LITERATURE REVIEW

An Evaluation of Novel Immunological Biomarkers for the Diagnosis of Neonatal Sepsis in the Emergency Department

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Abstract

Introduction: Sepsis, a common presentation to the Emergency Department (ED), is characterised by a dysregulated and rapidly progressive immunological response causing multi-organ dysfunction. Neonatal sepsis (NS) occurs in infants less than 28 days old and is one of the most common causes of paediatric death. The current gold standard for diagnosis is blood culture. However, a novel combinational approach looking at levels of biomarkers which elevate upon the onset of bacterial sepsis is recommended for diagnosis and prognosis.

Methods: A literature review on biomarkers of NS in the ED.

Results: MiRNAs exhibit altered patterns in NS, and due to their specificity and ease of detection, they also make for potential high yield markers in the ED.

Discussion: While new biomarkers hold promise, further studies are needed before standardisation and recommendation for clinical practice.

Keywords: Neonatal sepsis, Early-onset sepsis, Biomarkers, Late-onset sepsis, Presepsin, Procalcitonin, microRNA

Introduction

Sepsis is a severe medical condition characterised by a dysregulated immunological host response to infection causing multi-organ organ dysfunction[1]. Sepsis is a common presentation to the emergency department (ED) which can rapidly progress if left untreated. Neonatal sepsis (NS) is defined as sepsis presenting in infants less than 28 days old[2]. NS can be divided into early-onset sepsis (EOS), within the first 72 hours of life, and late-onset sepsis (LOS), which is between the first 72 hours and 28 days of life[2]. Every year, one million deaths occur from NS, with an overall mortality rate of 17.6%[3]. The rapid onset and progression of NS necessitates a timely and accurate diagnosis to enable pathogen-specific treatment. However, current diagnostic methods of NS have a high analytical time period and a low positive predictive value (PPV), which hinder an accurate and timely diagnosis of NS. As such, this paper presents a discussion into the current microbiological diagnostic methods and new promising biomarkers in the evaluation of NS. As EOS is usually diagnosed in the postnatal ward prior to discharge, this paper primarily focuses on LOS as it more commonly presents to the ED due to community-acquired infections.

Epidemiology and Risk Factors

Neonatal sepsis remains one of the most common causes of paediatric mortality and disability worldwide[4,5]. Research indicates that high-income countries present the lowest case fatality rates (CFRs), while the highest CFRs are found in low-income countries[6]. Risk factors associated with NS, particularly LOS, include immaturity, intravascular catheters, mechanical ventilation, prolonged parenteral nutrition, surgery, hospitalisation and underlying cardiovascular or respiratory diseases[7,8].

Pathophysiology and Presentation

Recognition by pattern recognition receptors (PRRs) is the initial step of the immune response in sepsis; the release of inflammatory and regulatory cytokines cause endothelial cell activation, increased adhesion, and localised recruitment of immune cells[10].

In neonates, soluble cytokine/receptor antagonists modulate inflammatory activity[10]. Presepsin is a soluble fragment of CD14, a receptor found on the surface of immune cells which has elevated levels in NS. Similarly, procalcitonin, a precursor molecule to the hormone calcitonin, rises a few hours after bacterial infection. Lastly, microRNAs may regulate gene expression to modulate immune homeostasis in response to NS.

The clinical manifestations of NS can be highly non-specific and encompass a range of symptoms such as fever, respiratory distress, lethargy or irritability, seizures, protruding fontanelles, aversion to feeding, jaundice, haemorrhage, abdominal swelling, and dysregulation of body temperature[10].

Common Causative Agents of Late-Onset Neonatal Sepsis

Compared to EOS, causative agents of LOS are typically acquired from the surrounding environment where transmission is primarily via the mother to their child[2]. The most common causative agent of LOS is coagulase-negative staphylococcal species (CoNS), culpable for more than 50% of LOS in high-income countries[2]. It is important to note that the common causative agents of LOS differ internationally; CoNS is less implicated in LOS in developing countries[10].

In terms of management, neonates promptly receive intravenous (IV) antibiotics, once clinical suspicion for sepsis arises[10,11]. Supportive therapy including cardiopulmonary support, IV nutrition, and blood product transfusion can also be utilised[13].

Diagnosis of Late-onset Neonatal Sepsis

As soon as LOS is suspected, blood samples should be taken via venepuncture, preferably at two peripheral sites, and cultured for both aerobic and anaerobic microbes[13]. Prompt treatment with empiric antibiotics with coverage for nosocomial infection (vancomycin plus an aminoglycoside are recommended) should be started even before confirmation with laboratory data[2].

The gold standard for confirmation of LOS has predominantly been a positive blood culture or polymerase chain reaction (PCR). However, these conventional microbiology laboratory techniques take a considerable amount of time, with blood cultures having a turnaround rate of between 48 and 72 hours[14]. Furthermore, CoNS-positive blood cultures should be interpreted with caution, as CoNS is part of the skin flora and is often a common contaminant[10]. The National Institute of Child Health and Human Development (NICHD) Neonatal Research Network published that in order to confirm a culture-proven diagnosis of CoNS sepsis, two positive blood cultures or one positive blood culture alongside elevated C-reactive protein (CRP) are required, due to the high likelihood of contamination in blood cultures[10].

Recent studies have also emerged that question the sensitivity of blood cultures in proving LOS infections. In a study conducted on post-mortem haematological findings of newborns, pre-mortem blood cultures were negative in 14% of newborns with confirmed infection at autopsy[15]. Additionally, another study reported that only 8.9% of 164,744 blood cultures obtained from very low birth weight (VLBW) infants with clinically suspected LOS were positive[15].

Wynn suggests that when diagnosing LOS there may be a significant risk of false negatives in blood culture[15]. Potential reasons for decreased sensitivity of blood cultures include low blood volume drawn, improper timing of blood collection, sub-optimal number of samples collected, prenatal antibiotic use, and limited laboratory capabilities[14]. Zea-Vera and Ochoa report that culture negative cases represent the majority of NS cases in developing countries[10]. The possible decreased sensitivity of blood cultures due to limited laboratory or hospital resources should be taken into consideration when interpreting negative blood cultures, especially in developing countries. Furthermore, regular optimal blood draws (≥1ml) may be hard to achieve in very low birth weight (VLBW) infants and may increase the need for blood transfusions, further adding to the limitations of blood cultures14. In addition, 68% of septic infants present with low level bacteremia (≤10 colony forming units (CFU)/ml), with 42% of septic infants yielding ≤1 CFU/ml. This only further increases the likelihood of a false negative result, with a study showing that low level bacteremia coupled with suboptimal blood draws (0.5ml) can yield false negative results in up to 60% of cases[10]. This may suggest that a negative blood culture alone, especially when conducted under suboptimal conditions, might be inadequate to rule out LOS. Additionally, the ratio of culture-confirmed to culture-negative sepsis cases in term-infants admitted to neonatal units in Norway was 91:1447 (1:16)[16], further questioning the reliability of a negative blood culture for ruling out LOS.

Other biomarkers commonly used in aiding the diagnosis of sepsis include C-reactive protein (CRP) and elevated immature to total (I:T) neutrophil ratio[2]. However, these biomarkers have a low PPV, and thus are typically used as "rule-out" tests[2,14,15]. CRP typically increases at six to eight hours after the onset of sepsis and peaks at 24 hours[2]. Continuously normal levels of CRP are indicative of an absence of bacterial LOS[2], and potentially could be used in determining the discontinuance of empirical antibiotics in neonates[14]. Higher I:T neutrophil ratios are also associated with progressively increasing odds of positive LOS infection[17]. Two normal I:T ratios along with a sterile blood culture have a maximum NPV of 100%, thus making I:T ratio laboratory investigations a useful tool in ruling out LOS[14]. However, the presence of elevated I:T ratios alone is not confirmatory for LOS; elevated I:T ratios are found in up to half of uninfected infants, and have a poor PPV of 25%[2].

The historical gold standard of LOS diagnosis, combined with the low PPV of current biomarkers indicate the need for further research into new diagnostic biomarkers with higher PPVs and faster turnaround rates, which would expedite reliable NS diagnoses in the ED.

Biomarkers

Procalcitonin

Procalcitonin (PCT) has emerged as a promising biomarker for sepsis. PCT is synthesised as a prohormone of calcitonin by thyroid C-cells and is an acute phase protein. Several tissues secrete PCT in response to diverse stimuli, including cytokines (IL-6, IL-1β and TNF-α) and

lipopolysaccharide (LPS). PCT acts as a chemoattractant for blood monocytes[18]. In healthy neonates, PCT levels increase until postnatal days 2–4. In contrast to CRP, PCT does not increase with local bacterial infections, non-infectious inflammatory reactions and viral infections. This is due to the ability of IFN-γ, commonly produced in viral infections, to downregulate PCT. PCT is a potential biomarker to discriminate between bacterial and viral aetiologies of infection, though this has yet to be conclusively established in neonates.

PCT levels rise rapidly 2-4 hours after an endotoxin challenge and peak within 6-8 hours, thus making it more sensitive than CRP as a biomarker for the early diagnosis of NS[19]. Along with a turnaround time of less than 1-2 hours (with assays performed on site), this rapid rise makes PCT a potentially useful biomarker for the early diagnosis of NS in the ED, allowing for timely therapeutic measures to be initiated whilst reducing sepsis-related mortalities[20,21]. Moreover, the increase in serum PCT appears to correlate with the severity of both disease and mortality. PCT levels also offer prognostic value, as response to antibiotic treatment leads to a rapid decrease in PCT levels, showing potential for use in PCT guided antibiotic therapy.[20]

More recent reviews have focused on comparing PCT with CRP. Eschborn et al. identified 39 studies directly comparing the two. They established that the mean sensitivity of PCT for LOS was 88.9%, compared to 77.4% for CRP. Specificity values were 75.6% for PCT and 81.7% for CRP respectively[22]. Therefore, PCT was found to be slightly more sensitive and specific in NS diagnosis. Furthermore, PCT appeared less affected by other factors such as mode of delivery or surgical procedures. This was confirmed by a 2021 systematic review which concluded that PCT showed better sensitivity, specificity, PPV and NPV than CRP, making it a sensitive, independent and useful biomarker for the early diagnosis of NS[23].

A 2023 meta-analysis highlighted the importance of biomarkers in enhancing diagnostic capability for NS in low- and middle-income countries, where disease burden is high and standard diagnostic modalities are potentially lacking[24]. A total of 23,179 neonates were included in this study, which assessed CRP, ESR, WBC and PCT. While both PCT and CRP displayed good discriminatory value for NS, their specificity alone was insufficient for a definitive diagnosis of sepsis.

Determining normal cut-off values for PCT is critical due to its physiological increase after birth. A meta-analysis identified a PCT cutoff of 2-2.5ng/ml as showing the highest sensitivity and moderate accuracy for neonates with suspected sepsis. While this cut-off has a high sensitivity, its specificity is low. The need to maintain a low false-negative ratio due to the high mortality rate of NS makes this acceptable[25]. Importantly, this meta-analysis suggests that the use of two different cut-offs could improve accuracy with higher cut-offs for neonates with EOS than those with LOS.

PCT's implication in bacterial NS makes it a promising diagnostic biomarker with prognostic value and the potential for PCT guided antibiotic therapy. These factors can help to shorten the NS diagnosis time, and reduce mortality rates in the ED.

Presepsin

Presepsin (sCD14-ST) is the truncated form of sCD14 (soluble cluster-of-differentiation 14), a glycoprotein associated with the membrane surface of various immune cells, such as monocytes, neutrophils and macrophages. CD14 acts as a high-affinity receptor for LPS complexes[26]. sCD14 is the soluble form of the receptor, and it is found in the plasma, where it is cleaved by circulating plasma proteases[27,28,29]. Upon bacterial infection, the CD14-LPS-LBP (lipoprotein binding protein) complex activates a TLR4-specific pro-inflammatory signalling cascade which results in the release of cytokines. The complex is then internalised into a phagolysosome, where cathepsin D cleaves its N-terminal. This results in the formation of presepsin, which is released into the bloodstream[26].

Several studies have shown that presepsin is a reliable biomarker for NS, as it is unaffected by confounding perinatal inflammatory factors. Clinical studies have demonstrated that the plasma levels of presepsin tend to increase significantly at onset of sepsis and septic shock. Compared to other biomarkers, presepsin seems to have a better sensitivity and specificity, a crucial feature for the diagnosis and treatment of NS[27,30].

In order to use presepsin as a first-line biomarker for NS, it is crucial to have clear reference values. In 2015, Pugni et al. conducted a hallmark study aimed at identifying reference ranges of presepsin in neonates. Upon collection of 100 µL of whole blood from each neonate, they were able to establish a reference for both term (466.5-791 pg/mL) and preterm neonates (503-864 pg/mL)[31]. These values are important for comparison with presepsin serum levels in EOS and LOS. Researchers have compared the two and concluded that initial presepsin levels in both EOS and LOS were significantly higher than in healthy neonates[32, 33].

Several studies have proven the diagnostic and prognostic reliability of presepsin, along with additional advantageous properties. For example, the concentration of presepsin increases very early in bacterial infection, and the measurement can be taken directly in the ED within 17 minutes[34]. Compared to the time-consuming microbiological isolation of blood cells, presepsin offers a faster, yet sensitive and specific opportunity to diagnose sepsis. Additional studies have also demonstrated its high pooled sensitivity (92%) and pooled specificity (86%), in regards to all NS cases[35].

A systematic review aimed to compare the diagnostic accuracy of PCT alone, CRP alone, PCT combined with CRP andand presepsin alone in the diagnosis of NS. It found that the pooled sensitivity of CRP was the weakest, and the pooled negative likelihood ratio (NLR) of PCT + CRP, as well as presepsin alone, were less than CRP alone. The area under the curve (AUC) for presepsin (was 99%,) was greater than PCT + CRP (96%), PCT (91%) and CRP (85%), highlighting that the combination of PCT and CRP or presepsin alone improves can improve the accuracy of diagnosis of NS[19]. This suggests that a combination of multiple biomarkers as well as clinical findings is best for decision making during the diagnostic process.

Furthermore, past studies on presepsin levels on arrival in the ED have shown that it can be useful for risk stratification. In fact, presepsin plasma levels within 24 hours upon arrival were noticeably lower among NS survivors. This correlation can be a useful therapeutic marker, as presepsin plasma levels tend to decline by the 7th day of treatment with an effective antibiotic therapy. Complications and increased mortality are associated with persistently high presepsin plasma levels[36]. Such a relationship can be attributed to the prominent role of CD14 during infection. It is also worth noting that these observations have primarily been made on adult patients[36]. Hence, it is necessary to test this hypothesis further on neonates.

One caveat applies to the use of presepsin as a diagnostic biomarker in NS. High concentrations of this molecule in the blood can also be a consequence of other clinical conditions, particularly renal failure[37]. There are other clinical conditions which can interfere with normal presepsin range values, such as translocation of the intestinal microbiome, but these have yet to be studied in neonates[37].

Interleukin-6

Interleukin-6 (IL-6) is another potentially useful biomarker for NS. IL-6 production increases immediately after exposure to bacterial endotoxins, and is primarily released by monocytes, endothelial cells and fibroblasts. Its concentration rises rapidly with the onset of the inflammatory response, but has a short half-life and normalises within 24 hours[38]. IL-6 has a pro-inflammatory effect, inducing CRP and PCT release, as well as T-cell differentiation and B-cell maturation. IL-6 has been found to be elevated in neonates with both EOS and LOS[39].

A meta-analysis found encouraging results for IL-6 as a diagnostic biomarker for NS. The pooled sensitivity and specificity were 79% and 84% respectively, with an area under the ROC curve (AUC) of 89%. This shows favourable accuracy of IL-6 for predicting NS, reinforced by the results of the meta regression analysis confirming that the diagnostic accuracy of IL-6 remains unaffected by confounding variables such as cut-off levels of IL-6 assay or birth weight[40]. Another meta-analysis found similar results[39]. IL-6 demonstrated a specificity of 88% and a sensitivity of 82%. In addition, the area under the summary receiver operating characteristics curve AUC was notably high at 92%[39]. This statistical technique can be used in meta-analyses of diagnostic tests, and the closer the value is to 1, the more accurate a diagnostic test is. This suggests that IL-6 holds promise as an accurate tool in diagnosing NS.

Ye et al. conducted a study evaluating numerous cytokines, including IL-6, as biomarkers compared to CRP. IL-6 levels (>12.5 pg/mL) and the IL-6/IL-10 ratio (>3.5) were shown to be as valuable in the diagnosis of NS as CRP. The interleukins with the highest specificity and sensitivity were IL-6 and the IL-6/IL-10 ratio at 94.1% and 100% respectively[41]. Another study recognised that distinct cut-off values must be used depending on neonatal age. It examined cut-off values of serum IL-6 at 80 pg/ml on day of life 1, 40 pg/ml on day of life 2–7 and 30 pg/ml after day of life 7[42]. A sensitivity of 75% and specificity of 81% for culture-confirmed sepsis was achieved, concluding that these IL-6 cut offs have a high accuracy for the detection of NS.

Interleukin-35

Interleukin-35 (IL-35) is an anti-inflammatory cytokine produced by immune cells including T-regulatory, dendritic, macrophages and monocytes as well as vascular endothelial, and smooth muscle cells during sepsis. Recent research aimed to assess the viability of IL-35 as a predictive biomarker for NS[43]. IL-35 levels were compared between neonates with and without sepsis, with septic neonates showing significantly higher IL-35 levels. The ROC curve analysis indicated an AUC of 89.5%, with an IL-35 cutoff >8.05 pg/ml showing 97% sensitivity[43].

Another study compared laboratory results between two groups: septic neonates and those unlikely to be infected with NS. Infected neonates had significantly higher levels of IL-35, PCT, CRP and white blood cell (WBC) counts compared to the likely uninfected group. IL-35 consistently showed higher levels in infected neonates across different time intervals. For predicting NS, IL-35 performed well with an AUC of 75.6%, while PCT, CRP, and WBC had lower AUC values[44]. These findings showcase the promise IL-35 holds as a predictive biomarker, however further research with larger sample sizes/ diverse populations is needed to confirm its utility, stability, and potential convenience for clinical diagnosis.

Angiopoietin

Prominent among the biomarkers studied in NS are angiopoietin-1 and -2 (Ang-1 and Ang-2). Ang-1 promotes vascular maturation and stability by binding to receptor Tie-2, a tyrosine kinase receptor primarily found in endothelial cells[45]. In general, Ang-1 can be viewed as a stabilising messenger, causing continuous Tie-2 phosphorylation, while Ang-2 acts as a destabilising messenger. Angiopoietins can directly stimulate both endothelial cells and neutrophils for an overall pro-inflammatory and pro-angiogenic response in sepsis. This balance between Ang-1 and Ang-2 is crucial in determining vascular development and maintenance[45].

Serum Ang-2 levels are associated with sepsis severity and are predictive of outcomes, however it is essential to quantify the reliability, cutoffs, and added value of Ang-2 over traditional markers before it can be used in clinical practice[9]. The angiopoietin-Tie-2 system shows some promise in sepsis diagnosis, but rigorous clinical studies are needed to confirm its utility as a biomarker, particularly in the ED, as testing is currently expensive as well as time consuming.

MicroRNA

MicroRNAs (miRNA) are small (20-24 nucleotides), endogenous non-coding RNA molecules[46-48]. If a miRNA is a perfect complement to its target mRNA, miRNA binds to the 3'-untranslated region of the target mRNA, leading

to destabilisation and degradation via deadenylation and capping[46-48]. If the miRNA is a partial complement, the molecule can inhibit translation, preventing conversion of mRNA to protein. Due to their precise intrusion into several molecular pathways and pathology-specific expression levels, miRNAs have been pinned as promising future diagnostic and therapeutic tools in disorders characterised by aberrant cellular signalling.

The literature directly implicates miRNAs in the modulation of NS pathology. MicroRNAs strongly regulate TNF pathways, one of the major signalling arms in sepsis[46]. Furthermore, stimulating macrophages and hepatic cells by bacterial lipopolysaccharide (LPS) produces increased miR-155 expression from these cell lines[46]. Similar experiments with LPS induction in monocyte cell lines have shown increased miR-150 and let-7a (a precursor miRNA) expression levels[46].

The structural stability and specificity suggest the clinical utility of miRNAs in the early diagnosis of NS. Furthermore, detection requires extremely low blood volumes which offers advantages in neonate diagnostics in the ED.

Initial work implicated several miRNAs as diagnostic and prognostic biomarkers in septic adult patients. However, the incomparable developmental and immunological states of neonate and adult septic patients does not make these results generalisable. Indeed, Cheng et al. have reported that the repertoire of miRNAs dysregulated in neonates is profoundly different to that in adult patients[49].

In neonates, miR-26a is found to be downregulated in the serum and blood mononuclear cells. Cheng et al. report that this change in miRNA expression is associated with IL-6 overexpression in septic neonates, especially those suffering from early onset NS[50]. Moreover, another study found that serum levels of both IL-6 and miR-26a may be indicative of the extent of inflammatory response to tissue injury[51].

Fatmi et al. studied miR-23b, a miRNA which plays a major role in attenuating the effects of pro-inflammatory cytokines, in both EOS and LOS patients. They found that EOS patients with positive haemoculture showed increased miR-23b levels, while both full-term and preterm LOS patients showed decreased levels[52]. The team also found that downregulation of miRNA-23b in NS has the potential as a prognostic biomarker, as decreased expression of miRNA was associated with neonatal death[52].

A recent meta-analysis has highlighted additional miRNAs worthy of future investigation[53]. El-Khazragy et al. highlight that miR-34a has a sensitivity of 89% and specificity of 97% for ruling out NS[53]. Downregulated miR-34a was also correlated with disease progression, while increased levels of miR-1 and miR-124 were associated with poor prognosis[53]. All three of these modulate aspects of the innate immune response such as the polarisation of the M1/M2 macrophage axis[53].

MicroRNA testing would be favourable in time-sensitive NS ED diagnoses, as the assay time has been estimated to be 2-2.5 hours[53]. The ease of use and the commendable specificity and sensitivity of the tests, further support the use of miRNAs in the early diagnosis of NS.

Overall, detection of these miRNAs in serum of patients may be a useful diagnostic and prognostic tool, possibly providing early diagnosis. A future research avenue could be to assess the practicality of using miRNA expression levels in guiding appropriate antibiotic prescription on a pathogen-specific basis.

Conclusion

While blood cultures play a useful diagnostic role, particularly in identifying the specific strain of bacteria, and thus guiding more targeted antibiotic therapy, other biomarkers should be used in adjunct. However, a combinational approach may be most useful in the diagnostic and prognostic roadmap to improving outcomes in NS. Exploration of the named biomarkers reveals intriguing findings related to vascular regulation and immune response during the neonatal phase of life.

Presepsin, a marker resistant to perinatal factors, exhibits rapid elevation upon bacterial infection, making it valuable in the ED. PCT responds swiftly to bacterial sepsis, showing a higher sensitivity EOS; this can help to ensure a definitive diagnosis, reducing hospital stays, antibiotic overuse and thus microbial resistance. The pronounced elevation of markers like IL-6 and Ang-2 levels in neonates with sepsis presents an intriguing prospect, potentially serving as a valuable biomarker for assessing sepsis severity and offering prognostic insights. MiRNAs, particularly miR-26a, miR-23b, and miR-34a, exhibit altered patterns in NS, and due to their specificity and ease of detection, they make for potential high yield markers in the ED.

While these biomarkers hold promise, further large-scale studies are needed before standardisation and recommendations for routine clinical practice are established. Overall, it is undeniable that these biomarkers would be a significant addition to our arsenal, enabling rapid diagnosis, accurate risk assessment, and improving prognostic outcomes. ◄

Declarations

This article was anonymised following submission and subsequently reviewed and accepted by an independent team of editors and peer reviewers as per the TSMJ's peer review and article acceptance protocol. The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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