REVIEW ARTICLE

Neonatal Sepsis; A New Paradigm in Laboratory Diagnosis

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ABSTRACT

Neonatal sepsis is a leading cause of morbidity and mortality worldwide, necessitating rapid and accurate diagnostic methods to initiate proper treatment. Traditional blood cultures, while considered the gold standard for diagnosing sepsis, have limitations such as low sensitivity and prolonged time to results, which can delay critical therapeutic interventions. Various biomarkers such as C-reactive protein (CRP), procalcitonin (PCT) and interleukin-8 are used for diagnosis of sepsis. However, these biomarkers may also be elevated in noninfectious conditions such as premature rupture of membranes, fetal distress and perinatal asphyxia, resulting in false positive results and low specificity for neonatal sepsis The amplification of the 16S ribosomal RNA (rRNA) gene by polymerase chain reaction (PCR) has emerged as a promising alternative due to its potential for rapid and sensitive detection of bacterial pathogens. The diagnostic test must be rapid and sensitive to improve the outcome associated with neonatal sepsis and to avoid over use of antibiotics.

INTRODUCTION

Neonatal sepsis is a systemic infection caused by bacteria, viruses, or fungi, leading to hemodynamic instability, clinical symptoms, and significant illness or death [1]. It is classified into two subtypes: early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS) [2]. While definitions vary, EONS typically occurs within the first 72 hours of life, whereas LONS develops after this period but before 28 days. [3]

In neonatal intensive care units (NICUs),it is difficult distinguish between infection and sepsis.The difference remains unclear. Current neonatal sepsis definitions differ from those used in older populations, often relying on the duration of antibiotic therapy or the presence of pathogens in blood cultures[4]. However, defining sepsis solely by bacteremia and treatment response fails to enhance clinical decision-making or research efforts aimed at reducing mortality. [5]

Epidemiology

Newborns are particularly vulnerable to illness due to their underdeveloped immune systems and exposure to various prenatal risk factors. This susceptibility increases the likelihood of neonatal sepsis and mortality. Globally, an estimated 2.5 million infants died within their first month of life in 2018, with significant regional disparities in neonatal mortality rates. According to the Levels and Trends in Child Mortality Report (2019), Sub-Saharan Africa and South Asia have the highest newborn death rates .[6]

Since 2000, global neonatal deaths have decreased by roughly 3.6 million per year, largely due to reduced cases of severe infections like pneumonia and diarrhea. However, despite its substantial impact on childhood mortality, neonatal sepsis receives far less international funding and attention as a public health priority compared to other life-threatening conditions .[7]

Neonatal Risk Factors

Prematurity and low birth weight considered the primary risk factors for neonatal sepsis. Preterm infants with low birth weight have 3 to 10 times higher sepsis risk compared to full-term, normal-weight newborns.[8]
Risk factors differ between early- and late-onset sepsis:

- Early-onset sepsis (EOS) is associated with fetal distress, low APGAR scores, neonatal resuscitation, and multiple pregnancies.[9]
- Late-onset sepsis (LOS) is often linked to invasive procedures (e.g., repeated blood draws, intubation, mechanical ventilation and catheter use), poor breastfeeding, prolonged parenteral nutrition, and surgical interventions.[9]

Maternal Risk Factors

Maternal group B streptococcal (GBS) colonization, chorioamnionitis, early rupture of membranes (>18 hours), intrapartum maternal fever (>38 ºC), birth before 37 weeks of gestation, and other factors that raise the newborn's risk of contracting GBS infection . [10]

Microbial Causes and Transmission Patterns of Neonatal Sepsis

The bacterial pathogens responsible for neonatal sepsis vary by region and have changed over time. In developing countries, *Staphylococcus aureus* and *Klebsiella pneumoniae* are the most common causes of both early-onset (EOS) and late-onset sepsis (LOS), as

reported by the National Neonatal Perinatal Database (NNPD). In contrast, developed nations predominantly report gram-positive organisms as the leading cause . [11]

A) Transmission Routes

- Early-onset sepsis (EOS) is primarily caused by vertical transmission from the mother. Pathogens can ascend through ruptured or intact membranes, leading to chorioamnionitis. However, placental transmission is also possible, particularly in cesarean-delivered infants without membrane rupture, as evidenced by severe symptoms and bacteremia at birth [12].
- Late-onset sepsis (LOS) usually results from horizontal transmission, including nosocomial or environmental exposure from caregivers .[13]

Pathogen-Specific Trends

- *Staphylococcus aureus* infections are strongly associated with catheter use. A UK study found that 50% of *S. aureus* sepsis cases occurred in infants with central catheters. Candida is a significant pathogen in very low birth weight (<1500g) infants, ranking as the third most common cause of LOS, especially in prolonged NICU stays .[14]

Viral Causes of Neonatal Sepsis

- Herpes Simplex Virus (HSV) is a leading viral cause of LOS, affecting 1 in 3,200 U.S. newborns. Despite diagnostic and treatment advances, it remains a major contributor to morbidity and mortality [15]. Enteroviruses can also cause LOS, presenting with non-specific symptoms such as lethargy, poor feeding, fever, jaundice, and severe complications like meningoencephalitis, myocarditis, and hepatitis .[16]

Diagnostic Challenges

- Early and accurate diagnosis is critical for survival but remains challenging due to non-specific early symptoms, requiring high clinical suspicion, especially in preterm infants [17]. In addition to overlapping signs with other conditions, making differentiation difficult .[18]

Clinical Findings in Neonatal Sepsis

Neonatal sepsis has non-specific signs and symptoms. Differential diagnosis is therefore crucial [17] .In the respiratory system, neonatal sepsis may manifest as groaning, nasal wing breathing, cyanosis, tachypnea, and contraction of the accessory muscles of respiration; in the cardiovascular system, bradycardia/tachycardia, peripheral circulatory disturbance, hypotension, and prolonged capillary refill time; in the digestive system, nutritional intolerance, difficulty sucking, vomiting, abdominal distention, hepato-splenomegaly, and jaundice; in the skin, sclerema, cutis marmaratus, pustule, abscess, petechiae, and purpura; and in the central nervous system, lethargy, hypotonicity, sleepiness, weak or high-pitched crying, bulging fontanelle, irritability, convulsion, hypoactivity and body temperature regulation issues . [19]

Pathophysiology

– ***Neonatal Immune Defence Mechanisms***

The innate and acquired immune systems make up neonatal immunity. The complement cascade and phagocytes are the main drivers of innate immunity, the newborn's initial line of defense against infection.the innate system also works with T and B cells from the acquired immune system to produce memory responses to antigens that the body has already encountered . [20]

The slower but more focused immune response known as acquired immunity is fueled by cells and antibodies that are acquired from the mother. The newborn is more susceptible to infection due to a number of immunological abnormalities in both of these systems . [21]

– ***Innate Immunity***

The epithelium, several cell types, cytokines, and the complement cascade are all components of the innate immune system, which is mostly used in the first few postnatal days. A physical barrier that prevents pathogen invasion is provided by the skin and the epithelial membranes of the gastrointestinal and respiratory systems. Immune cells phagocytize the pathogen, interact with the acquired immune system as antigen-presenting cells (APCs), and release cytokines to attract more immune cells . [22]

– ***Acquired Immunity***

Exposure is necessary for the acquired immune system to function well. Through the development of cellular memory to encountered infections, the neonate's acquired immune system starts to create a response in the extrauterine environment. If the same infection is encountered in the future, this memory leads to a more robust and effective immune response .[22]

Effector CD4+ T cells, which produce cytokines to activate different immune cells, and suppressor CD8+ T cells, which have a cytotoxic function, are responsible for cell-mediated immunity. Th1 or Th2 cells are subtypes of CD4+ cells, also referred to as T helper or Th cells. The proinflammatory response against microbial infections is significantly influenced by Th1 cells. In response to allergens and parasites, Th2 cells produce cytokines and initiate an anti-inflammatory reaction . [10]

B cells play a key role in humoral immunity because they produce antibodies, activate CD4+ cells by acting as APCs, and react to known antigens when they are exposed again. B cells create antibodies that directly block infections, start a complement system pathway, and activate innate system cells . [23]

Diagnosis of Neonatal Sepsis:

Research aiming at improving sepsis detection and management in this population has been identified as a cost-effective strategy for a number of reasons, including the newborn's lengthy life expectancy.

Although only a small percentage of infants are ultimately impacted by sepsis, the high death rates in neonates, as in older children and adults, prompt the early beginning of antibiotic treatment when sepsis is suspected. This suggests that the increased use of healthcare resources will have a major economic impact. Furthermore, when medicines are prescribed inappropriately, this approach may aid in the development of antibiotic resistance. However, if treatment is delayed in situations of genuine sepsis, surviving preterm children may experience lifelong consequences like neurodevelopmental delay, along with all the related social and financial expenses. Consequently, since delayed treatment raises the risk of death, an early diagnosis of sepsis is crucial .[24]

– ***Microbiological culture methods***

The "gold standard" for confirming the diagnosis of newborn sepsis is still conventional culture methods. The time to organism detection has been shortened to 24–48 hours with the advent of automated technologies that identify the existence of growth from bacterial CO_2 generation. The volume of blood obtained, the time of collection, and the number of samples taken are all variables that could affect the pathogen recovery from the blood . [25]

Blood culture sensitivity may be reduced in newborns with low or sporadic bacteremia and maternal intrapartum antibiotic exposure. Bacterial antibiotic resistance and a delay in focused antimicrobial therapy may result from increased exposure to broad-spectrum antibiotics caused by delays in pathogen identification and antibiotic susceptibility testing. [25]

In the newborn era, the microorganism's growth in blood culture is diagnostic; however, its absence does not rule out the diagnosis. Lack of culture growth could be caused by a small sample size, the mother's usage of antibiotics, the dosage of antibiotics given prior to sampling, low blood bacterial counts, or transient bacteremia .[26]

Ninety six percent of cultures acquired prior to antibiotic administration were positive at the end of the 48th hour, and 98% at the end of the 72nd hour, according to results obtained using manual laboratory methods .[27]

Recent years have seen the development of a number of automated devices that effectively identify bacterial growth before traditional culture techniques. The automated blood culture system BacT/Alert is one example of such a system. Compared to the traditional approach, the BacT/Alert system enables greater yields and shorter detection times for the different species[(28)] . According to Wynn[19], the following crucial elements affect blood cultures' capacity to identify key organisms:

- Volume of blood drawn.
- Dilution: the ratio of blood to culture medium in the blood culture bottle.
- Number of cultures taken.
- The blood culture method, which includes selecting the culture site and preparing the skin.
- Timing of culture.
- The selection of the blood culture system and bottle, including whether it detects anaerobic or aerobic organisms more preferentially.

Hematological Markers in Neonatal Sepsis Diagnosis

Complete blood count, white blood cell count (WBC), absolute neutrophil count, and the ratio of immature neutrophil count to total neutrophil count (I/T) are among the many tests that have been done on the diagnosis of newborn sepsis . [17]

The time and location of sample collection, the baby's gestational week, and variables other than sepsis can all have an impact on the normal WBC number, despite its extremely broad range. Conditions including preeclampsia, intraventricular hemorrhage, fetal asphyxia, meconium aspiration, pneumothorax, convulsions, and continuous crying are among the additional variables besides sepsis that alter the WBC value . [19]

The neutrophil count is another metric used to evaluate sepsis among full blood counts. When diagnosing sepsis, neutropenia is more useful than neutrophilia, particularly within the first 48 hours after birth.[8]

One non-specific late finding of newborn sepsis is thrombocytopenia. It was discovered that sepsis is linked to platelet counts below 100,000/mm3 during the first 10 days of the postnatal period and below 150,000/mm3 in subsequent periods. Viral infections can also cause thrombocytopenia, which is more common in conjunction with bacterial infections . [18]

▪ ***Inflammatory Biomarkers***

The scientific community has been swiftly and accurately guided to look for new diagnostic tools or particular approaches due to the severity of the illness and the challenge faced by caregivers in diagnosing newborn sepsis [25]. The use of biomarkers provided a highly bright outlook for the subject. The perfect biomarker should be highly accurate in promptly identifying whether newborn sepsis is present or not . [29]

– ***C-reactive Protein***

The biomarker that has been examined the most is C-reactive protein (CRP). In response to bacterial infections, serum CRP concentrations increase within 10 to 12 hours and peak 36 to 48 hours later; the values are correlated with the severity of the illness. It is unreliable for early identification of newborn sepsis (poor sensitivity) due to the elevation delay. Additionally, high CRP levels can also be caused by various non-infectious maternal and newborn diseases.

Serial measurements of CRP at 24 to 48 hours after the onset of symptoms have been demonstrated to increase its sensitivity and negative predictive value, and they may be helpful for tracking treatment response in infected neonates receiving antibiotics .[25]

– ***Procalcitonin***

As a response to cytokine stimulation, monocytes and hepatocytes produce procalcitonin, a prohormone of calcitonin. It rises until postnatal day's two to four after birth. Interferon-γ, a cytokine frequently generated in viral infections, downregulates PCT. As a result, PCT has become a viable biomarker for bacterial infection diagnosis that could help distinguish between viral and bacterial causes. PCT is a more sensitive test than CRP for the early detection of newborn sepsis because it rises quickly within 2-4 hours and peaks within 6-8 hours after exposure to bacterial endotoxins .[25]

– ***Serum Amyloid A***

Proinflammatory cytokines control the production of serum amyloid A (SAA), another acute phase reactant produced by hepatocytes, monocytes, endothelium, and smooth muscle cells 8–24 hours after bacterial contact. The highest amounts of SAA are found in old age, whereas the lowest levels are found in umbilical cord blood. SAA levels rise with age. SAA levels can rise up to 1000 times higher than baseline in response to infection or injury, but they can also be greatly impacted by the patient's nutritional state and hepatic function .[25]

– ***Cytokines and Chemokines***

Throughout the course of neonatal sepsis, cytokine levels fluctuate quickly. The main cause of the rise of cytokines, including IL-6, IL-1b, SIL2R, IL-8, and TNF-a, is bacterial infection. Clinical symptoms or positive results from routine diagnostic tests are preceded by this increase. Additionally, the risk of infection is indicated by the newborn's cytokine levels during the first few hours of life .[30]

The fact that more than 3000 sepsis biomarker studies have been published, with nearly 200 potential biomarkers assessed, reflects the difficulty of biomarker identification. Nevertheless, no one biomarker possesses adequate diagnostic precision to identify newborn sepsis. Combining different biomarkers or measuring them one after the other could be ways to improve the accuracy of the diagnosis .[25]

Molecular Methods

Methods of nucleic acid analysis are crucial for identifying the morphological, metabolic, or cytopathic characteristics of microorganisms that grow slowly or cannot be cultured. In order to diagnose sepsis, molecular technologies are being researched to enable quick identification of both gram-positive and gram-negative bacteria. There are several ways to amplify nucleic acids, including signal duplication (branched-probe DNA determination), replication of the nucleic acid probe (ligase chain reaction), and replication of the target nucleic acid (polymerase chain reaction) . [25]

Conserved portions of the 16S ribosomal RNA gene, which is unique to bacteria and absent from other organisms, are the target of polymerase chain reaction. PCR analysis was carried out both before and after the start of anti-biotherapy in clinically suspected sepsis cases in the study by Dutta et al. The sensitivity, specificity, positive, and negative predictive values of PCR were determined to be 96.2%, 96.3%, 87.7%, and 98.8%, respectively. Patients who tested positive for PCR in the sample obtained prior to anti-biotherapy continued to test positive 12 hours after anti-biotherapy [31].

Real-time PCR has been used by researchers in a number of studies to diagnose neonatal sepsis, and a recent meta-analysis suggests that PCR could be a useful diagnostic technique. For instance, Jordan et al. have carried out multiple investigations employing broad-range 16S ribosomal RNA gene PCR to quickly identify sepsis in a neonatal population at low risk. In another intriguing study, researchers were able to predict the more severe course of gram-negative sepsis by effectively using a gram-specific PCR to differentiate between gram-positive and gram-negative sepsis [32] .

CONCLUSIONS

Traditional culture methods, while considered the gold standard, fail to provide actionable data within the critical early hours of sepsis onset. Emerging technologies like real-time PCR and automated culture systems (BacT/Alert) demonstrate the potential to slash detection times from days to hours. Biomarker panels (e.g., PCT + IL-6 + SAA) offer improved specificity over isolated CRP testing but lack standardization. The future lies in algorithms integrating multi-omics data with clinical variables to generate risk scores tailored to gestational age and birth context.

Abbreviations:

Early onset neonatal sepsis(EONS),Late onset neonatal sepsis(LONS),Neonatal intensive care units(NICUS),group B streptococcal(GBS),National Neonatal Perinatal Database(NNPD),United kingdom(UK),Staphylococcus aureus(S.aureus),Herpes simplex virus(HSV),Antigen presenting cells(APCs),T helper cells(Th cells),Carbon dioxide(Co2),Whitebloodcells(WBC),Immature neutrophil count to total neutrophil count(I/T),Creactiveprotein(CRP),Procalcitonin(PCT),Serum amyloid A(SAA),Interleukin-6(IL-6),Interleukin-8(IL-8),Interleukin-1b(IL-1b),soluble interleukin 2R(SIL-2R),Tumor necrosis factor-a(TNF-a),Polymerase chain reaction (PCR),Deoxyribonucleic acid(DNA),Ribonucleic acid(RNA).

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