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Biomarkers for the diagnosis of neonatal sepsis and necrotizing entercolitis: Clinical practice guidelines

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ABSTRACT

Sepsis and necrotizing enterocolitis are major contributors to morbidity and mortality in neonates, especially in those born preterm. While therapeutic interventions are available for both (for e.g. antibiotics), a major dilemma is early diagnosis so that these interventions can be done in a timely manner. As clinical evaluation alone is unreliable in identifying infants in the early stages of neonatal sepsis or necrotizing enterocolitis, there is a need to find specific biomarkers associated with these conditions to improve diagnostic capabilities. Optimal use of biomarkers in the identification and management of affected neonates requires an understanding of the properties of each marker within the timeline of the inflammatory response. We propose that early- and mid-phase markers such as neutrophil CD64 and procalcitonin should be combined with the late-phase biomarker C-reactive protein for maximal diagnostic benefit. Appropriately powered trials evaluating the serial measurements of these markers in decisions related to antibiotic stewardship in the neonatal population are indicated, in addition to more studies investigating other potentially useful biomarkers.

1. Introduction

Sepsis and necrotizing enterocolitis (NEC) are regarded as two of the most significant contributors to mortality and morbidity in the neonatal population [1]. Neonatal sepsis (NS) can be classified either as early onset sepsis (EOS) occurring in the first 72 h of life and late onset sepsis (LOS) which occurs on or after postnatal day 4 [2]. These definitions are made in an effort to separate transplacental, ascending or intra-partum transmission of infection from transmission occurring in the home, hospital or community [3].

In North America, EOS has been shown to effect 0.76–0.98 infants/1000 live births with overall mortality rates of up to 24.4% [4,5] . Case fatality rate has been shown to be 54% in infected infants with gestational age (GA) between 22 and 24 weeks and 30% of infected infants between 25 and 28 weeks GA [5]. NEC is a potentially devastating multifactorial condition with a prevalence of 5–7% in very low birth weight infants [6]. Severity of disease ranges from mild involvement that can be managed medically with antibiotics and bowel rest to extensive necrosis of the intestinal wall necessitating surgical intervention. Despite advances in the understanding of the pathogenesis of NEC, mortality rates remain significant and relatively unchanged [7]. Furthermore both NEC and NS are associated with increased long-term morbidity including adverse neurodevelopmental outcomes in survivors [8,9,10].

The devastating nature of both NS and NEC make it tempting for clinicians to have a low threshold for treatment; however, antibiotic therapy is not without risk. Although treatment has the potential to be life-saving, non-selective, over-zealous use of antibiotics can actually increase the risk of NEC through disruption of the intestinal microbiome [6]. Extended courses of antibiotics also has the potential to generate the selection of resistant organisms thus making it more challenging to effectively manage infections in the future [11,12]

The lack of a single "gold standard" in the diagnosis of infection is another important concern and leads to inconsistencies between studies. Given the challenges of obtaining an adequate blood volume and exposure to antenatal antibiotics, the absence of a positive blood culture does not reliably exclude a diagnosis of sepsis in the neonatal population [13]. Furthermore, a vast array of clinical symptomatology can signal the presence of NS [14]. While the diagnosis of NEC can be suggested by the appearance of a systemic and local (abdominal) symptoms and signs, and confirmed by the presence of pneumatosis intestinalis on X-ray (Stage 2; modified Bell's criteria [15,16], early diagnosis of NEC (Stage 1) is still non-specific.

Timely identification of both sepsis and NEC is critical; however, clinical diagnosis is challenging as the presentation of both conditions is often subtle and non-specific, as mentioned above. As clinical evaluation alone is unreliable in identifying infants in the early stages of NS or NEC, there is a need to find specific biomarkers associated with these conditions to improve diagnostic capabilities. In this context a biomarker can be defined as any measurable parameter that provides meaningful information regarding the diagnosis of sepsis and/or NEC [17]. The

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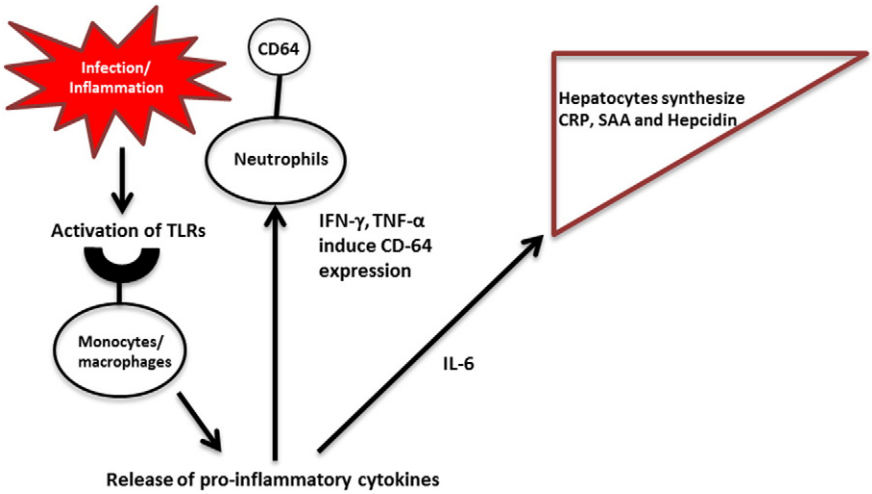

Fig. 1. Source of selective biomarkers. Infection/inflammation activates toll-like receptors (TLRs) on the surface of the monocytes/macrophages, triggering the release of pro-inflammatory cytokines. Among the latter, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) allow the CD64 antigen to be presented on the surface of the neutrophils. Interleukin-6 (IL-6) stimulates the release of C-reactive protein (CRP), serum amyloid A (SAA) and hepcidin into the blood stream from the hepatocytes in the liver.

ideal biomarker should be both highly sensitive so as not to miss potential cases and specific to avoid over-treating infants who are not affected. The perfect biomarker should also rise early during the course of an infection and decline in a meaningful pattern that can help guide decisions regarding duration of therapy. An ideal biomarker should be reliable and precise with a high predictive value. Other useful features include the ability to discern between infection with different pathogens, to be low cost with a rapid turn-around time and be dependent on technology that is easily accessible to the practicing clinician [18].

Effective research into the use of biomarkers is challenged by the multitude of factors that can affect the serum concentration of a specific marker making comparison between studies difficult [19]. Concentration fluctuations may be population specific (GA of subjects, birth weight, perinatal history), related to the causative pathogen, severity of infection as well as technical factors such as mode and timing of collection and measurement techniques. In addition, one of the main limitations in the markers commonly in use such as white blood cell indices and C-reactive protein (CRP) is that they rise relatively late in the course of disease and hence cannot be used to identify unaffected infants in the early stages.

In this review we will outline various biomarkers (Fig. 1) that can be used in the diagnosis of NS and NEC within the timeline (Fig. 2) of the inflammatory response. We searched the PubMed database, restricting our investigation to studies written in English using the search words "newborn" or "neonatal" and "sepsis" and "necrotizing enterocolitis" and "biomarkers". We have chosen to focus on articles written in the last 8 years selecting what we consider to be the most potentially useful biomarkers to the practicing clinician. After discussing the utility of each marker briefly, we will then put forward suggested guidelines for the use of biomarkers in clinical practice.

2. Hematological indices

Hematological indices such as total white blood cell count, absolute neutrophil count (ANC), absolute band count (ABC), immature to total white blood cell ratio (I:T ratio) and platelet count are commonly used in the evaluation of both suspected NS and NEC. In a large retrospective cross-sectional study, culture proven EOS was shown to be associated with both a low white blood cell count (WBC) and ANC and a higher band count [20].The diagnostic accuracy of WBC indices in the prediction of EOS improved with postnatal age, with low WBC being most indicative of infection. WBC indices appeared most useful when samples were obtained at or after 4 h of life. The authors concluded that if the complete blood count (CBC) is to be used as a decision making tool, it may be beneficial to postpone antibiotic therapy and testing until a minimum of 4 h of life, providing the infant is well appearing [20].

In the context of LOS, a large study showed an association between both high and low WBC counts, high ANCs, high I:T ratios and low platelet counts and infection [21]. Positive likelihood ratios were found to be highest for WBC <1000 mm^3 (4.1) and platelet counts < 50.000/mm^3 (3.5); however, sensitivity values for all indices were deemed to be too low to reliably rule out infection [21].

Hematological scoring systems have also been used for the early diagnosis of NS, especially when blood cultures are negative. Such composite scoring systems have been found to have somewhat improved sensitivities in the diagnosis of NS [14,22]. However, while these

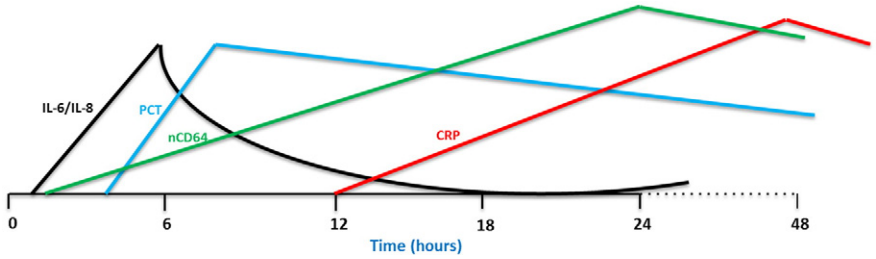

Fig. 2. Timeline of the release of selective biomarkers. Interleukins-6 and -8 (IL-6/IL-8) are released within 2 h of the infection, peaking at approximately 6 h, and then declining over the following 24 h. Neutrophil CD64 (nCD64) can be detected within 1–6 h, while procalcitonin (PCT) is usually detected within 3–4 h of the inflammatory response, remaining at high levels for at least 24–48 h. C-reactive protein (CRP) is released at 12–24 h into the inflammatory response, and peaks at 48 h.

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