects neutrophil count⁵⁵. A literature review by Sharma et al reported that leucopenia (WBC count $<5000/\text{mm}^3$) has a low sensitivity (29%) but high specificity (91%) for diagnosis of neonatal sepsis⁵⁶. Additional studies highlighted that leucopenia is more predictive of sepsis than leukocytosis (WBCs $>20,000/\text{mm}^3$) at more than 4 h⁵⁷. Neutrophil/lymphocyte (NLR) of 1.24 to 6.76 and platelet/lymphocyte (PLR) ratios of 57.7 to 94.05 may be diagnostic of neonatal sepsis^{58,59}.

Absolute Neutrophil Count (ANC)—Neutrophil counts are commonly evaluated in neonates with presumed sepsis but can be affected by maternal and infant risk factors^{54,55}. Neutropenia (ANC $<1,000/\text{mm}^3$ at 4 h) is considered more specific for early onset neonatal sepsis as opposed to neutrophilia (ANC $10,000/\text{mm}^3$)^{51,56,60}. Interpretation of ANC, however, must take into consideration the neonate's gestational and postnatal age as the lower limit of ANC decreases with lower GA. Furthermore, an analysis of 30,354 CBCs obtained in the first 72 h of life demonstrated that ANC peak later in early preterm neonates <28 weeks' gestation as compared with neonates 28 weeks' gestation (24 h of life vs 6–8 h, respectively)⁵⁴. Mean neutrophil volume >157 arbitrary units had sensitivity and specificity as 79% and 82% while sensitivity and specificity of CRP were 72% and 99%, respectively⁶¹. In 141 neonates with neonatal sepsis, cut-off level of delta neutrophil index (DNI) was calculated as 4.6 with 85% sensitivity and 80% while CRP had 81% sensitivity and 82% specificity⁶².

Immature to Total Neutrophil (I:T) Ratio—Compared to other hematological markers, I:T ratio may be the most sensitive indicator of neonatal sepsis⁶⁰, but this parameter also varies with GA and postnatal age. In healthy newborns, the I:T ratio peaks at 0.16 during the first 24 h and gradually declines over days. Gandhi et al., propose that I:T ratio >0.27 in term newborns and >0.22 in preterm neonates favor the diagnosis of neonatal sepsis⁶⁰. Murphy et al. demonstrated that two normal I:T ratios correlated with a sterile blood culture had a maximum NPV of 100%⁶³.

Red cell distribution width—Red cell distribution (RDW) width shows increased red blood cell production in inflammatory and infectious diseases. Elevated RDW has been shown to be associated with increased mortality from sepsis in both adult and neonates^{64,65}. In neonates, RDW was significantly higher in sepsis and among non-survivors⁶⁶. Cut-off levels as 16.3 and 19.5 had sensitivity (70–87%) and specificity (66.1–81%) in neonatal sepsis and gram-negative LOS, respectively^{64,67}.

Thrombocytopenia—Thrombocytopenia is associated with neonatal sepsis⁶⁸. Platelet volume increases while being more active and associated with cytokines and inflammatory mediators. A meta-analysis that included 11 studies and 932 patients, reported that MPV was higher in neonatal sepsis with a cut-off level between 8.6–11.4^{69–71}.

4. Inflammatory Biomarkers

Acute phase reactants—Acute phase reactants are produced by the liver in response to cytokines, which are induced by infection and tissue injury. TNF α , CRP, PCT, fibronectin, haptoglobin, pro-adrenomedullin (pro-ADM) and SAA have been evaluated in neonatal sepsis.

a. C-reactive Protein: C-reactive protein (CRP) has been the most studied biomarker¹⁶. Serum CRP concentrations rise within 10 to 12 hours in response to bacterial infections and peak after 36–48 hours, with concentrations that correlate with illness severity⁷². Due to the delay in elevation, it is unreliable for early diagnosis of neonatal sepsis (low sensitivity)¹⁵. Furthermore, other non-infectious maternal and neonatal conditions may also result in elevated CRP levels, thus making it a nonspecific biomarker^{72,73}. A systematic review of biomarkers for neonatal sepsis concluded that serial measurements of CRP at 24 to 48 hours after onset of symptoms has been shown to increase its sensitivity and negative predictive value and may be useful for monitoring response to treatment in infected neonates receiving antibiotics¹⁶. This suggests that CRP may be more useful for ruling out infection and discontinuing antibiotics when serial measurements are obtained.

Procalcitonin—Procalcitonin is synthesized in monocytes and hepatocytes as a prohormone of calcitonin in response to cytokine stimulation. After birth, it increases until postnatal day 2–4⁷⁴. PCT is downregulated by interferon- γ , a commonly produced cytokine in viral infections^{72,75,76}. Thus, PCT has emerged as a promising biomarker for the diagnosis of bacterial infections that may be useful in discriminating between bacterial and viral etiologies. After exposure to bacterial endotoxin, PCT levels rapidly rise within 2–4 hours and peak within 6–8 hours, thus making it a more sensitive marker than CRP for early diagnosis of neonatal sepsis⁷⁷. This increase often correlates with the severity of the disease and mortality. However, in early onset neonatal sepsis, PCT measurements at birth may initially be normal; a serial PCT measurement at 24 h of age may be more helpful for early diagnosis⁷⁸. Furthermore, serial PCT determinations allow to shorten the duration of antibiotic therapy in term and near-term infants with suspected early-onset sepsis⁷⁹. However, before this PCT-guided strategy can be recommended, its safety and reliability must be confirmed in a larger cohort of neonates.

In a meta-analysis with 1959 patients, sensitivity and specificity of PCT were reported to be 81% (95% CI: 74–87%) and 79% (95% CI: 69–87%), respectively⁸⁰. Studies in the meta-analysis used different cut-off thresholds (0.8–2.4 µg/L). Positive and negative likelihood ratios (PLR and NLR) were 7.7 and 0.11 for LOS while 3.2 and 0.3 for EOS indicating that diagnostic accuracy is better in LOS⁸¹. Cord blood PCT >0.7 µg/L in the diagnosis of sepsis showed 69% sensitivity and 70% specificity and PCT has been used in combination with other biomarkers in EOS⁸². Canpolat et al. reported that PCT (>1.74 ng/ml) and CRP (>0.72 mg/dL) had 76% and 58% sensitivity and 58% and 85% specificity respectively on the 3rd day of life in neonates with preterm premature rupture of membranes⁸³. Eschborn et al. evaluated 29 studies comparing PCT with CRP and found that mean sensitivity for EOS, LOS and EOS+LOS was 73.6%, 88.9% and 76.5% for PCT; 65.6%, 77.4% and 66.4% for CRP while mean specificity for EOS, LOS and EOS+LOS was 82.8%, 75.6% and 80.4% for PCT; 82.7%, 81.7% and 91.3% for CRP, respectively⁷². Authors concluded that performance of both biomarkers will be better with serial measurements, and correlation with clinical findings is needed for decision making.

Serum Amyloid A—Serum Amyloid A (SAA) is another acute phase reactant synthesized by hepatocytes, monocytes, endothelial and smooth muscle cells in 8–24 h after bacterial exposure and is regulated by proinflammatory cytokines. SAA levels increase with age, with the lowest levels seen in umbilical cord blood and highest levels seen in the old age⁸⁴. In response to infection or injury, SAA levels rapidly increase up to 1000 times higher than baseline but can be significantly influenced by the patient's hepatic function and nutritional status⁸⁵. In a study by Arnon et al, when compared with healthy infants at 0, 8, and 24 hours, SAA levels in septic infants were significantly higher ($p < .01$) at all time points⁵³. When compared with CRP, SAA had an overall better diagnostic accuracy for predicting EOS. Cetinkaya et al. also determined that SAA concentrations had better sensitivity and area under the curve when compared with CRP and PCT, though the difference was not statistically significant⁸⁶. Different cut-off points between 1–68 mg/L were reported with a pooled 78% sensitivity and 92% specificity⁸⁷.

Proadrenomedullin is a stable precursor of ADM, which modulates circulation, has antimicrobial properties and protects against organ damage⁸⁸. High sensitivity (86.8%), specificity (100%), PPV (100%) and NPV (83.9%) with a cut-off value 3.9 nmol/L of pro-ADM were observed in 76 neonates with neonatal sepsis⁸⁹. Higher pro-ADM levels were associated with increased sepsis severity and mortality⁹⁰.

Adipokines are released from adipose tissue and may initiate secretion of inflammatory and anti-inflammatory cytokines. Visfatin (>10 ng/mL) and resistin (>8 ng/mL) had sensitivity and specificity over 90% in 62 septic neonates⁹¹. Subsequent studies reported lower sensitivity and specificity for resistin, but levels were positively correlated with IL-6 and CRP^{92,93}. Hecpudin, progranulin, stromal cell-derived factor 1, endocan and pentraxin-3 are less studied APRs which have a role in inflammation, chemoattraction, complement activation, angiogenesis and future studies are needed to evaluate diagnostic accuracy of these markers^{94–98}

Vascular Endothelium—Vascular endothelium interacts with leukocytes, soluble mediators, PAMPs and DAMPs, which have a role in sepsis pathogenesis. E-selectin, L-selectin, sICAM-1 and sVCAM-1 and angiopoietin 1–2 were studied in diagnosis of neonatal sepsis⁹⁹. But limitation of these markers includes no normative data in neonates, physiological increase in the first month of life and lack of large studies.

Interleukins—IL-6 increases immediately after exposure to pathogens and normalizes in 24 h¹⁰⁰. IL6 has a proinflammatory effect inducing CRP, fibronectin and SAA release from liver, T cell differentiation and B cell maturation¹⁰¹. IL-6 has been studied more than other cytokines and found to be increased in neonates with EOS and LOS, and various cut-off levels between 18 and 300 pg/mL were reported in 31 studies with 1448 septic neonates¹⁰². The pooled sensitivity and specificity of IL-6 were 88% and 82% while PLR and NLR were 7.03 and 0.2, respectively. Combination of IL-6 with other markers such as CRP, pro-ADM, PCT showed better diagnostic accuracy^{19,89,103}.

Cortes et al. evaluated diagnostic accuracy of IL-6 and CRP in EOS and LOS¹⁰⁴. Authors concluded that IL-6 (>17.75 pg/mL) showed greater accuracy in EOS while CRP (>0.53 mg/dL) was more accurate in LOS. Kurul et al. showed that IL-6 (>580 pg/mL) and PCT (>0.94 ng/mL) were associated with 7-day mortality while CRP was not¹⁰⁵.

Ye et al. evaluated utility of cytokines in 420 neonates with neonatal sepsis¹⁰⁶. Interleukin-2, IL-4, IL-6, IL-10, TNF- α and INF- γ were measured and compared with CRP. Interleukin-6 (>12.5 pg/mL) and IL-6/IL-10 ratio (>3.5) were found as valuable as CRP while most sensitive and specific ILs were IL-6 (94.1%) and IL-6/IL-10 ratio (100%), respectively. Celik et al. observed that a cut-off level of 202 pg/mL for IL-6 differentiated gram negative (n=73) from gram positive (n=82) sepsis with 68% sensitivity and 58% specificity¹⁰⁷. In a later study, IL-6 (>400 pg/mL) alone or combination with TNF- α (>32 pg/mL), IL-8 (>200 pg/mL), G-CSF (>1000 pg/mL) had 100% sensitivity, specificity, NPV and 38 to 69% PPV to differentiate gram negative neonatal sepsis¹⁰⁸.

IL-8 is another proinflammatory cytokine promoting chemotaxis and activation of granulocytes and increases within 1–3 h with a half-life <4 h. Diagnostic accuracy was evaluated in a meta-analysis with 8 studies, 548 neonates (cut-off levels between 0.65 and 300 pg/mL), which reported a pooled sensitivity and specificity of 78% and 84% similar to CRP¹⁰⁹.

TNF- α is secreted from natural killer cells by IL-2 to induce T cell proliferation, vasodilatation and neutrophil adhesion¹¹⁰. In a systematic review, (where TNF- α cut-off values was ranged from 1.7 to 70 pg/mL) at a mean cut-off value of 18.94 pg/mL, the sensitivity was 79% and specificity was 81% and better accuracy in LOS than EOS¹¹¹. Meta-analyses of data from neonates show variable sensitivity and specificity for of IL-6, IL-8, and TNF- α with only moderate accuracy in diagnosing neonatal sepsis^{16,109,111}. However, when combined with other cytokines or late proinflammatory markers, such as CRP, sensitivity and specificity increase^{106,112,113}. Currently, measuring cytokines for diagnosis of neonatal sepsis may not be practical or cost-effective because enzyme immunoassays are expensive and time consuming.

CELL ADHESION MOLECULES

Leukocyte antigens are upregulated after bacterial exposure and can be quantified by flow cytometry^{114,115}. These markers increase in minutes after infection and levels were not affected by GA, timing of sepsis onset, type of microorganism or non-infectious diseases^{116,117}. Limitation of these markers are need of high technology and non-standardized normal ranges.

Cluster differentiation molecule-64 (CD64) expressed from neutrophils and monocytes facilitates phagocytosis and intracellular killing of opsonized microorganisms. Increased levels can be detected in 1 hour and stable for 24 hours. Shi et al. performed a meta-analysis of CD64 levels from 17 studies including 3478 neonates and found that pooled sensitivity, specificity, PLR and NLR were 77%, 74%, 3.58 and 0.29, respectively¹¹⁸. Serial measurements and combination with other markers have been reported with varying diagnostic accuracy^{119,120}. Increased CD11b expression was found both in EOS and LOS with high sensitivity and specificity up to 100%¹¹². In a recent meta-analysis including 9 studies with 843 neonates showed that CD11b is a promising biomarker with sensitivity, specificity, PLR and NLR as 82%, 93%, 11.51 and 0.19, respectively¹²¹.

Soluble CD14 fragment (presepsin) is a specific and high affinity receptor complexes of lipopolysaccharides and activates TLR to proinflammatory cytokine secretion. Both meta-analysis revealed that presepsin was as accurate as PCT and CRP in the diagnosis of neonatal sepsis^{77,122}.

Gram negative infections lead to higher sCD14 levels¹²³. Cord blood presepsin levels were evaluated in 288 preterm infants with premature rupture of membranes for EOS and a cut-off level 1370 pg/mL yielded a odds ratio of 12.6 (95% CL 2.5–28.1)¹²⁴. Presepsin, PCT, IL-6 and IL-8 were compared in diagnosis of EOS and presepsin was found as the most accurate biomarker with 88.9% sensitivity and 85.7% specificity¹²⁵.

Soluble triggering receptor expressed on myeloid cells-1 (sTREM1) regulates the innate immune system and inflammation by promoting the release of proinflammatory cytokines. Increased levels were found in neonatal sepsis with a cut-off value of 310 pg/mL although higher levels were reported in culture proven sepsis¹²⁶. Urine sTREM-1 >78.5 pg/mL had 90% sensitivity, 78% specificity, 68% PPV and 94% NPV in 62 neonates with sepsis, respectively¹²⁷. A meta-analysis including 8 studies with 667 neonates reported that sensitivity and specificity of sTREM-1 were 95% and 87%, respectively¹²⁸. Limitations include small number of studies and different cut-off levels between 77.5 and 1707 pg/mL¹²⁸.

The challenge of biomarker identification is reflected by the fact that over 3000 sepsis biomarker studies have been published with almost 200 candidate biomarkers evaluated¹²⁹. However, there is not a single biomarker that has sufficient diagnostic accuracy for diagnosis of neonatal sepsis. Combination of biomarkers or their serial measurements may be strategies to enhance diagnostic accuracy. Combination of IL-6, sTREM-1, and PCT has been suggested, as each biomarker represents a different component in the pathophysiology of sepsis¹³⁰. Others propose that early- and mid-phase markers such as neutrophil CD64

and procalcitonin should be combined with the late-phase biomarker CRP for maximal diagnostic benefit⁴⁰. A recent literature review summarizes the utility of combining both early and late biomarkers for neonatal sepsis¹³⁰.

5. Strategies for the future

Mass spectrometry for identification of pathogens from blood culture

specimens—Matrix-assisted laser desorption-ionization/time-of-flight (MALDI-TOF) mass spectroscopy is a relatively new approach that can identify microorganisms within 30 min after blood culture positivity¹³¹. Meta-analyses have found that use of MALDI-TOF for diagnosis of infection from culture bottles has acceptable sensitivity and specificity¹³² and with higher sensitivity in gram negative infections compared to gram-positive infections¹³³.

Point-of-care devices for diagnosis of neonatal sepsis—Rapid tests done at the bedside that could confirm diagnosis or provide prognostic information have the potential to improve patient outcomes and decrease healthcare costs (Figure 3). Novel techniques such as analysis of volatile organic compounds in the breath has been demonstrated to be reasonably sensitive and specific¹³⁴ and capable of distinguishing sepsis from inflammation in rat models¹³⁵, yet to be validated in human studies. Point-of-care (POC) devices using a variety of biomarkers including blood plasma protein quantification and leukocyte monitoring are being evaluated for the diagnosis of sepsis¹³⁶.

Omics technologies and personalized medicine—Omics technologies provide data on genome-wide gene expression, protein translation and metabolite production that are differentially regulated in neonatal sepsis^{137,138}. Proteomics measures protein components released after infection or inflammation. Cord blood and amniotic fluid proteomics have provided information regarding the fetal response to intra-amniotic inflammation and have successfully predicted EOS with >92% accuracy^{139,140}. Proteomics including neutrophil defensin 1–2, cathelicidin, S100A12, S100A8, pro-apolipoprotein C2, apolipoprotein A-E-H, β -2 microglobulin, haptoglobin, desarginin from amniotic fluid, cord blood, plasma were found to be valuable in diagnosis of EOS and LOS^{141–143}.

Metabolomics by nuclear magnetic resonance imaging (NMR) and mass spectrometry (GC-MS) has also been investigated in adult sepsis with favorable results¹⁴⁴. Urinary metabolomics profile of adult pneumococcal pneumonia, for example, has been found to be distinctly different from viral and other bacterial causes of pneumonia¹⁴⁵. This indicates that evaluation of urinary metabolite profiles may be useful for effective diagnosis and lead to faster targeted antibiotic treatment. Urine samples of neonates with sepsis were evaluated with H-NMR and GC-MS showed increase in glucose, maltose, lactate, acetate, ketone bodies, D-serine and also normalization of variations with treatment¹⁴⁶.

A prospective observational study comparing genome-wide expression profiles of 17 VLBW infants with bacterial sepsis identified distinct clusters of gene expression patterns in gram-positive and gram-negative sepsis when compared with controls¹⁴⁷. Genomic analysis may determine sepsis risk, treatment response and prognosis while evaluating gene variants responsible for PRPs, signaling molecules and cytokines^{143,148}.

Machine Learning—Machine learning and artificial intelligence are increasingly used to sort transcriptomic, proteomic and metabolomic data for biomarker screening, developing prognostication models and for identifying the right patients for specific therapies (personalized medicine). One example is the Pediatric Sepsis Biomarker Risk Model (PERSEVERE), which was developed and validated as a prognostic enrichment tool for pediatric septic shock and in predicting mortality^{138,149}. Ongoing research is investigating the application of the PERSEVERE model in neonatal sepsis prognostication¹⁵⁰.

Reduced heart rate variability and transient decelerations were detected in hours to days before diagnosis of sepsis^{151,152}. In these studies, early diagnosis of sepsis and reduced mortality has been reported. Recently predictive models using machine learning were developed. These models use the vital signs, clinical and laboratory features of patients. Mithal et al. calculated a triggering score 5 by using heart rate, respiratory rate, temperature, desaturation and bradycardia events. Authors found that LOS was diagnosed 43.1±79 h before culture positivity with 81% sensitivity, 80% specificity, 57% PPV and 93% NPV in 72 patients¹⁵³. Clinical findings such as birth weight, gender, catheter use and laboratory findings such as blood gas parameters, CBC were also integrated into prediction models and found valuable in diagnosis of sepsis^{154,155}.

New Genetic Techniques—Non-coding RNAs (transcriptomics) including microRNAs (miRNA), circular RNAs (circRNAs) regulate many cell signaling pathways including cell proliferation, differentiation, development, metabolism, apoptosis and proinflammatory cytokine production¹⁵⁶. Both increased (miRNA 15-16a-23b-451) and decreased (miRNA 25-129-132-181a-223) expression were reported while 80–89% sensitivity and 79–98% specificity were found in diagnosis of neonatal sepsis^{157,158}. Exosomes and neutrophil extracellular traps (NETs) released during inflammation may be therapeutic targets in the future.

Conclusions—Identification of an ideal biomarker to diagnose neonatal sepsis is still the holy grail but advances in technology have given us a glimpse of the promising tests for the future. Inflammatory markers such as CRP and PCT as well as other hematological indices used currently have limited value in neonates. Serial measurements of an ideal combination of biomarkers have shown to increase diagnostic accuracy but remain expensive and cumbersome for clinical practice. Molecular diagnostic tools such as PCR and sequencing, and mass spectrometry offer promise for more rapid and sensitive detection of disease. Omics technology and machine learning may provide us diagnostic and prognostic models that could be personalized for the future.

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Impact

1. Reviews the clinical relevance of currently available diagnostic tests for sepsis
2. Summarizes the diagnostic accuracy of novel biomarkers for neonatal sepsis
3. Outlines future strategies including the use of omics technology, personalized medicine and point of care tests.

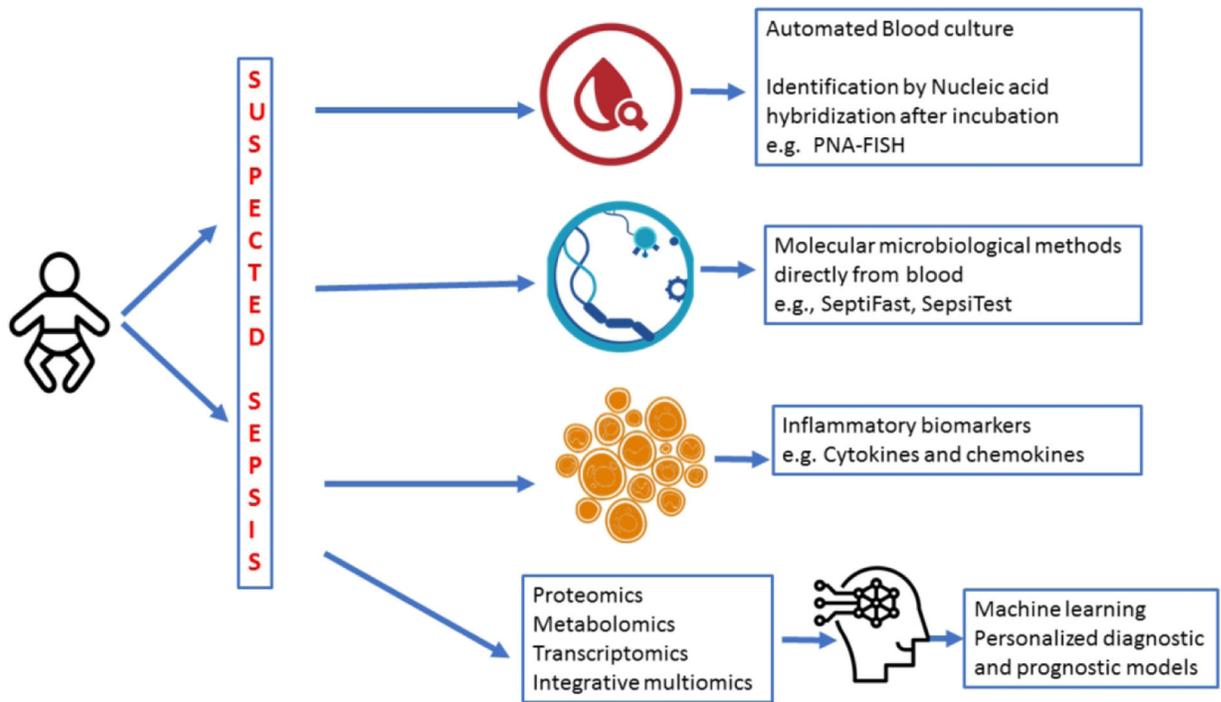


Figure 1: A Schematic on the categories of diagnostic tests available for neonatal sepsis
 Traditional methods of blood cultures have changed to automated blood culture monitoring for bacterial growth by CO₂ detection. Newer tests involve rapidly identifying organisms from positive cultures by fluorescent in situ hybridization techniques. Molecular microbiological diagnostics using PCR for bacterial and fungal genes can be applied directly to blood specimens. Inflammatory biomarkers including CRP, procalcitonin and cytokines are another category of adjunctive diagnostic tests. Multiomic technology enables us to scour genome wide gene expression, protein and metabolites for developing diagnostic tests and prognostic models.

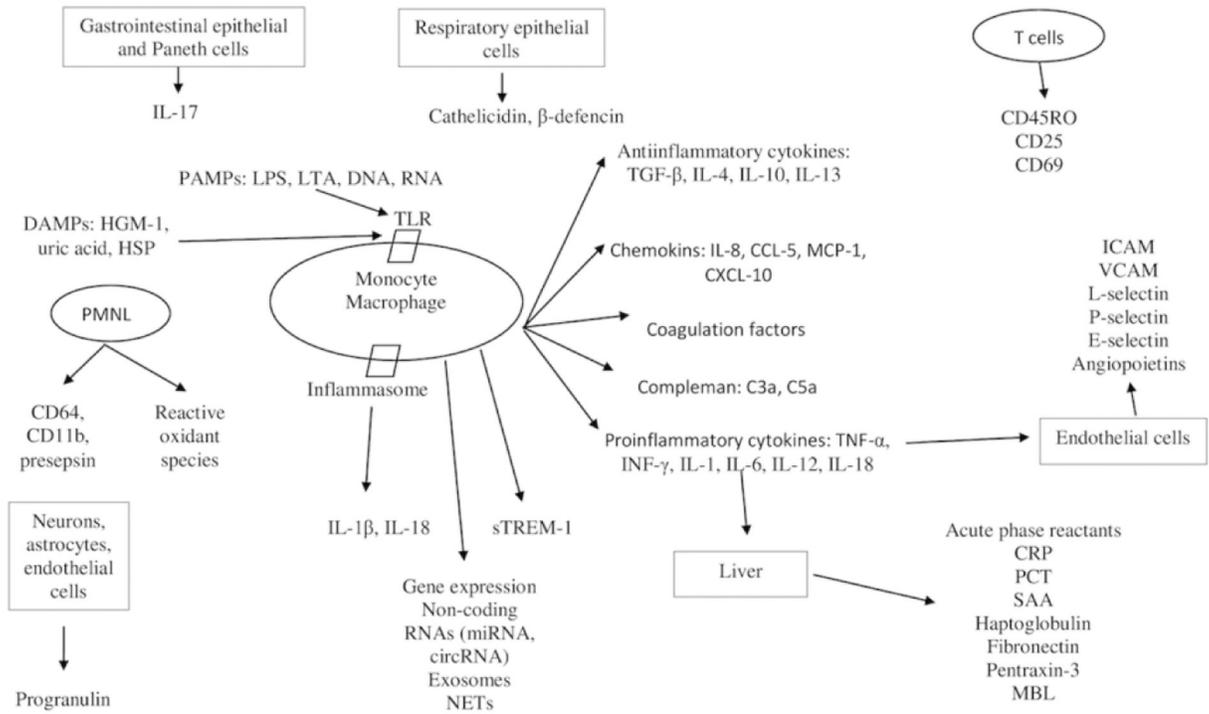


Figure 2. The relationship between host immunity and biomarkers

CD, cluster of differentiation; sTREM-1, soluble triggering receptor expressed on myeloid cells-1; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; DAMPs, damage associated molecular patterns; HGM-1, high mobility group box 1; LPS, lipopolysaccharide; LTA, lipoteichoic acid; NETs, neutrophil extracellular traps; TLR, toll-like receptor; HSP, Heat shock protein; TNF- α , tumor necrosis factor- α ; INF- γ , interferon- γ ; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; CXCL-10, chemokine ligand-10

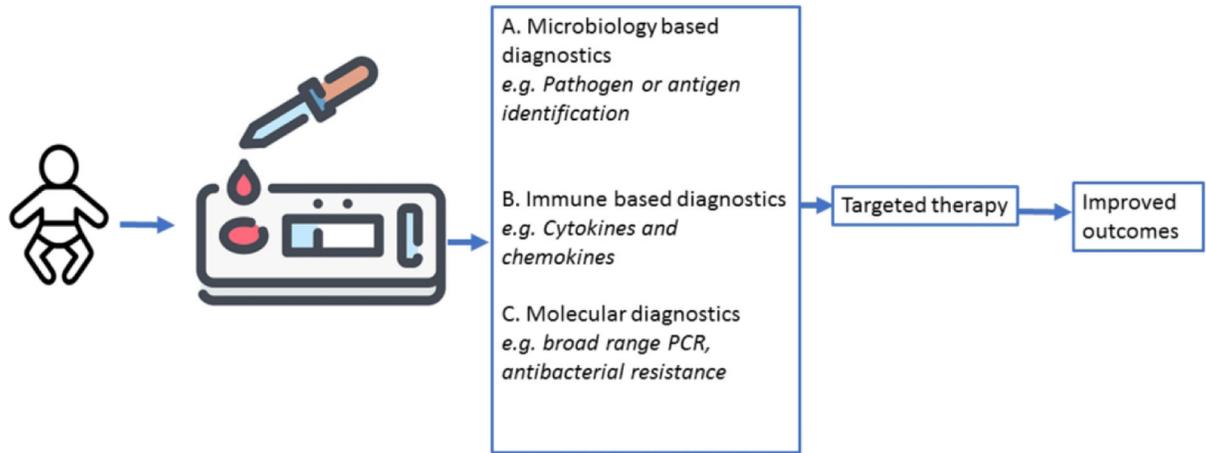


Figure 3. Point of care testing for diagnosis of neonatal sepsis

Blood samples are drawn on suspicion of infection on laboratory chips that are microbiology, immune, or molecular based diagnostics. The results enable us to initiate targeted therapy. The rapid results and targeted therapy will improve clinical outcomes.

Table 1.

Most studied and promising biomarkers in diagnosis of neonatal sepsis

Biomarker	Patient characteristics	Performance							Comments
		Cut-off	Sensitivity	Specificity	PPV	NPV	PLR	NLR	
Complete blood cell count ⁵¹									
WBC	EOS LOS	-	0.3–18 0.1–23	79–99 80–99	36 13–100	94– >99.8 74–96	-	-	Leukocyte (<5000, 20000) and absolute neutrophil count (<1000, 5000) were traditionally used parameters. But these parameters are affected by gestational age, postnatal hours and days and clinical conditions such as maternal hypertension, perinatal asphyxia, intraventricular hemorrhage, etc.
ANC	EOS LOS	-	8–68	95–99	14–21	74–96	-	-	
I/T ratio	EOS LOS		22–62 33–54	74–96 62–100	2.5 12–100	99 66–96	-	-	The I/T ratio has better sensitivity than WBC and ANC. Disadvantage of this ratio is the interreader difference. The ratio >0.2 has been traditionally used.
Platelet count	EOS LOS		0.8–4 8–48	97–99 89–98	13–14 99	- 94	-	-	Low platelet count can be found in neonatal sepsis, especially gram negative and fungal sepsis but often remain decreased during sepsis process.
MNV, MNC, MNS	76 proven, 126 clinical sepsis, 98 control All gestations (mean 30±5 w), early and late onset sepsis ⁶¹	>157 au <159 au <127.5 au	79 66 60	82 64 65	90 80 21	65 47 55	-	-	No statistical difference between early and late onset sepsis; proven and clinical sepsis. Combination with IL-6 and CRP gave better diagnostic performance. Levels normalized with treatment.
DNI	110 proven, 31 clinical sepsis, 87 control All gestations (median 30 (23–41) w), early and late onset sepsis ⁶²	4.6	85	80	87	77	-	-	DNI was insignificantly higher in late onset sepsis. Proven sepsis had significantly higher DNI levels. Levels normalized with treatment. Mortality was predicted with DNI.

Biomarker	Patient characteristics	Performance							Comments
		Cut-off	Sensitivity	Specificity	PPV	NPV	PLR	NLR	
CD64	Meta-analysis of 17 studies including 3478 neonates, all gestations, EOS and/or LOS, proven and/or clinical sepsis ¹¹⁸	1.8–4.3 CD64 index; 1010–6010 \$	21–100	59–100	9–96.2	73–100	1.84–47.1	0.06–0.48	Pooled sensitivity, specificity, PLR and NLR were 77%, 74%, 3.58 and 0.29, respectively. The pooled DOR was 15.18 (95% CI: 9.75–23.62). Proven sepsis group had better diagnostic performance than clinical sepsis group. Term infants had higher sensitivity, specificity, PLR and DOR.
CD11b	Meta-analysis of 9 studies including 843 neonates, all gestations, EOS and/or LOS, proven and/or clinical sepsis ¹²¹	12.6–600 MFI	65–100	56–100	50–100	61–100	2.1–156	0.01–0.49	Pooled sensitivity, specificity, PLR and NLR were 82%, 93%, 11.51 and 0.19, respectively. The pooled DOR was 59.50 (95% CI 4.65 to 761.58). The diagnostic accuracy of was higher in early-onset sepsis.
Presepsin	Meta-analysis of 11 studies including 793 neonates, all gestations, EOS and/or LOS, proven and/or clinical sepsis ¹²²	650 650–850 850	91 91 90	85 97 86	-	-	-	-	The pooled DOR: 71.78 (7.46–690.56) 542.72 (156.62–1880.60) 75.60 (8.32–686.53)
	Meta-analysis of 28 studies including 2661 neonates, all gestations, EOS and/or LOS, proven and/or clinical sepsis ¹⁷⁷		67–98	75–100	-	-	-	-	Pooled sensitivity, specificity, PLR and NLR were 85%, 98%, 50.8 and 0.06, respectively. The pooled DOR was 864.
STREM-I	Meta-analysis of 8 studies including 667 neonates, all gestations but mostly term infants, EOS and/or LOS, proven and/or clinical sepsis ¹²⁸	77.5–1707.35 pg/mL	70–100	48–100	34–93.3	62–90	1.6–9.33	0.07–0.48	Pooled sensitivity, specificity, PLR and NLR were 95%, 87%, 7 and 0.05, respectively. The pooled DOR was 132.49 (95% CI 6.85–2560.70).
IL-6	Meta-analysis of 31 studies including 1448 septic neonates ¹⁰¹	3.6–300 pg/mL	54–100	45–100	-	-	1.63–88.79	0.03–0.50	Pooled sensitivity, specificity, PLR and NLR were 88%, 82%, 7.03 and 0.2, respectively. The pooled DOR was 29.54 (95%CI: 18.56–47.04)
IL-8	Meta-analysis of 8 studies including 548 neonates, all gestations, EOS and/or LOS, proven and/or clinical sepsis ¹⁰⁸	0.65–100 pg/mL	34–94	66–100	64–100	59–95	2.22–80.49	0.06–0.76	Pooled sensitivity, specificity, PLR and NLR were 78%, 84%, 4.58 and 0.25, respectively. The pooled DOR was 21.64 (95% CI: 7.37 to 63.54).

Biomarker	Patient characteristics	Performance							Comments
		Cut-off	Sensitivity	Specificity	PPV	NPV	PLR	NLR	
TNF- α	Meta-analysis of 15 studies including 1201 neonates ¹¹¹	0.18–180 (20000 in 2 studies)	21–100	43–100	-	-	-	-	Pooled sensitivity, specificity were 66%, 76%, respectively. The pooled DOR was 7.43 (95%CI 3.47–15.90). Diagnostic accuracy was found slightly better in LOS than EOS.
CRP	Review of 27 studies including 4996 neonates ¹¹²	2.5–100 mg/L	22–100	59–100	31–100	38–96	-	-	
PCT	Meta-analysis of 16 studies including 1959 neonates, all gestations, EOS and/or LOS, proven and/or clinical sepsis ⁸⁰	0.5–5.75 μ g/L	57–100	50–100	19–100	56–100	-	-	Pooled sensitivity, specificity, PLR and NLR were 81%, 79%, 3.9 and 0.24, respectively. The pooled DOR was 16 (95% CI 8–32). Diagnostic accuracy was better in LOS than EOS.
SAA	Meta-analysis of 9 studies including 823 neonates, all gestations, EOS and/or LOS, proven and/or clinical sepsis ⁸⁷	1–68 mg/L	23–100	33–100	57–100	57–100	-	-	Pooled sensitivity, specificity were 84%, 89%, respectively. The pooled DOR was 91.84 (95% CI, 16.78–502.80). CRP has higher pooled sensitivity and DOR than SAA.
Pro-ADM	31 proven, 41 clinical sepsis and 52 control, preterm and term infants, EOS and/or LOS ⁸⁹	3.9 nmol/L	86	100	100	83	-	-	Diagnostic accuracy of pro-ADM was similar with IL-6 and CRP. Higher pro-ADM levels was found in gram negative sepsis.
Hepcidin	27 neonates with LOS and 17 control, VLBW infants ⁹⁴	92.2 mg/dL	76	100	100	87	-	-	Diagnostic performance was better than CRP and combination with CRP did not give better performance than hepcidin alone
Programulin	2 studies: Neonates >34 w at risk of EOS, proven and clinical sepsis (n:152), (n:121) ⁹⁵	1.39–37.86 ng/ml	67–94	80–51	76–61	67–91	3.4–1.95	0.16–0.11	Programulin was found efficient to predict EOS. Combination with CRP, PCT, IL-6 gave better diagnostic performance.
Vascular endothelium	74 infected, 118 non-infected samples of 149 neonates, preterm and term infants with EOS or LOS ⁹⁹	228 ng/mL 132 mg/L 1 mg/L	76 54 23	75 82 92	66 66 66	83 73 66	-	-	LOS group had higher sICAM-1 and SAA while lower sE-Selectin levels. Combination of these biomarkers with hsCRP altogether gave sensitivity, specificity, PPV and NPV as

Biomarker	Patient characteristics	Performance							Comments
		Cut-off	Sensitivity	Specificity	PPV	NPV	PLR	NLR	
Molecular assays (Meta-analysis ^{5,2})	All molecular tests (35 studies) Broad range PCR (9 studies) Real-time PCR (9 studies) Post-PCR processing (5 studies) Multiplex PCR (6 studies) LOS (10 studies) EOS and LOS (23 studies) Preterm (5 studies) Preterm and term (30 studies)		90 (38–100) 97 86 97 76 81 79 94 89 90	93 (32–100) 93 94 96 81 84 92 87 94	-	-	-	-	90%, 67%, 64% and 91%, respectively. Molecular tests have the advantage of rapid results and can be used as add-on tests. Molecular assays, including PCR and hybridization methods and have rapid detection times compared to blood cultures (6–8 h vs 20–36 h). Costs, availability of equipment and need for technical skills are disadvantages.
Future									
Omics approach	Metabolomics: sugars, lipids, small peptides, vitamins including glucose, maltase, lactate, acetate, ketone bodies, D-serine, acylcarnitines, acetoacetate, creatine ¹⁴⁶								
Nanotechnology	Proteomics: neutrophil defensin 1–2, cathelicidin, S100A12, S100A8, proapolipoprotein C2, apolipoprotein A-E-H, β -2 microglobulin, haptoglobin, desarginin ^{141, 142}								
Machine learning	Magnetic, gold, fluorescent and lipid based nanoparticles for contrast agents and biosensors ^{45,46}								
Heart rate variability	Reduced heart rate variability and transient decelerations were associated with early diagnosis of sepsis in 633 neonates ¹⁵² while reduced mortality due to different clinical problems including sepsis was reported in 2989 VLBW infants ¹⁵¹								
Vital signs	Heart rate, respiratory rate, temperature, desaturations, bradycardias of 155 neonates between 23–32 w with LOS ¹⁵³	81	80	57	93	-	-	LOS was diagnosed 43.1±79 h before culture positivity	
Clinical findings	Fever, apneas, platelet counts, gender, bradypnea, band cells, catheter use, birth weight and maternal age, cervicovaginitis in 238 neonates ¹⁵⁴	93	80	82	92	-	-	25 potential maternal and neonatal features were studied. Predictive model was created with combination of clinical, laboratory and demographic features.	
	Blood pressure, temperature and saturation were found vital candidate markers out of heart rate, respiratory rate, systolic blood pressure, diastolic blood pressure in 7870 neonates ¹⁵⁵								
New genetic techniques	Non-coding RNA's: miRNA, circRNA, miRNA-181a ¹⁵⁷ miRNA-16a ¹⁵⁸ miRNA-451 ¹⁵⁸	83 88 64	84 98 60	- 95 61	- 88 62	-	-	Study groups included term infants, both EOS and LOS, proven and clinical sepsis. miRNA levels were correlated with WBC, CRP and respiratory discomfort.	

WBC, White blood cell count; EOS, early onset neonatal sepsis; LOS; late onset neonatal sepsis; ANC, absolute neutrophil count; I/T ratio, immature/total neutrophil count ratio; MNV, mean neutrophil volume; MNC, mean neutrophil conductivity; MNS, mean neutrophil scatter; DNI, delta neutrophil index; CD, cluster of differentiation; sTREM-1, soluble triggering receptor expressed on myeloid cells-1; IL₆, interleukin; TNF- α , tumor necrosis factor- α ; CRP, C-reactive protein; PCT, procalcitonin; SAA, serum amyloid A; pro-ADM, proadrenomedullin; sICAM-1, Soluble intercellular adhesion molecule-1; sE-Selectin, Soluble endothelial leukocyte adhesion molecule-1; PCR, polymerase chain reaction; VLBW, very low birth weight infants; RNA, ribonucleic acid; miRNA, micro RNA; circRNA,

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circular RNA; NET, neutrophil extracellular traps; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; δ cAntibody-phycoerythrin molecules bound per cell; DOR, diagnostic odds ratio; MFI, mean fluorescence intensity

Table 2-

Microbial identification from blood cultures and molecular non-culture techniques

Technique	Target Pathogen	Resistance Typing	Turnaround time	Sensitivity	Specificity
Culture based Technique					
Blood culture	All culturable microbes	Yes	48-72 hours	-	-
Automated identification	All culturable microbes	Yes	24-48 hours	-	-
Nucleic Acid-Based Identification					
PNA-FISH	Differentiates between <i>Staphylococcus aureus</i> and CoNS; <i>Enterococcus faecalis</i> and <i>Enterococcus</i> species; <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , and <i>Pseudomonas aeruginosa</i> ; and <i>Candida</i> species.	No	1.5-3 hours ^a	96% – 100%	96% – 100%
QuickFISH	<i>S. aureus</i> , CoNS, <i>E. faecalis</i> , other Enterococci, <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>	No	<30 min ^a	96% – 100%	96% – 100%
MALDI-TOF	GP and GN bacteria, yeast, fungi, filamentous fungi, mycobacteria	In development	10-30 min ^a	-	-
Gene Xpert MRSA/SA	<i>S. aureus</i>	<i>mecA</i> for methicillin resistance	<1 hour ^a	98.3% – 100% for MRSA and MSSA	98.6% – 99.4% for MRSA and MSSA
Verigene gram-positive	<i>Staphylococcus</i> spp., <i>S. aureus</i> , <i>S. epidermidis</i> , <i>S. lugdunensis</i> , <i>Streptococcus</i> spp., <i>S. pyogenes</i> , <i>S. agalactiae</i> , <i>S. anginosus</i> group, <i>S. pneumoniae</i> , <i>E. faecalis</i> , <i>E. faecium</i> , and <i>Listeria</i> spp.	<i>mecA</i> for methicillin resistance and <i>vanA/B</i> genes for vancomycin resistance	2.5 hours ^a	92.6% – 100%	95.4% – 100%
Verigene gram-negative	9 bacterial targets including <i>E. coli</i> , <i>Shigella</i> spp., <i>K. pneumoniae</i> , <i>K. oxytoca</i> , <i>P. aeruginosa</i> , <i>Serratia marcescens</i> , <i>Acinetobacter</i> spp., <i>Proteus</i> spp., <i>Citrobacter</i> spp., <i>Enterobacter</i> spp.	KPC, NDM, CTX-M, VIM, IMP, OXA	2 hours ^a	97.1%	99.5%
FilmArray	27 targets, including staphylococci, streptococci, <i>Enterococcus</i> , <i>Listeria</i> , <i>Acinetobacter</i> , <i>Neisseria meningitidis</i> , <i>P. aeruginosa</i> and members of the Enterobacteriaceae family, as well as <i>Candida</i> spp.	<i>mecA</i> , <i>vanA/B</i> , and <i>K. pneumoniae</i> carbapenemase (KPC) genes	1 hour ^a	>90%	-
Culture-Independent Diagnostic Tests					
SeptiFast	25 pathogens, (10 GN, 9 GP, 6 fungi)	-	4-6 hours	63-83%	83-95%
SepsiTest	>345 pathogens, 13 fungi	-	8-12 hours	11-87%	83-96%
T2 MR Candida	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. krusei</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i>	In development	3-6 hours	90%	98%

^aTurn-around times are after culture turns positive.

CONS-coagulase-negative staphylococci, GP- gram positive, GN- gram negative