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Neonatal sepsis
An old problem with new insights

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Abbreviations: CDC, Centers for Disease Control and Prevention; GBS, group B streptococcus; EOS, early-onset sepsis; IAIP, inter alpha inhibitor protein(s); LOS, late-onset sepsis; NEC, necrotizing enterocolitis; NICHD, Eunice Kennedy Shriver National Institute of Child Health and Human Development; NICU, neonatal intensive care unit; VLBW, very low birth weight

Neonatal sepsis continues to be a common and significant health care burden, especially in very-low-birth-weight infants (VLBW <1500 g). Though intrapartum antibiotic prophylaxis has decreased the incidence of early-onset group B streptococcal infection dramatically, it still remains a major cause of neonatal sepsis. Moreover, some studies among VLBW preterm infants have shown an increase in early-onset sepsis caused by *Escherichia coli*. As the signs and symptoms of neonatal sepsis are nonspecific, early diagnosis and prompt treatment remains a challenge. There have been a myriad of studies on various diagnostic markers like hematological indices, acute phase reactants, C-reactive protein, procalcitonin, cytokines, and cell surface markers among others. Nonetheless, further research is needed to identify a biomarker with high diagnostic accuracy and validity. Some of the newer markers like inter α inhibitor proteins have shown promising results thereby potentially aiding in early detection of neonates with sepsis. In order to decrease the widespread, prolonged use of unnecessary antibiotics and improve the outcome of the infants with sepsis, reliable identification of sepsis at an earlier stage is paramount.

Neonatal Sepsis

Neonatal sepsis remains one of the leading causes of morbidity and mortality both among term and preterm infants.[1] Although advances in neonatal care have improved survival and reduced complications in preterm infants, sepsis still contributes significantly to mortality and morbidity among very-low-birth-weight (VLBW, <1500 g) infants in Neonatal Intensive Care Units (NICUs).[2,3]

The signs and symptoms of neonatal sepsis are nonspecific.[4] These include fever or hypothermia, respiratory distress including cyanosis and apnea, feeding difficulties, lethargy or irritability, hypotonia, seizures, bulging fontanel, poor perfusion, bleeding problems, abdominal distention, hepatomegaly, gauiac-positive stools, unexplained jaundice, or more importantly, "just not looking right".[5,6] Infants with hypoxia–acidosis may gasp in utero and lead to pneumonia and meconium aspiration.[7]

The incidence of neonatal sepsis or bacteremia in asymptomatic infants is low, but not negligible.[4] Voora et al. reported a 1% prevalence of fever in term newborns with 10% of the febrile (≥37.8 °C rectal or core body temperature) infants having culture-proven sepsis.[8] While term newborns were described as being more likely to react to a bacterial infection with fever, preterm newborns were more likely to react with hypothermia, because of transitional difficulty with temperature control especially in the first 2 d.[9,10] In contrast, the lack of clinical relevance of body temperature in diagnosing sepsis later in preterm infants might be attributable to the use of incubators.[11] However, neonates with core body temperature elevation sustained for more than 1 h, not due to environmental causes and greater than 39 °C are more likely to have bacteremia, meningitis, pneumonia, and also associated with viral disease, particularly herpes simplex encephalitis and therefore evaluation should include lumber puncture.[12] Respiratory distress including tachypnea, grunting, nasal flaring, and retraction of respiratory muscles can be the sole manifestation of sepsis with or without pneumonia and can be confused with transient tachypnea of newborn initially. Rapid clinical deterioration ensues unless prompt antibiotic management is started in neonates with sepsis. Neonatal sepsis can be complicated by metastatic foci of infection, disseminated intravascular coagulation, congestive heart failure and shock.[13]

Necrotizing enterocolitis (NEC) is an acute inflammatory necrosis of the bowel and may be the underlying cause of neonatal sepsis. The probability of the latter is high when a neonate presents with gram-negative sepsis and has nonspecific intestinal and radiological signs.[14-16] Chaaban et al. reported 12 of 51 neonates with nonspecific abdominal findings had positive blood cultures.[17] Rates are especially higher in premature sick infants. Thirty-four percent of infants with <1000 g birth weight and 51% of infants with <29 week gestational age had concurrent bloodstream infections in a study of NEC.[15] In fact, depending on severity, 40–60% of NEC cases have concurrent bloodstream infections.[18-20] Gram-negative bacteremia and sepsis are the most common.[14,15]

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Table 1. Microbial pathogens and risk factors associated with neonatal sepsis

Neonatal sepsis	Microbial pathogens	Risk factors
Early-onset	• Group B streptococci • *Escherichia coli* • *Streptococcus viridans* • Enterococci • *Staphylococcus aureus* • *Pseudomonas aeruginosa* • Other gram-negative bacilli	• Maternal Group B streptococcal colonization • Chorioamnionitis • Premature rupture of membranes • Prolonged rupture of membranes (> 18 h) • Preterm birth (< 37 weeks) • Multiple gestation
Late-onset	• Coagulase-negative Staphylococci • *Staphylococcus aureus* • *Candida albicans* • *Escherichia coli* • *Klebsiella pneumoniae* • Enterococci • *Pseudomonas aeruginosa* • Group B streptococci	• Prematurity • Low birth weight • Prolonged indwelling catheter use • Invasive procedures • Ventilator associated pneumonia • Prolonged antibiotics

Modified from reference 1.

Based on the timing of the infection neonatal sepsis has been classified into early-onset sepsis (EOS) and late-onset sepsis (LOS).[2] This classification helps to guide antibiotic therapy as it implies differences in the presumed mode of transmission and predominant organisms. EOS is defined as onset of sepsis in the first 3 d and is mostly the result of vertical transmission of bacteria from mothers to infants during the intrapartum period.[21] LOS is defined as infection occurring after 1 week of life is attributed to the horizontal transmission of pathogens acquired postnatally and is often more insidious in onset.[2] One investigative group classified neonatal sepsis into early-onset (≤4 d), late-onset (5–30 d), and late, late-onset (>30 d) according to the infant's age when positive blood culture obtained.[2] VLBW preterm infants are at particularly high risk for LOS in part because of immaturity of the immune system, prolonged mechanical ventilation, prolonged hospitalization, use of indwelling catheters, endotracheal tubes, and other invasive procedures.[22]

Microbiology of Neonatal Sepsis

Longitudinal trends in the demographics, pathogens, and outcome were observed in a single-center database on neonatal sepsis at Yale–New Haven Hospital from 1928.[2] *Streptococcus pneumoniae* and group A streptococci were the major causes of neonatal sepsis from 1933 to 1943. From the late 1940s to the mid-1960s, Gram-negative organisms, especially *Escherichia coli* (*E. coli*), were the most common causes of neonatal sepsis.[23] Thereafter, group B streptococci infections emerged as the foremost cause of EOS in the1970s.[1]

Organisms associated with early-onset sepsis (EOS)

Group B streptococcus (GBS, *Streptococcus agalactiae*) is a gram-positive encapsulated bacterium and remains the leading cause of neonatal sepsis and meningitis in the United States. Stoll et al. has recently described *Escherichia coli* (*E. coli*) to have emerged as the major pathogen of neonatal sepsis in preterm infants and the second most common cause in term infants.[24] *E. coli* is frequently associated with severe infections and meningitis and is the leading cause of sepsis related mortality among VLBW infants (24.5%).[25] GBS and *E. coli* together account for about 70% of cases of EOS in the neonatal period.[2,26] Although less common, *Listeria monocytogenes* is associated with invasive disease in the newborn, spontaneous abortions or stillbirth if acquired during pregnancy.

Organisms associated with late-onset sepsis (LOS)

With improved survival of preterm infants, LOS has become an important cause of morbidity and mortality among low birth weight infants.[27] LOS is mainly associated with the organisms acquired from the environment after birth. In a study on 6215 infants admitted to National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN) centers, 70% of first episode late-onset infections were caused by gram-positive organisms, with coagulase-negative staphylococci accounting for 48% of the infections.[22] Death rates were highest for infants infected with *Pseudomonas aeruginosa*, *Candida albicans*, *Serratia marcescens*, and *E. coli*.[28] The incidence of late-onset GBS disease has remained unchanged despite intrapartum antibiotic prophylaxis. Meningitis remains a common presentation of late-onset GBS disease, with serious neurologic sequelae and permanent impairment among many survivors.[29,30] The microbial pathogens and risk factors associated with neonatal sepsis are shown in **Table 1**.

Prevention of Early-Onset Group B Streptococcal (GBS) Disease

Early onset GBS infection has a case fatality of 5–20%; a multistate active surveillance system demonstrated that 6% of early-onset GBS infections resulted in death.[31] Neonatal infection can occur when GBS ascends from the vagina to the amniotic fluid after the onset of labor or rupture of membranes.[32,33] It is more commonly the result of vertical transmission from mother to infant in women with recto-vaginal colonization. Colonization with GBS occurs in roughly 10–30% of pregnant woman in their vagina or rectum.[34]

The only intervention proven to decrease the incidence of early-onset neonatal GBS sepsis is maternal treatment with intrapartum intravenous antibiotics. Adequate prophylaxis is exposure to penicillin (preferred agent), ampicillin, or cefazolin given

for >4 h before delivery.[31,35] Erythromycin is no longer recommended for prophylaxis because of high resistance rates. In those women with a non-serious penicillin allergy, cefazolin is the drug of choice. For a mother with a history of life-threatening penicillin allergy (anaphylaxis, rash, angioedema, respiratory symptoms), clindamycin is the substitute for penicillin, but should only be used if the rectovaginal GBS isolate is tested and found to be susceptible. If the clindamycin sensitivity is unknown or the GBS isolate is resistant to clindamycin, vancomycin is the substitute for prophylaxis. Intrapartum antibiotics are indicated in the following situations:[35]

1) Positive antenatal cultures or molecular testing at admission for GBS (except for women who have a cesarean delivery without labor or membrane rupture),

2) Unknown maternal colonization status with gestation <37 weeks, rupture of membranes >18 h, or temperature >100.4 °F (>38 °C),

3) GBS bacteriuria during current pregnancy,

4) Previous infant with invasive GBS disease.

Risk-Based Approach vs. Universal Screening Approach

The epidemiology and management of neonatal GBS disease has evolved significantly over the last two decades. Initially, screening approaches based on risk factors for EOS were tested. Later, the effectiveness of universal screening was compared with risk-based approaches in preventing early-onset GBS disease in a multistate retrospective cohort study. The risk of early-onset GBS sepsis was notably lower in the infants of women who underwent universal screening than among those in the risk-based group.[36] With universal screening, it is possible to identify GBS colonized woman even without obstetric risk factors and reach more of the population at risk than with the risk based approach. After controlling for risk factors associated with early-onset GBS disease (preterm delivery, prolonged ROM, young maternal age, black race), the protective effect of the universal screening approach has been shown to be robust in subsequent prospective studies.[37] Intrapartum antibiotic prophylaxis is approximately 90% effective in preventing early-onset GBS disease.[37]

Changes in Microbial Pathogens in the Post Chemoprophylaxis Era

Decreased incidence of neonatal group B streptococcal (GBS) disease

After the nationwide implementation of intrapartum antibiotic prophylaxis, a striking 80% decline in the incidence of invasive early-onset GBS disease was observed.[35] Before prophylaxis, the incidence of GBS in the United States was 2–3 cases per 1000 live births.[38] After the 1996 GBS prevention guidelines were issued, incidence of early-onset GBS sepsis declined significantly in the following 2 y and then reached a plateau of approximately 0.5 cases per 1000 live births during the period from 1999–2001.[35] Upon issuance of the 2002 guidelines published in the landmark *Morbidity and Mortality Weekly Report* (MMWR) by CDC, the incidence further declined to 0.3–0.4 cases per 1000 live births.[35] This additional decline is consistent with the transition from the 1996 prevention strategy to the universal screening approach recommended in 2002.

Increased proportion of non-group B streptococcal pathogens

A potential increase in false-negative neonatal blood cultures as well as sepsis caused by pathogens other than GBS is a potential concern with the extensive use of maternal intrapartum antibiotics. Such a change would be important as several studies have demonstrated increased severity of disease and risk of death in the neonates with gram-negative infections.[24] In a study conducted by the NICHD between 1998 and 2000 on 5447 VLBW infants (those weighing between 401 and 1500 g), there was a significant reduction in the incidence of EOS caused by GBS. However, there was also a significant increase in the proportion of *E. coli* infections among VLBW infants.[23] Though there has been a dramatic decline in the incidence of EOS due to GBS, the increasing incidence of ampicillin-resistant neonatal sepsis among VLBW infants is concerning.[39] Nonetheless, the benefits from the use of antepartum antibiotic chemoprophylaxis still offset the risks of resistant bacterial infections.[36]

Neonatal Early-Onset Sepsis Risk Algorithms

Pediatricians currently use the CDC 2010 and American Academy of Pediatrics Committee on the Fetus and Newborn (COFN) 2012 algorithms for evaluation and management of infants at risk for EOS born at or near term gestation.[35,40] While both sources acknowledge maternal chorioamnionitis as a significant risk, they do not offer a standard definition of this clinical diagnosis.[24] Additionally, there are differences in their recommendations for the evaluation of infants who received inadequate intrapartum GBS prophylaxis and with the rupture of membranes at least equal to 18 h. Puopolo et al. proposed a multivariate predictive model of EOS risk developed for infants born at or above 34 weeks' gestation based on objective clinical data available at the time of birth.[41,42] This could decrease the number of infants evaluated and empirically treated for EOS but studies are needed for validation of this computational model.

Biomarkers of Neonatal Sepsis

Since sepsis is a systemic inflammatory response to infection, isolation of bacteria from blood is considered the gold standard for the diagnosis of sepsis.[43] However, it takes 24–48 h for culture results. Inoculation of only 0.5–1.0 ml of blood decreases its sensitivity, as approximately 60–70% of infants have a low level of bacteremia.[44] Theoretically, for optimal results, 6 ml of blood would be required which is not feasible.[45] Sepsis cannot always be excluded even when blood cultures are found to be negative. Conversely, isolation of bacteria in a blood culture may reflect asymptomatic bacteremia or contamination. Non-culture dependent methods based on proteomics, in situ hybridization, gene arrays, mass spectroscopy, and polymerase chain reaction (PCR) methods to screen blood for bacteria, and other supplemental

diagnostic tests based on evaluation of the immune system are being evaluated to help resolve ambiguities in these situations.[46]

Complete blood count (CBC)

In order to improve the outcome associated with neonatal sepsis, it is necessary for a diagnostic test to be rapid and sensitive to decrease delay in treatment. At the same time in order to avoid unnecessary exposure to antibiotics and invasive procedures, a test with higher specificity is needed. A large number of studies have been performed to evaluate the use of complete blood count (CBC), differential count, and immature to total leukocyte ratio (I:T) for the diagnosis of neonatal sepsis. Although the CBC has a poor predictive value, serial normal values can be used to enhance the prediction that bacterial sepsis is not present.[47,48]

Low WBC and absolute neutrophil counts, as well as high immature-to-total neutrophil ratio (I:T) are associated with an increased risk of infection (odds ratios 5.38, 6.84, and 7.90 respectively).[44] However, the sensitivity for detection of sepsis is low. Two serial normal CBCs, performed 8 to 12 h apart, and a negative blood culture at 24 h improve the predictive power to rule out EOS in the first 24 h after birth.[49] This strategy has been associated with a negative predictive value as high as 100%, but the specificity and positive predictive values may be too low to guide therapy decisions.[49] Components of the white cell count, including absolute neutrophil count (ANC) and immature to total neutrophil ratio (I:T) have also been shown to be more useful for excluding infants without infection rather than identifying newborns who are infected.[40] The maximal (I:T) ratio in uninfected newborns is 0.16 in the first 24 h, which by 120 h decreases to 0.12.[50] I:T ratio of >0.2 is suggestive of sepsis. However, the I:T ratio can be affected by various noninfectious processes like labor, prolonged induction with oxytocin, and even prolonged crying.[49]

A total leukocyte count of <5000 to 7500/mm^3 can be used to infer the diagnosis of neonatal sepsis.[6] Many infected newborns may have higher counts. However, the sensitivity of a low leukocyte count is 29%, though the specificity is as high as 91%.[6] There are important gestational age effects on the leukocyte count in the newborn period. In newborns >36 weeks gestation, the lower limit of normal for ANC at birth is 3500/mm^3. The lower limit of normal in infants born between 28–36 weeks is 1000/mm^3 and 500/mm^3 in infants <28 weeks gestation.[51] Total neutrophil counts rise after birth and reach their peak levels at 6 to 8 h of life. The lower limits of normal at that time are 7500/mm^3, 3500/mm^3, and 1500/mm^3 for infants born at >36 weeks, 28–36 weeks, and <28 weeks respectively.[51] Thus it is more effective to obtain total leukocyte counts at 6–12 h after birth, as they are more likely to be reliable. Factors such as maternal hypertension or perinatal asphyxia may cause neutropenia or an elevated I/T ratio.[50] Also, leukocyte counts may be normal in the early course of neonatal sepsis. In summary, the WBC, ANC, and I/T ratio have significant limitations in the diagnosis of neonatal sepsis.

C reactive protein (CRP)

CRP is one of the most extensively studied, most available, and most frequently used laboratory tests for the diagnosis of neonatal sepsis.[52] CRP is an acute phase reactant synthesized by the liver. It has a half- life of 24–48 h. It takes 10–12 h for CRP to change significantly after onset of infection. Serial determination of CRP 24–48 h after the onset of symptoms increases its sensitivity.[52,53] Serial CRP measurements may also be helpful in monitoring the response to treatment in infected neonates and thus may help clinicians guide the duration of antibiotic therapy.[53-55] The specificity and positive predictive value of CRP ranges from 93–100%.[56] Thus, CRP can be considered as a "specific" but "late" marker of neonatal infection. If the CRP levels remain persistently normal, it correlates strongly with the absence of infection thereby guiding safe discontinuation of antibiotic therapy.[57]

Preterm infants have lower CRP baseline values and a lower rise in response to infection. A variety of non-infectious conditions like meconium aspiration syndrome, traumatic or ischemic tissue injuries, hemolysis, or histologic chorioamnionitis may cause an elevation in the CRP levels.[52] Because it takes 10–12 h to change significantly after the onset of infection; the sensitivity of CRP is low during the early phase of sepsis. Due to noninfectious CRP elevations, the influence of gestational age and birth weight on kinetics of CRP, and the lack of reliable age specific reference values, the use of CRP requires further research to cover these pitfalls and falls short as an ideal marker.

Procalcitonin (PCT)

PCT is an acute phase reactant produced both by hepatocytes and macrophages that has been studied since the mid-1990s. Serum concentrations of PCT begin to rise 4 h after exposure to bacterial endotoxin, peak at 6 to 8 h, and remain elevated for at least 24 h.[58] The half-life is about 25–30 h, and the serum concentration is not affected by gestational age. Nonetheless, in noninfected newborns, serum PCT concentrations vary widely. It is low soon after birth, rises to a peak at 24 h and returns to baseline at 48 h.[56] Serum PCT concentrations increase appreciably in the presence of systemic bacterial infection and necrotizing enterocolitis during early- and late-onset neonatal infection.[59,60] The PCT response is more rapid than the elevation of CRP, thus it is an attractive alternative for the detection of EOS. Because PCT levels remain high compared with TNF-α and IL-6, PCT is also useful in predicting severity of infection, response to treatment, and outcome.[59,60] In contrast to CRP, infants with trauma, viral infections, meconium aspiration, and hypoxemia have normal or minimal elevation in PCT.[60] The sensitivity and specificity of PCT varies between 83–100% and 70–100% respectively.[61] The sensitivity and specificity of PCT is greater than CRP or interleukin 6 if different cutoff points at birth and at 24 h and 48 h of life are used.[61,62]

Nonetheless, PCT has its own limitations as it is increased in newborns requiring neonatal resuscitation and in infants born to mothers with chorioamnionitis in the absence of neonatal infection.[61] In healthy neonates it has been shown that PCT concentrations are affected by maternal GBS colonization and prolonged rupture of membranes ≥18 h.[63] Therefore, PCT needs to be studied further in larger groups of infants so as to improve its diagnostic accuracy.

Cytokines

Changes in the blood levels of cytokines occur rapidly in the setting of neonatal sepsis even before that of acute phase reactants. Several cytokines like interleukin-1β (IL-Iβ), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-2 soluble receptor (SIL2R), and tumor necrosis factor α (TNF-α) have been shown

to rise early in response to bacterial infection in neonates. The rise occurs before the newborn develops signs or symptoms of sepsis and even before previously described laboratory tests become positive.[57] Cytokines do not cross placental barrier and elevations have been found in umbilical cord blood suggesting the possibility of predicting infants who are going to develop sepsis in first few hours of their life.[64,65] In addition, cytokine analysis may be useful in predicting late-onset infection.[66]

IL-6

In response to exposure to bacterial endotoxins, IL-6 concentrations rise before that of CRP.[67] Umbilical cord IL-6 is consistently increased in newborns with EOS.[65,68] The sensitivity of cord blood IL-6 in predicting neonatal sepsis was found to be 87–100%, with the negative predictive value of 93–100% in some cohorts.[65,68-70] However, the half-life of IL-6 is very short and the levels fall to undetectable values quickly with treatment; thus sensitivity falls at 24 and 48 h (67% and 58% respectively).[71] Therefore, IL-6 can be considered as an early and sensitive marker of neonatal infection.[56] Diagnostic accuracy is further improved by combining IL-6 (early and sensitive) and CRP (late and specific) in first 48 h of presumed clinical sepsis.[72]

TNF-α

The concentrations of TNF-α were shown to be significantly higher in infected as compared with non-infected newborns in multiple studies.[71,73,74] TNF-α has very similar kinetics to IL-6.[67] Silveira et al. observed the diagnostic accuracy of TNF-α was equivalent to PCT.[74] Sensitivity and specificity increases to 60% and 100% respectively when TNF-α and IL-6 levels are combined for the diagnosis of neonatal sepsis.[75]

IL-8

IL-8 is a pro-inflammatory cytokine which aids in the activation and chemotaxis of neutrophils.[76] IL-8 not only serves as a marker for sepsis but is also associated with severity of infection. It is produced as a result of infection by monocytes, macrophages, and endothelial cells with kinetics similar to IL-6.[76] IL-8 has sensitivity and specificity ranging from 80 to 91% and 76 to 100% respectively.[67,70] In a study performed by Boskabadi et al. in 93 neonates greater than 72 h of age, serum concentrations of IL-8 in non-surviving neonates were 3.3 times higher than surviving neonates.[77] The combination of IL-8 and CRP is more reliable for early diagnosis of neonatal sepsis, with a sensitivity and specificity of 91% and 73% respectively.[55,65,67] Thus, combining CRP and IL-8 may reduce excessive use of antibiotics.[55]

Though IL-6 and IL-8 increase very rapidly with bacterial invasion, their levels promptly normalize in serum (within the first 24 h), limiting their ability to be used as ideal markers. Therefore, operational difficulties in detection of cytokines, elevation of cytokines in non-specific settings, and lack of availability in many centers are limitations for their use in day-to-day practice.[65] Studies with larger sample sizes are needed before cytokines can be endorsed as valid diagnostic markers.

Cell Surface Markers

With the advances in flow cytometric technology, cell surface antigens can be detected in blood cells. Such tests are readily performed requiring a very low volume (0.05 ml) of whole blood. Neutrophil cluster of differentiation (CD) CD11β and CD64 have been found to be reliable markers for detecting early- and late-onset neonatal sepsis respectively with a high sensitivity and specificity.[56] Their expression increases within minutes following exposure to bacterial products. Further, as the biological activities of the cytokines may not be revealed by their circulating concentrations, measuring the cellular response to cytokines may be a better way of recognizing an early immunological response to infection.

CD11β

CD11β is the α-subunit of the β2-integrin adhesion molecule involved in neutrophil adhesion, diapedesis, and phagocytosis. It is detectable within 5 min in response to bacterial infection.[78,79] The sensitivity and specificity are as high as 96–100% and 100% in two studies.[80,81] It has better diagnostic accuracy for early than late-onset neonatal sepsis.[56] The variable diagnostic accuracy of CD11β in late-onset neonatal sepsis may be related to different infant population being evaluated, time interval between phlebotomy and sample processing and at which phase of infection blood is obtained.[82,83]

CD64

The high affinity antibody receptor CD64 is expressed at a very low level on the surface of neutrophils in the absence of an infection.[84,85] The expression of CD64 on activated neutrophils markedly increases after an episode of bacterial infection.[86-88] CD64 has a sensitivity of 95–97% and a negative predictive value of 97–99%.[56,73] The addition of IL-6 or C-reactive protein to CD64 further enhances its sensitivity and negative predictive value to 100%, with the specificity and positive predictive value exceeding 88% and 80%, respectively.[82] With the use of CD64 it may be possible for clinicians to discontinue antibiotics within 24 h in non-infected newborns.[82]

In a prospective study performed by Streimish et al. at Yale University, using a cut-point CD64 index value of 2.38 for EOS, the test had a sensitivity of 100%, a specificity of 68%, and an NPV of 100%; while using a cut-point value of 3.62 for LOS sepsis, the test had a sensitivity of 75%, a specificity of 77%, and an NPV of 96%. Due to the large sample size, this study was able to demonstrate the potential of CD64 to influence the initiation and duration of antibiotic therapy.[89] However, the cost, the need for sophisticated equipment and the processing time are barriers to the use of these markers in clinical practice.

Genomics, Proteomics, and Nucleic Acid-Based Molecular Techniques

Esparcia et al. employed a gene-based molecular technique using 16S rDNA for diagnostic accuracy of bacterial meningitis and early-onset neonatal sepsis.[90] Ng et al. used a score based on proapolipoprotein CII and a des-arginine variant of serum amyloid to withhold antibiotics in 45% of infants with suspected infection and to discontinue antibiotics in 16%.[91,92] Kasper et al. recently found that sensitivity of multiplex real-time PCR (Roche SeptiFast®) was 0.90 (95% CI 68.2–98.3) but specificity was low (0.80; 95% CI 58.7–92.4%) for late-onset nosocomial sepsis

Table 2. Diagnostic performance of adjunctive tests of neonatal sepsis

Diagnostic test	Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[b] (%)	Likelihood ratio (+)	Likelihood ratio (–)
WBC[c]	44	92	36	94	5.5	0.60
I:T ratio[d]	54.6	73.7	2.5	99.2	2.07	0.61
Platelets[e]	22	99	60	93	2.72	0.78
CRP[f]	70–93	78–94	7–43	97–99.5	3.18–15.5	0.07
PCT[g]	83.3	88.6	83.33	88.57	6.9	0.188
IL-6[h]	87	93	76	97	12.42	0.14
IL-8[i]	91	93	91	97	13	0.10
TNF-α[j]	75	88	67	51	6.25	0.28
IAIP[k]	89.5	99	95	98	89.5	0.106

[a]PPV, positive predictive value; [b]NPV, negative predictive value; [c]white blood cell (WBC) counts ≤5000 or ≥25 000, 30 000, or 21 000 per mm^3 at birth, 12–24 h and day 2 or after, respectively[57]; [d]I:T ratio (ratio of immature to absolute neutrophil count) >0.2[44]; [e]platelets <150 000 cells/mm^3[,57]; [f]C-reactive protein (CRP) >1 mg/dl[4]; [g]procalcitonin (PCT) >5.38 ng/ml at 24 h of life[62]; [h]interleukin-6 (IL-6) >100 pg/ml[70]; [i]interleukin-8 (IL-8) >300 pg/ml[70]; [j]tumor necrosis factor α (TNF-α) >13 pg/ml[70]; [k]inter α inhibitor proteins (IAIP) ≤177 mg/L[98]

in premature infants.[93] The limitations of these studies includes failure to provide information about antibiotic resistance, inability to differentiate the false-positive results because of potential contamination during blood sampling or processing from true positive cases and high cost. Prospective evaluation is needed to determine accuracy and safety of these exciting new approaches. Therefore, these are currently adjunctive methods with the exception of HSV PCR, which remains gold standard for the diagnosis of HSV encephalitis.[1]

None of the markers including hematologic indices, acute phase reactants, cytokines, and cell surface markers have shown sensitivity, specificity, positive and negative predictive value that are sufficiently powerful to guide the clinical management of neonatal sepsis.[4,94] Different biomarkers have been used to diagnose neonatal sepsis, but with inconclusive results, because of small sample size, lack of clear reference values and lack of homogeneity in the study group. Thus, there remains a need for a marker with high sensitivity, specificity, positive and negative predictive accuracy which is able to detect infection at an early stage.

Inter α inhibitor proteins (IAIP)

The inter alpha inhibitor family of proteins (IAIP) are serine protease inhibitors which provide protection from the increased protease activity associated with systemic immune system activation that accompanies sepsis and inflammation. They are involved in extracellular matrix stabilization, inflammation, wound healing, and play an important anti-inflammatory and regulatory role in infection.[95] IAIP is one of the important serine protease inhibitors secreted by the liver. IAIP is a heterotrimeric, 250 kd protein complex composed of two heavy chains and one light chain held together by glycosaminoglycan bonds. The light chain, Bikunin, has a molecular weight of 30 kd and is the active, anti-protease component. In the presence of serine proteases, Bikunin is released and it provides protective effects.[95,96] The half-life of Bikunin is very short and it is rapidly excreted by the kidneys.

IAIP concentration is independent of gestational age, postnatal age, and is similar to adult levels.[97] However, IAIP levels are significantly lower in septic neonates as compared with non-septic age matched controls. Receiver operating curve analysis has shown IAIP measurement to have a sensitivity of 89.5%, a specificity of 99%, a positive predictive value of 95% and a negative predictive value of 98% in a pilot study of 573 neonates.[98] The levels of IAIP not only decrease in neonatal sepsis but also rise in response to antibiotic treatment.[97] Yang and colleagues showed that low levels of IAIP are highly predictive of mortality in septic adult patients.[99] Because the levels of IAIP decrease with severe sepsis, measurement may also help to guide the prognosis as lower levels are associated with adverse outcome.[100] Chaaban et al. demonstrated that the levels of IAIP also decrease significantly in patients with necrotizing enterocolitis (NEC, stage II/III according to modified the Bell criteria) and thus can be useful to diagnose patients with NEC at an early stage.[17] Singh et al. showed an immunomodulatory and protective role of administration of IAIP in a septic newborn mice.[101] This underscores potential role of IAIP as a theranostic marker in infants with sepsis. The diagnostic performance of IAIP and other adjunctive tests of neonatal sepsis is shown in **Table 2**.

Conclusion

Systemic bacterial infection in the newborn creates a significant burden due to its impact on neonatal mortality and long-term morbidity. In spite of ongoing efforts in early diagnosis, treatment, and prevention, neonatal sepsis still remains an enigmatic area for neonatologists due to changes in epidemiology and the lack of ideal diagnostic markers. The need for a biomarker with high diagnostic accuracy and reliability is paramount as a guiding tool for physicians to assess the risk of infection and need for antibiotic therapy. Studies are currently ongoing in the search of a novel marker for neonatal sepsis. Inter α inhibitor proteins are among the candidates with significant promise.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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