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Recent biomarkers of neonatal sepsis; review article

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Abstract

Neonatal sepsis is a serious infection that is caused by bacterial, fungal or viral infection. Neonatal sepsis is a common cause of morbidity and mortality in neonatal intensive care unit (NICU). Neonatal sepsis is classified into early-onset sepsis (EOS) and late-onset sepsis (LOS) according to timing of presentation and mode of acquisition with early-onset sepsis symptoms manifesting in the first 72 hours of life. The diagnosis of newborn sepsis is still difficult. Due to the nonspecific signs and symptoms of neonatal sepsis that overlap with other conditions or even normal birth processes; different early objective diagnostic tests particularly in preterm infants, make it difficult to diagnose neonatal sepsis. The search for an ideal biomarker for diagnosis of neonatal sepsis is still ongoing. For a biomarker to be ideal it needs to diagnose neonatal sepsis early. Blood culture is the cornerstone of the investigations used for diagnosis of neonatal sepsis. This article aimed to describe the recent biomarkers available and their role in the early diagnosis, treatment, and prognosis of neonatal sepsis. It also explores the possible advances and future prospects of these biomarkers. Traditional haematological and microbiological techniques used to detect newborn sepsis remain ineffective in the face of high mortality and severe morbidity. The possible utilization of novel technological advances and changing awareness of present biomarkers' strengths and limits has improved biomarkers that offer early specific and reliable identification of the neonate at risk of the infection and aid decision of the optimal timing of antibiotic initiation and stoppage.

Keywords: c-reactive proteins (crp), infection, metabolomics, serum biomarkers, neonatal sepsis

Full length article **Corresponding Author*, e-mail: manar.anwar@mu.edu.eg

1. Introduction

Neonatal sepsis, or illness caused by systemic bacterial infection, is a major cause of pediatric morbidity and mortality. The 2015 Global Burden of Disease study identified neonatal sepsis as the third most common cause of newborn mortality (336300 total deaths per year) and the 16th greatest contributor to years of lost life across all age groups. [1]. Clinically, EOS and LOS present with the same constellation of features. In both, an early phase of mild and easily missed signs will- if left untreated- progressto severe illness with vital sign instability, central nervous system manifestations such as irritability, lethargy, or seizures, and, ultimately, multi-organ system dysfunction and failure. [2]. Neonatal sepsis is a condition that continues to puzzle us with its variety of symptoms, its unpredictability, and the difficulty of correctly diagnosing it. Thereis to date neither solitary biomarker nor combination of biomarkers available for correctly discriminating neonatal sepsis from trauma, tissue damage or even the normal birth process, especially in preterm infants [3]. Diagnostic tests other than blood culture have poor positive predictive value and are not helpful in deciding which newborns require antibiotic treatment. A complete blood count is also recommended, in particular for the leucocyte and platelet counts, as well as the leucocyte differential. [4]. Inflammatory markers are also non-specific but can be drawn at the time of the blood culture to set a baseline value to trend over time. C reactive protein (CRP) is an acute-phase reactant that peaks within 24-48 hours of infection onset. The negative predictive value of two normal CRP values (the first 8-24 hours after birth and the second 24 hours later) is 99.7%. [4]. One might argue that a combination of a fast acting and a slow acting biomarker solves this issue, but even though the fast-acting biomarkers rise quickly enough, they also generally drop below the cut-off limit before the slow acting biomarkers have even begun to rise. Aim of the work was to evaluate the diagnostic value of serum amyloid A and salivary C-reactiveprotein in diagnosis of neonatal sepsis.

2. Epidemiology

In 2010 worldwide, 7.6 million children less than 5 years old died, predominantly due to infectious causes including sepsis; neonatal deaths (in the first 28 days of life), accounted for 40% of the total lives lost [5]. In 1990, both the United Nations (UN) and World Health Organization (WHO) prioritized a 2/3rd reduction in the unacceptable child mortality rate by 2015. However, in 2013, 44% of deaths in children under the age of five occurred during the neonatal

period, up from 37% in 1990. Despite major advances in neonatal care and increasing research, in developed countries, four of every ten infants with sepsis die or experience major disability including significant permanent neurodevelopmental impairment [6]. A study from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Neonatal Research Network documented rates of culture-confirmed early-onset sepsis among almost 400000 livebirths at network centers [7]. The overall rate of early-onset sepsis, defined as a positive blood or CSF bacterial culture at less than 72 hours of age, was 0.98 infections per 1000 livebirths, with rates inversely related to birthweight (10·96 per 1000 livebirths for 401–1500 g birthweight, 1·38 for 1501–2500 g birthweight, 0·57 for >2500 g birthweight) [7]. 2·5 million sepsis-related hospital admissions (30.8 per 1000 livebirths) were noted from 1988 to 2006 in infants less than 3 months of age in a cross-sectional study of records contained in the US National Hospital Discharge Survey [8]. The authors noted that episodes of clinical neonatal sepsis declined following the widespread implementation of intrapartum antimicrobial prophylaxis (IAP) that paralleled declines in GBS early-onset neonatal sepsis. A modest but steady decline occurred in hospital admission rates for full-term infants, and less so for preterm infants during the surveillance period [8]. By comparison, a retrospective study from the Canadian Neonatal Network of early-onset sepsis, defined as a bacterial isolate from culture of blood or CSF obtained from infants in the first 72 h of life who were admitted to neonatal intensive care units, revealed an early-onset neonatal sepsis rate of 6.8 per 1000 admissions from 2003 to 2005 and 6.2 per 1000 admissions from 2006 to 2008. Similar to observations in the USA, the authors noted a significant reduction in GBS and an increased isolation rate of non-GBS organisms as possible causes of early-onset neonatal sepsis [9]. Risk factors for EOS and LOS vary by the nature of pathogen acquisition, though the primary characteristic that places neonates at greatest risk for infection is decreased gestational age, regardless of the mechanism of transmission. Neonates of extreme prematurity and very low birth weight (VLBW), defined as less than 1500 g, and are more likely than term infants to be diagnosed with sepsis [10]. While maternal factors primarily influence risk of EOS, neonatal characteristics primarily influence risk of LOS. Neonatal factors include prematurity, VLBW status, and the presence of congenital anomalies. Infants with these factors often require invasive devices, delayed enteral feeding, medications, and complex management in a neonatal intensive care unit [10]. Central venous catheters and endotracheal tubes, both commonly required in these groups of neonates, allow for direct pathogen entrance. Delayed enteral feedings and the administration of certain medications (ie, antibiotics, histamine receptor antagonists, and proton pump inhibitors) affect the neonate's microbiome and contribute to pathogenic vulnerability [11]. In addition to neonatal characteristics, external factors have been shown to contribute to the occurrence of LOS. A high acuity unit with increased workload can lead to decreased compliance with infection prevention measures and a significant elevation in LOS risk [12].

3. Clinical diagnosis of neonatal sepsis and its challenges

Although a formal consensus definition of neonatal sepsis has not been developed, it is generally defined as an infection causing organ dysfunction because of a dysregulated response. As shown, there are many causes of organ dysfunction in infants, particularly those who are premature. Therefore, a careful evaluation for infection and organ dysfunction is indicated before assigning sepsis as the cause of these clinical signs [13].

3.1. Neonatal Sequential Organ Failure Assessment (nSOFA) Score

This particular score, nSOFA, is an objective, automated electronic data capture related to neonatal sequential organ failure for predicting mortality from LOS among preterm, very-low-birth-weight (VLBW) neonates. Neonates with an nSOFA score of more than 4 had a higher mortality compared to those with a score less than 4 [15, 16]. (nSOFA) scores are useful for defining organ dysfunction associated with mortality risk and are not affected by pathogen, sex, hospital, or extreme prematurity. This score has scope of providing a foundation for building a consensus definition for sepsis among preterm neonates [15, 16].

4. Biomarkers for the diagnosis of neonatal sepsis

4.1. Microbiological culture methods

Conventional culture techniques remain the "gold standard" to confirm the diagnosis of neonatal sepsis. 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**.** 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 [17].

4.2. 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 [18]. 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) [19] 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 staining [20]. 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 genes [18]. Recent advances in molecular techniques enable amplification of microbial pathogens directly from whole blood samples in fewer than 12 h without relying on initial microbial growth in blood cultures [18]. A systematic review concluded that molecular diagnostics had value as adjunctive tests with an overall sensitivity of 90% and specificity of 96% [21]. 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 [22].

4.3. Hematological Indices

Leukocyte (<5000 or ≥20000/mm3), absolute neutrophil (<1000 or ≥5000/mm3) 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 sepsis **[23]**.

4.3.1. White blood cell count (WBC)

Leukocyte count starts between 6000 and 30000/mm^3 in the first day of life and decreases to 5000–20000 mm^3 later. Neutrophil count tends to be lower at lower gestational ages (GA) and peaks 6–8 h after birth [24]. A literature review by Sharma et al reported that leucopenia (WBC count <5000/mm3) has a low sensitivity (29%) but high specificity (91%) for diagnosis of neonatal sepsis [25]. 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 [26]. 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 30354 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) [24]. Mean neutrophil volume >157 arbitrary units had sensitivity and specificity as 79% and 82% while sensitivity and specificity of CRP were 72% and 99%, respectively [27].

4.3.2. Immature to Total Neutrophil (I:T) Ratio

Compared to other hematological markers, I:T ratio may be the most sensitive indicator of neonatal sepsis **[**28], 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 sepsis [28].

4.3.3. 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 [29].

4.4. Inflammatory Biomarkers

4.4.1. Acute phase reactant

Acute phase reactants are produced by the liver in response to cytokines, which are induced by infection and tissue injury. TNFα, CRP, PCT, fibronectin, haptoglobin, pro-adrenomedullin (pro-ADM) and SAA have been evaluated in neonatal sepsis [22].

4.4.2. C - reactive protein

C-reactive protein (CRP) has been the most studied biomarker. 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 severity [30]. 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 antibiotics [30].

4.4.3. 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 2–4 [32]. Furthermore, serial PCT determinations allow shortening the duration of antibiotic therapy in term and near-term infants with suspected early-onset sepsis [33]. However, before this PCT-guided strategy can be recommended, its safety and reliability must be confirmed in a larger cohort of neonates [22].

4.4.4. 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 age [34]. 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 status [35].

4.4.5. Proadrenomedullin

It is a stable precursor of ADM, which modulates circulation, has antimicrobial properties and protects against organ damage, 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 sepsis [36].

4.4.6. Adipokines

These 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 neonates [37].

4.4.7. 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 sepsis [38]. But limitation of these markers includes no normative data in neonates, physiological increase in the first month of life and lack of large studies [22].

Table 1. Common symptoms of neonatal sepsis [14]

System	Symptoms
General	Fever, temperature instability; "not doing well", or poor feeding
Gastrointestinal system	Abdominal distension, vomiting, diarrhea, or hepatomegaly
Respiratory system	Apnea, dyspnea, tachypnea, retractions, flaring, grunting, or cyanosis
Renal	Oliguria
Cardiovascular	Pallor, mottling, cold, clammy skin, tachycardia, hypotension, or bradycardia
Central nervous system	Irritability, lethargy, tremors, seizures, hyporeflexia, or hypotonia

4.4.8. Interleukins

IL-6 increases immediately after exposure to pathogens and normalizes in 24 h 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 neonates [39]. Kurul et al., [40] showed that IL-6 (>580 pg/mL) and PCT (>0.94 ng/mL) were associated with 7-day mortality while CRP was not. 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 CRP [41]. 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/m/L, the sensitivity was 79% and specificity was 81% and better accuracy in LOS than EOS [42].

5. Cell adhesion molecules

GA, timing of sepsis onset, type of microorganism or non-infectious diseases [43], did not affect these markers increase in minutes after infection and levels. Limitation of these markers are need of high technology and non-standardized normal ranges [22]. Cluster differentiation molecule-64 (CD64) expressed from neutrophils and monocytes facilities 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, respectively [44]. Increased CD11b expression was found both in EOS and LOS with high sensitivity and specificity up to 100% (Delanghe & Speeckaert, 2015)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, respectively [45]. The challenge of biomarker identification is reflected by the fact that over 3000 sepsis biomarker studies have been published with almost 200 candidate biomarkers evaluated [46]. 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 sepsis [47].

6. Strategies for the future

6.1. 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 positivity [48]. Meta-analyses have found that use of MALDI-TOF for diagnosis of infection from culture bottles has acceptable sensitivity and specificity and with higher sensitivity in gram negative infections compared to gram-positive infections [49].

6.2. 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. Novel techniques such as analysis of volatile organic compounds in the breath has been demonstrated to be reasonably sensitive and specific and capable of distinguishing sepsis from inflammation in rat models **[**50], 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 sepsis [51].

6.3. 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 [52]. 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 [53]. 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 treatment [53]. 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 controls [55]. Genomic analysis may determine sepsis risk, treatment response and prognosis while evaluating gene variants responsible for PRPs, signaling molecules and cytokines [53].

6.4. 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 [56]. 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 [57]

6.5. 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 [58].

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