Clinica Chimica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Translational research and biomarkers in neonatal sepsis

Joris R. Delanghe [a,*], Marijn M. Speeckaert [b]

[a] Department of Clinical Chemistry, Ghent University Hospital, Ghent, Belgium
[b] Department of Nephrology, Ghent University Hospital, Ghent, Belgium

ARTICLE INFO

Article history:
Received 11 August 2014
Received in revised form 24 January 2015
Accepted 24 January 2015
Available online xxxx

Keywords:
Biomarkers
Necrotizing enterocolitis
Neonatal sepsis
Metabolomics
Proteomics

ABSTRACT

As neonatal sepsis is a severe condition, there is a call for reliable biomarkers to differentiate between infected and noninfected newborns. Although blood culture has been considered as the gold standard, this analysis is still too slow and limited by false negative results. Use of CRP is hampered by a physiological 3-day increase, resulting in a low sensitivity to detect sepsis at an early stage. A moderate diagnostic accuracy of other acute phase proteins has been demonstrated (serum amyloid A, procalcitonin, lipopolysaccharide binding protein, mannose binding lectin and hepcidin).

In neonatal sepsis, changed chemokine/cytokine levels are observed before those of acute phase reactants. High IL-6, IL-8, IL-10 and TNF-α concentrations are detected in infected infants. Soluble interleukin-2 receptor has been used to identify bacteremia, whereas low plasma RANTES concentrations are characteristic for septicemia. Several cell adhesion molecules contribute to the pathogenesis of sepsis. As an upregulated CD64 expression on granulocytes is found within 1–6 h after bacterial invasion, serial CD64 measurements could guide antibiotic therapy. An increased CD11b/CD18 density can improve the diagnosis, and a positive correlation between CD11b and the severity of systemic inflammation has been reported. An early increase in sCD14-ST presepsin is also observed during sepsis, whereas high sTREM-1 values in early-onset neonatal sepsis (EOS) have been associated with mortality.

Biomarkers resulting from proteomics are also promising. A 4-biomarker 'mass restricted' score has been validated as diagnostic for intra-amniotic infection and/or inflammation. S100A8 in amniotic fluid is a strong predictor of an increased incidence of EOS. Proteomic analysis of cord blood has revealed altered protein expression patterns. The ApoSAA score is useful for identifying sepsis and could guide prescription of antibiotics. ^{1}H-NMR and GC–MS metabolomics allow to diagnose septic shock, which is associated with increased concentrations of 2-hydroxybutyrate, 2-hydroxyisovalerate, 2-methylglutarate, creatinine, glucose and lactate.

Contents

* Corresponding author at: Department of Clinical Chemistry, Ghent University Hospital, De Pintelaan 185 B-9000 Ghent, Belgium. Tel.: +32 9 332 29 56; fax: +32 9 332 49 85.
E-mail address: joris.delanghe@ugent.be (J.R. Delanghe).

http://dx.doi.org/10.1016/j.cca.2015.01.031
0009-8981/

1. Introduction

Sepsis is a major cause of morbidity and mortality in neonates, most frequently seen in preterm infants with comorbidities or prolonged hospitalization and in very low birth weight (VLBW) infants. This clinical condition is classified as early-onset neonatal sepsis (EOS, infection ≤ 72 h of life), occuring in 1.5–2% of VLBW infants [1], and late-onset neonatal sepsis (LOS, infection > 72 h of life), with a prevalence of up to 21% in VLBW infants [2]. Accurate diagnosis of neonatal sepsis is scientifically challenging. For decades, blood culture has been considered as the gold standard. However, false negative results are not rare as bacteremia is often of low density and intermittent, and the obtained blood samples are frequently small in volume. Antibiotic treatment prior to blood culture may further reduce the diagnostic performance of blood culture [3].

At this moment, no single marker has a significant advantage over the others to diagnose neonatal sepsis. As the diagnostic utilities [sensitivity, specificity, positive predictive value and negative predictive value] determine the usefulness of a clinical test, a diagnostic method should possess an acceptable sensivity (±80%), an excellent sensitivity and a NPV of ± 100%. An ideal biomarker should rise rapidly and should have a good diagnostic window [4,5]. Several hematological indices (amount of white blood cells, neutrophil count, immature to total neutrophil count ratio) have already been used during the previous decades. In this manuscript, an overview of old and novel non-hematological diagnostic biomarkers in neonatal sepsis and necrotizing enterocolitis will be presented.

2. Acute phase reactants

2.1. C-reactive protein

Despite the development of new biomarkers, C-reactive protein (CRP) is the most extensively studied acute-phase protein in EOS [6]. Being present in bacterial cell walls, biological membranes and lipopolysaccharides, phosphocholine is the major ligand to CRP [7]. After bacterial infection, IL-6 and other proinflammatory cytokines stimulate the hepatic synthesis of CRP (peak at 48 h), which is followed by activation of the complement system, increased phagocytosis, activation of macrophages and monocytes, and elevated production of proinfammatory cytokines [8].

The use of this acute phase protein in the first days of life is hampered by a nonspecific physiological 3-day increase, which is related to the stress of delivery and some other noninfectious perinatal and maternal factors. Different sensitivities and specificities (Table 1) have been published, which can be explained by variations in definitions of sepsis, test methodologies, reference values, cutoff points (most used upper limit of 10 mg/L), patient charateristics, inclusion criteria, sampling times and number of collected samples. CRP has an unacceptable low sensitivity to detect neonatal sepsis at an early stage due to a delayed induction of its hepatic synthesis. A combination with another biomarker (IL-6, procalcitonin, …) could increase the sensitivity during the early phases of sepsis. Serial measurements, 24–48 h after onset of the infection, are associated with better sensitivities (74–98%) and specificities (71–94%), and are used to guide the antibiotic treatment in neonatal sepsis [6].

2.2. Serum amyloid A

Serum amyloid A (SAA) is an acute phase reactant with a hepatic synthesis, regulated by proinflammatory cytokines [interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)]. However also smooth muscle cells, macrophages, adipocytes en endothelial cells are involved in the production of this family of 12–14 kDa polymorphic apolipoproteins. Multiple fuctions such as chemotaxis, immunomodulation and tissue regeneration have been attributed to SAA [9,10]. A 1000-fold increase in the serum concentration of SAA has been reported during neonatal sepsis [10]. However, technical issue have hampered the use of SAA assays in clinical practice.

In a meta-analysis [11], consisting of a total of nine studies [10, 12–19] with 823 neonates, a moderate diagnostic accuracy of SAA for EOS and LOS (8–96 h after onset of infection) was reported, which was comparable with the diagnostic accuracy of CRP and procalcitonin. Differences in age of the study population groups, differences in the SAA assay and differences in cutoff point (1–68 mg/L, depending on time point of analysis) could explain the heterogeneity between the published studies. In comparison with CRP, SAA was characterized by a pooled sensitivity of 78% (95% CI: 73–83%) versus 67% (95% CI: 62–73%), and a pooled specificity of 92% (95% CI: 89–95%) versus 89% (95% CI: 84–92%) [11]. In addition, the value of SAA for the diagnosis of newborns with necrotizing enterocolitis has also been demonstrated. However, its value in the follow-up of those young patients should be further investigated [9].

2.3. Procalcitonin

Hepatocytes and monocytes are the most important producers of procalcitonin, which is the 116-aminoacid peptide prohormone of calcitonin. In neonates, procalcitonin is characterized by a marked physiological increase after birth, which limits its value in the first 2–4 days of life and calls for age-specific cutoff values in this period [20–22].

During a bacterial or fungal infection, a more rapid rise (within 3–4 h) in the serum procalcitonin concentration is observed in comparison with CRP, with elevated levels at least 24–48 h after onset of infection and with a maximum serum concentration at 18–24 h (halflife of ± 24 h) [21]. Bacterial infections are associated with increased serum procalcitonin concentrations up to 1000 μg/L, which are correlated with severity of disease and mortality [22]. In a small study comparing different biomarkers for neonatal sepsis, the order of the markers according to sensitivity and specificity was CRP > procalcitonin > TNF-α > SAA at the time of diagnosis [23]. However other studies showed that procalcitonin is a better diagnostic sepsis marker than CRP and

Please cite this article as: Delanghe JR, Speeckaert MM, Translational research and biomarkers in neonatal sepsis, Clin Chim Acta (2015), http://dx.doi.org/10.1016/j.cca.2015.01.031