Biomarkers in early-Onset Neonatal Sepsis: An Update

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Abstract

Globally, an estimated 500,000 newborns die each year from serious neonatal infections, which account for about 15% of all neonatal deaths. “Suspected sepsis” is one of the most common reasons for admission to the neonatal intensive care unit (NICU). The challenge to the physician has always been trying to differentiate newborns with early onset sepsis from other non-infectious pathology whose clinical features overlap with sepsis. Current diagnosis of early onset sepsis is a combination of clinical presentation, non-specific biomarkers and blood culture. Traditional biomarkers such as complete blood count (CBC) and C-reactive protein (CRP) have poor sensitivity and positive predictive value. Current literature suggests the potential utilities of novel biomarkers such as cell surface markers (CD11B, CD64), genomics, proteomics and advanced molecular techniques in the diagnosis of neonatal sepsis. An ideal biomarker or a combination of biomarkers will help clinicians in the judicious use of antibiotics in early onset sepsis.

ABBREVIATIONS

EOS: Early Onset Sepsis; CRP: C-reactive protein; PCT: Procalcitonin

INTRODUCTION

Sepsis continues to present a significant problem in the neonatal intensive care units (NICU) worldwide [1]. Especially, in this age of advanced neonatal care, sepsis burden assumes even greater significance as extremely preterm infants are increasingly being cared for in the NICUs. Sepsis in premature infants is an important cause of morbidity and mortality [2,3]. The risk of sepsis increases with decreasing gestational age and birth weight. Neonatal sepsis has traditionally been classified as early-onset sepsis (EOS), if the onset of infection is in the first 72 hours, and late-onset sepsis (LOS) if the onset of infection occurs after the first 3 days of life. This classification is helpful in targeting the presumed bacterial pathogens based on the timing of their acquisition. Typically, EOS is attributed to pathogens acquired by vertical transmission from the maternal genital tract and LOS is caused by pathogens acquired by horizontal transmission from the caregivers and environment.

Epidemiology

Neonatal infections account for the majority of neonatal deaths worldwide [1]. Globally, an estimated 500,000 newborns die each year from serious neonatal infections, which account for about 15% of all neonatal deaths [4]. The incidence of EOS is ~ 0.7-1/1000 live births in developed world [5,6]. Based on few hospital based studies performed in developing countries, the incidence of EOS ranges anywhere from 2.2 to 9.8 per 1000 live births [7,8]. Neonatal sepsis also increases short term and long term morbidities such as necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA), prolonged ventilation, neurodevelopmental impairment and prolonged hospital stay [9-12]. Shatrov et al in their meta-analysis showed significant association between chorioamnionitis (clinical or histological) and cerebral palsy in preterm and term children [13].

Enigma of “Suspected Sepsis”

“Rule out sepsis” or “Suspected sepsis” is one of the most common reasons for admission to a NICU. The challenge to the physician has always been trying to differentiate newborns with sepsis from other non-infectious pathology whose clinical features overlap with sepsis. Given the morbidities associated with delayed identification of sepsis, the number of sepsis evaluations performed in this population is very high. Several studies have attempted to identify neonates who are infected at birth [14-17]. These studies focused on laboratory tests, including total and differential white blood counts (WBC), C-reactive protein (CRP), erythrocyte sedimentation rate and clinical symptoms to select infants at high risk for sepsis. Similar to clinical signs of sepsis, the conventional laboratory tests are also less reliable in the newborn period. In the absence of an unequivocal evidence to prove or disprove infection, empirical use of antibiotics is widely prevalent. But widespread use of antibiotics is not without potential adverse effects. In a study from the Neonatal Research Network, prolonged empirical antibiotic use in extremely low birth weight infants (ELBW) was associated with NEC and
death [18]. The other major problem worldwide as a result of a widespread antibiotic use is antimicrobial resistance [19,20] and hence there is an increased focus on antibiotic stewardship. American Academy of Pediatrics (AAP) in its latest guidelines has advocated withholding antibiotics in low risk neonates pending blood culture results [21]. A myriad of studies have been conducted on various biological markers that may be useful in the early diagnosis of sepsis [17,27,43,45,46]. This review will focus on the utility of the biomarkers that have been investigated in early onset sepsis in neonates.

**Biomarkers**

The sepsis work upon neonates includes complete blood count (CBC), blood culture, and lumbar puncture [22]. Acute phase reactants, especially CRP and procalcitonin are also part of the sepsis workup in some units. Depending on the clinical scenario, tracheal aspirate culture in intubated patients, urine culture and chest x-ray are also used in the evaluation of EOS.

**Complete Blood Count:** WBC counts and the components of WBC, absolute neutrophil count (ANC), absolute band count and the ratio of immature to total neutrophils (I/T) in the blood are parameters that are commonly used as screening tests for the diagnosis of sepsis.

Total leukocyte counts (5000/mm$^3$ to 30000/mm$^3$) although commonly used have a poor positive predictive value and have poor diagnostic accuracy (low sensitivity and specificity) in sepsis, [23,24]. Neutropenia is a more reliable marker for neonatal sepsis, but the definition of neutropenia is dependent on gestational age, delivery method (cesarean delivery without labor result in lower counts than vaginal delivery) and altitude (elevated altitudes have higher total neutrophil counts), [14,25,26]. The definition of neutropenia in term and late preterm infants is a neutrophil count <1800/mm$^3$ at birth and <7800/mm$^3$ at 12–14 hours of age based on Manroe et al. [14]. Based on Schmutz et al, neutrophil counts increased over the first several hours following delivery, with peak values occurring between 6 and 8h for infants more than 28 weeks gestation and between 12 and 24h for infants delivered at less than 28 weeks [25].

Immature to total neutrophil count (I/T) ratio is considered to be more specific than total leukocyte count in sepsis. In contrast to absolute neutrophil count (ANC) and band count, I/T ratio is at its peak (0.16) at birth and decreases to 0.12 with increasing postnatal age. A single cut off value of 0.3 has a high negative predictive value (NPV) (99%) but a poor positive predictive value (25%) [27]. I/T ratio of >0.2 is suspicious for sepsis. Notable inter-reader differences in identification of bands in the blood smear decreases its reliability in clinical practice. A retrospective study by Murphy et al. showed that a negative blood culture at 24h of age along with two serial normal WBC screens separated by 8 to 12 hours had a very high negative predictive value (100%) in neonates empirically started on antibiotics at birth [28]. Overall, low ANC, high band count and high I/T ratio are associated with an increased risk of infection. In practice, it may be advantageous to wait at least 6h after birth to obtain CBC, as multiple studies have shown WBC and ANC to increase for the first 6h after birth and also an established inflammatory response is a requisite for inducing a noticeable leftward shift in the WBC [14,29,30].

In reality, components of WBC, i.e. ANC and I/T ratio have been shown to be more useful for excluding infants without infection rather than identifying newborns who are infected [21].

**Platelet counts:** Platelet counts are characterized by poor sensitivity and specificity for the diagnosis of neonatal sepsis and hence are unreliable for initiating or discontinuing antibiotics [31,32]. Mean platelet volume is also a poor marker of neonatal sepsis [33].

**C-reactive protein (CRP):** CRP is an acute phase reactant produced in the liver and an extensively studied biomarker in neonatal sepsis. Inflammatory triggers like sepsis induces the release of the cytokine IL-6 from various immune cells which in turn stimulates the release of CRP. This increase in CRP is noted within 8h of infection and peaks at 24h [34,35]. CRP levels change significantly from baseline at 10-12h after onset of infection. Hence a rising CRP is a strong predictor of infection. CRP should be obtained 12-24h after onset of infection. Most studies use a value of 10mg/L as a cut-off [36-38]. In viral infections the levels usually do not rise above 5mg/L [38,39]. A low CRP (<10mg/L) at 18h has a negative predictive value of 93% in EOS [40]. Benitz et al. showed that serial normal CRP values are a strong evidence to rule out bacterial sepsis [38]. The pitfalls of using CRP are; it is a “late” marker of sepsis and it is elevated in non-infective conditions like meconium aspiration, hemolysis and ischemia. In preterm infants, baseline CRP values are lower than term infants [41].

**Procalcitonin:** Procalcitonin (PCT) is a propeptide of calcitonin, produced by macrophages and hepatocytes. Serum PCT levels increase rapidly in 2-4h and peaks at 6-12h in response to bacterial infection [42]. PCT levels are elevated in early and late onset neonatal sepsis and necrotizing enterocolitis [43]. A meta-analysis of 29 studies revealed a sensitivity of 81% (74–97%) and specificity of 79% (69–87%) [44]. The advantages of PCT over CRP in sepsis include: rapid rise in response to infection (useful in EOS), levels decline with control of infection (half-life is 24h), not typically affected by viral infections (specific for bacterial sepsis) and it correlates with severity of infection [45].

PCT also has its own limitations. PCT is increased in newborns requiring neonatal resuscitation, maternal GBS colonization and prolonged rupture of membranes ≥18h [43,46]. PCT in healthy neonates increases gradually from birth to its peak at 24h and decrease by 48h [43]. Therefore, to improve its diagnostic accuracy, specific cutoff values needs to be established with respect to different gestational age and postnatal age. Overall, in EOS, PCT might be a better biomarker than CRP [47,48].

**Cytokine Profile:** Cytokines play a vital role in sepsis. They are produced by neutrophils and various other immune cells in response to sepsis. It is well established that levels of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-8 are elevated in the serum in sepsis. Studies in neonates have shown that proinflammatory cytokines such as IL-6, IL-8, and TNFα level were higher in septic neonates than controls [49,50]. IL-6 is an inducer of CRP and has been extensively studied among all the cytokines in sepsis. IL-6 is a very early marker with a very high sensitivity (75-90%) in EOS [51,52]. The limitation is that it has short half-life and hence less useful...
if not obtained within the first few hours of life [51]. In current scenario, measuring cytokines for diagnosis of EOS may not be practical or cost-effective because enzyme immunoassay is expensive and time consuming. An easily available, inexpensive automated assay in the future might make cytokines an attractive tool in the evaluation of neonatal sepsis. Evaluation of a composite set of markers involving acute phase reactants, leukocytes and cytokines/chemokines may increase sensitivity and specificity in the diagnosis of sepsis, for e.g. CRP and IL-6 [53], CRP and leukocyte indices [54]. The lack of widespread hospital laboratory availability of the tests and the need for serial measurements may be limiting issues for their routine use.

**Cell surface markers (Neutrophil CD64 and Neutrophil/Monocyte CD11b):** Various cell surface antigen markers are activated by infection and their detection is made possible by advances in flow cytometry technologies [55,56]. Neutrophil CD64 and neutrophil/monocyte CD11b are such cell surface antigenic markers that are activated by bacteria and therefore could be a potential tool in the diagnosis of neonatal sepsis. A recent study by Genel et al. showed that the expressions of CD64 and CD11b were significantly enhanced in the infection group compared to the non-infective group and the controls. There was no difference between the non-infected and control groups [57]. There may be several advantages in using CD64: a) Studies require minimal blood (50 µL of whole blood), b) results are obtained in (few hours within 4 hours), c) the persistent expression of CD64 for at least 24 hours gives the marker a wide diagnostic window (in contrast to IL-6) and d) reliability is proven in neonatal sepsis [58,59]. High cost and availability of resources are likely the barriers for its use in clinical practice.

**Molecular Techniques for Early Detection of Neonatal Sepsis:** Molecular techniques for diagnosis of infection may be useful in infants whose mothers have received intrapartum antibiotics, may provide rapid results and have better sensitivity compared to blood cultures. Amplification methods such as polymerase chain reaction (PCR) for the bacterial 16S rRNA gene or hybridization methods such as microarrays have been evaluated in clinical studies in neonates. In a meta-analysis of 23 included studies on PCR-based molecular methods, the summary estimates of sensitivity and specificity with 95% CI intervals were generated using the bivariate random effects model [60]. Mean sensitivity and specificity were 0.90 (95% CI, 0.78 to 0.95) and 0.96 (95% CI, 0.94 to 0.97) respectively. The authors conclude that molecular assays do not have sufficient sensitivity to replace microbial cultures in the diagnosis of neonatal sepsis but may perform well as ‘add on’ tests.

**Genomics and Proteomics:** In the quest to discover novel biomarkers with high sensitivity and specificity in sepsis, the fields of proteomics and genomics are helping us understand the fundamental mechanisms of sepsis. In sepsis, genomics is used to identify genes that are preferentially upregulated in infection using sophisticated DNA sequencing methods and bioinformatics [61]. Host genetic background is an important factor influencing the host response to infection. Genomics potentially could uncover this host genetic variability that likely results in the variable clinical presentation and outcome of neonates infected with similar pathogens [62]. Proteomics is used to analyze the structure, function and interaction of specific proteins that are elevated in blood or other samples as a result of infection using mass spectrometric methods. Proteomic methods have identified several proteins, that are increased in neonatal sepsis [63]. Proteomic analyses of amniotic fluids have also provided useful information regarding the fetal response to intra-amniotic inflammation [64]. Currently, novel methods using genomics and proteomics are being researched that can be translated to the bedside in the future, to guide therapy decisions.

**Suggested pragmatic approach to common clinical scenarios in EOS [65]**

[i] Asymptomatic neonates born to mothers diagnosed and or treated for chorioamnionitis:

- **Intervention:** Obtain CBC, CRP/PCT (optional) at 6-12h after birth and blood culture. Start empirical antibiotics based on institutional antibiotic protocols.

  a) If normal labs, negative blood culture and well appearing newborn: Discontinue antibiotics at 48h. In preterm infants, consider discontinuing antibiotics after 48-72h, given the higher risk of infection in premature infants.

  b) If abnormal labs, negative blood culture and well appearing newborn: There is a dichotomy in expert opinion in this scenario due to paucity of scientific evidence. The two approaches are as follows.

    - **Physician may elect to continue antibiotics for a longer period of time (5-7 days)** if there are strong risk factors for infection, especially in the setting where mothers received antibiotics, which make postnatal blood cultures less sensitive.

Some clinicians may choose to discontinue antibiotics after 48-72h. The rationale to discontinue antibiotics despite ‘abnormal’ labs is that the supplementary tests for neonatal sepsis are better at excluding sepsis but not very reliable in predicting the presence of sepsis (40% or less) [54]. Although a 40% risk of sepsis cannot be written off, it is highly unlikely for a well appearing newborn who is feeding well with normal physical examination at 48h to have sepsis [66, 67].

[ii] Asymptomatic term and late preterm neonates with an antenatal risk of infection (prolonged rupture of membranes>18h, foul smelling liquor) may be observed for 48h with no antibiotics after obtaining blood culture at birth. Infants not started on antibiotics must be monitored closely for at least 48 hours (vitals check every 2-4h) before discharge.

[iii] Symptomatic term neonates with no antenatal risk of infection and with evidence of rapidly improving symptoms in the first few hours of life (4-6h), may have their antimicrobial therapy withheld and monitored clinically. As mentioned above, clinical features of neonatal sepsis at birth, such as tachypnea overlap with those of non-infectious etiologies such as transient tachypnea of newborn (TTN).

**SUMMARY AND CONCLUSION**

Despite several studies on various diagnostic markers, further research is needed to identify a rapid, reliable and an inexpensive biomarker. CBC, CRP and procalcitonin are currently the most
commonly used biomarkers in clinical practice in the setting of EOS. In the absence of an ideal marker to rule in or rule out an infection, physicians need to rely on clinical symptomatology and temporal course of the symptomatology and monitoring. More reliable biomarkers will help clinicians in the judicious use of antibiotics that will decrease antimicrobial resistance. The goal of sepsis evaluation is not only to avoid sepsis related morbidity and mortality but also to avoid the unwanted exposure to antibiotics.

REFERENCES


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