ACUTE PHASE REACTANTS AND CYTOKINES IN THE EVALUATION OF NEONATAL SEPSIS

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Abstract

In the latest years, biochemical markers are important in research areas in neonatal infections. Inflammatory cascade as response to an infection comprise many elevated markers, frequently used for diagnosis and monitoring of sepsis. White blood cells release cytokines and chemokines and others mediators which regulate inflammatory process. Interleukin-6 (Il-6) and interleukin-8 (Il-8) are considered markers of *early-onset neonatal sepsis*. Late-onset neonatal sepsis in newborn infants is sepsis that occurs after the first 72 hours of life and is a major cause of infant mortality. C-reactive protein, procalcitonin and hepcidin, released as a consequence of increased cytokine activity are considered markers of *late-onset neonatal sepsis*. Mannose-binding lectin (MBL) is a collagenous protein involved in innate immunity of neonates and a useful marker of infection. Early diagnosis and prompt intervention are essential to prevent morbidity and mortality in neonates (<28 days of age) and infants (>28 days of age) with sepsis.

Key words: interleukin 6, interleukin 8, procalcitonin, cytokines

Introduction

Neonatal sepsis occurs from 1 to 21 newborns out of 1 000 live births with mortality rates as high as 30% up to 69% and the "gold standard" for the neonatal diagnosis of sepsis is a positive microbiological culture (1,2). Neonatal sepsis is defined as a clinical syndrome characterized by bacteremia with systemic signs and symptoms of infection in the first 4 weeks of life (3). The diagnosis of sepsis includes also urine, cerebrospinal fluid, or bronchial fluid specimens and usually takes 24 to 48 hours (4,5). Data from the National Institute of Child Health and Human Development Neonatal Research Network reported mortality rates with gram-negative infections at 36% and 32% with fungal infections (6). In clinical practice a rapid diagnosis of neonatal sepsis is difficult, because the clinical manifestations of this condition can overlap with those of non-infectious conditions, such as the meconium aspiration syndrome, respiratory distress syndrome, and hemodynamic instability of various underlying etiologies (7).

Newborns are frequently evaluated after the principle "rule out sepsis". In United States, it was reported that 15% of term newborn undergo this evaluation (7,8). Based on the timing of presentation, neonatal sepsis is classified as either early or late. In the literature, however, there is no definitive consensus as to what age limits apply, with early-onset sepsis ranging from 48 hours to 6 days after delivery (9). Late-onset sepsis generally occurs beyond the first week of life. The clinical relevance of this distinction is that early-onset disease is often due to organisms acquired during delivery (10).

Although no significant sex difference has been reported, it was observed that male infants had a higher incidence of neonatal sepsis than females, which may be related to X-linked immunoregulatory genes (11). The bacteria that cause neonatal sepsis are acquired shortly before, during, and after delivery (12). The results of blood culture are usually available only after a delay of about two days, thus necessitating initial empirical treatment for suspected cases (13). The adaptive immune system of neonates, particularly of preterm infants, is severely impaired because of immature B and T cell function (14,15).

An earlier diagnosis by laboratory markers could reduce the incidence and high mortality rate through neonatal sepsis (4). CRP, white blood cell count, absolute neutrophil count, and immature/total neutrophil ratio are the most widely used tests in the diagnosis of neonatal sepsis (5). Procalcitonin and mannose-binding lectin are probably more sensitive and specific for detecting the early stages of bacterial infections than traditional inflammation indicators such as Erythrocyte Sedimentation Rate or CRP (17). The advantages of peripheral circulatory measurements as diagnostic markers are that 1) they can be done rapidly and noninvasively, 2) the measurements are reproducible shortly after birth, and 3) the result of the test is available immediately (18).

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Furthermore, a number of other acute-phase proteins including fibronectin, granulocyte colony–stimulating factor, and α_1-antitrypsin have been evaluated as diagnostic markers for neonatal sepsis. Although all could be used as markers for diagnosing sepsis, none has been routinely studied on a large scale or in the clinical setting (6).

Acute Phase Reactants

C-Reactive Protein

C-reactive protein (CRP) is one of the most studied and used laboratory tests for neonatal sepsis. The utility of CRP for the diagnosis of early-onset neonatal infection has been the subject of controversy because of its unsatisfactory sensitivity (19). The delayed synthesis during the inflammatory response accounts for its low sensitivity during the early phases of the disease (20). Upon resolution of the inflammation, CRP levels rapidly decline with an elimination half-life of 19 hours (21). A study compared six inflammatory mediators: CRP, interleukin-6 (IL-6), soluble tumor necrosis factor receptors (p55 and p75) and soluble adhesion molecules (ICAM-1, E-selectin) as early diagnostic tests for neonatal sepsis, and studied the possible benefit of combining parameters (22). CRP proved to be best diagnostic test for neonatal sepsis, the diagnostic accuracy was improved by combining CRP and IL-6, but other parameters (p55, p75, ICAM-1 and E-selectin) added no further diagnostic information (22). The cut-off levels of CRP may vary among authors from 5 to 50 mg/l (23-25). Another study evaluated CRP levels in four hundred and twenty neonates with clinical suspicion of sepsis over a 6 month period. They concluded that the qualitative method of estimating CRP has moderate sensitivity, specificity and negative predictive value (21). Another recent study tried to find out the role of hematologic scoring system (HSS), CRP and haptoglobin in the early diagnosis of neonatal septicemia. They observed that HSS and CRP are useful test to differentiate the septicemic from non septicemic neonates and help in decisions regarding judicious use of antibiotic therapy, but haptoglobin level was not found useful for screening of sepsis (26). Recently, a study compared the diagnostic accuracy of neutrophil CD_{64} and CRP as a single test for the early detection of neonatal sepsis and the authors observed that the diagnostic accuracy of CD_{64} is superior to CRP when measured at the time of suspected sepsis (27). On the contrary, a study reported that implementation of an algorithm based on the determination of IL-6 and CRP, in the initial assessment of the newborn with clinical suspicion of infection, could reduce unnecessary antibiotic therapy.

Procalcitonin

Lately, procalcitonin (PCT) has acquired, especially in Europe, an important role in the diagnosis of bacterial infection in both pediatric and adult population (29). PCT, one of the precursors of calcitonin, is a 116 amino acid peptide, physiologically produced and secreted in the thyroid gland and secreted into the blood circulation during infection, without increasing calcitonin (25). PCT is detectable in the plasma as early as 2 h after the exposure to the bacterial products; its level rises for 6 to 8 h, reaches a plateau after 12 h, and then decreases to a normal level after 2 to 3 days. A cut-off of 0.5 ng/ml starting from the third day of life appears to be capable of ensuring good test sensitivity and specificity. Furthermore, non-infective perinatal events, such as intracranial hemorrhage, perinatal asphyxia, respiratory disorders, and fetal distress, may increase PCT concentrations.

A study that compared the diagnostic value of PCT and CRP in neonatal sepsis, observed that PCT is a useful, sensitive and independent biomarker of neonatal sepsis, but measurement of both CRP and PCT may increase the specificity (30). A study reported that the serum PCT concentration showed a good diagnostic value for the early detection of neonatal sepsis of vertical transmission compared to traditional inflammatory mediators, such as IL-6 and CRP values. On the contrary, some studies found that the sensitivity of PCT is low (70%–80%) to rule out sepsis at birth (4,5). PCT and CRP thresholds for the diagnosis of sepsis were 5.38 ng/ml (sensitivity 83.3%, specificity 88.6%) and 12 mg/l (sensitivity 76.4%, specificity 78.9%) at 24 h of age (31). Although CRP and PCT are accepted sepsis markers, there is still some debate concerning the correlation between their serum concentrations and sepsis severity (8). The measurement of procalcitonin can also help differentiation between bacterial and viral infection, therefore it has been introduced in many European protocols for the management of febrile children (31).

Hepcidin

Hepcidin is an acute-phase reactant that plays a critical role in inflammation and iron homeostasis (33). Although hepcidin has an important role in anemia, there is a known relationship between iron metabolism and innate immunity. It is already known that synthesis of hepcidin is up-regulated by lipopolysaccharide and interleukin-6. Recently, it was reported that serum hepcidin concentration may be a useful adjunct test, in addition to blood culture and other markers of infection, in the evaluation of late-onset sepsis in very low birth weight infants.

Mannose-binding lectin (MBL)

Mannose binding lectin (MBL) is an acute phase protein produced by the liver that activates the lectin pathway of the complement system by binding to various microorganisms, which leads to enhanced phagocytosis. Furthermore, MBL binds to mannose and other sugar residues present on the cell wall of bacteria, viruses and parasites with high affinity. A study investigated the relationship between MBL gene polymorphism and early neonatal outcome in preterm infants. They found out that MBL gene polymorphism was associated with early neonatal sepsis and increased frequency of patent ductus arteriosus in infants. Single-nucleotide polymorphisms in exon 1 of the MBL_2 gene are responsible for altered MBL serum levels and impaired function (7). Other study suggested that low MBL concentrations are a risk factor for sepsis associated with infections with Gram-positive but not Gram-negative bacteria (14). A study showed that low MBL levels and presence of B allele of MBL exon 1 gene are

important risk factors for development of both neonatal sepsis and pneumonia, especially in premature infants. Recently was reported that newborns with low MBL levels appear to have culture-confirmed sepsis more frequently than MBL-sufficient newborns.

Cytokines and Chemokines

Cytokines, small molecules secreted by lymphocytes and monocytes are thought to be endogenous mediators of the immune response to bacterial infections, while chemotactic cytokines, collectively known as chemokines, appear to have the capacity to control the movement of leukocytes and are important elements in this process. Elevated levels of cytokines and chemokines can be detected in umbilical blood of the neonates with sepsis and are considered sensitive and specific markers of acute infection. Elevated serum levels of some cytokines like interleukin-6, interleukin-8 or tumor necrosis factor α may precede the increase of CRP and may stimulate the hepatocytes for increased CRP production.

Interleukin-6

Another marker that has gained much attention more recently is interleukin-6 (Il-6). Il-6 is produced by monocytes, endothelial cells and fibroblasts (24). The advantage of measurement of Il-6 is that it rises at the onset of infection while CRP reaches the maximum concentration with a noticeable delay. Cord blood Il-6 rather than CRP is a better predictor to initiate treatment in neonates with prenatal infectious risk factors immediately after birth. Furthermore, elevated concentrations of Il-6 and CRP are risk factors for preterm birth < 32 weeks. A recent study found that the optimal cut-off value of Il-6 for diagnosis of neonatal sepsis is 24.65 pg/ml (30). On the contrary, another study found out that the optimal cut-off value of Il-6 for diagnosis of neonatal sepsis is 40.5 pg/ml. The sensitivity of Il-6 assay ranged from 0.61 to 0.96 (23). Higher serum level of PCT, hs-CRP, and Il-6 were reported in neonates with sepsis compared to those without sepsis (25). Furthermore, the concentration of Il-6 in preterm and term infants does not seem to be influenced by gestational age or maternal cytokine concentration (24). IL-6, IL-10 and oxidative parameters in umbilical cord blood contribute as an indicator of neonatal sepsis in recognized high-risk neonates (3). Therefore, Il-6 can be used as an important marker for *early-onset neonatal sepsis* in neonatal care units.

Interleukin-8 (IL-8)

IL-8 is a pro-inflammatory cytokine predominantly produced by monocytes, macrophages, and endothelial cells, has a role in release, activation and chemotaxis of neutrophils and rises early in the course of neonatal bacterial infections. The cut-off value for diagnosis of neonatal sepsis is IL-8 > 60 pg/ml. Interleukin-8 is considered to be an accurate marker, with sensitivities ranging from 80% to 91% and specificities from 76% to 100% (6).

It was reported that the use of multiple markers as CRP, PCT, IL-6 and Il-8 is useful both to early (24-48 h) diagnose of neonatal sepsis, and to monitor the antibiotic treatment while waiting for the results of cultural examinations (2). It was reported that IL-8 may be a valid and early predictive marker of neonatal infection that is associated with severity of infection.

Tumor necrosis factor α (TNF-α)

TNF-α is one of the primary agents which sets in motion the exaggerated cellular, metabolic, and vascular responses of sepsis. TNF binds to specific receptors (p55, p75) on target cells, which also exist as soluble isoforms (22). A study detected that sensitivity, specificity and diagnostic efficacy values of IL-6, CRP and IL-8 are lower than PCT and TNF-α (18).

Cytokines and chemokines such as Il-6 and Il-8 have good diagnostic utilities as early phase markers, while acute phase reactants such as C-reactive protein, procalcitonin, hepcidin and mannose-binding lectin have superior diagnostic properties during the later phases.

Conclusions

Combination assay of serum levels of cytokines like interleukin-6, interleukin-8 or tumor necrosis factor α and acute-phase reactants like C reactive protein, procalcitonin, hepcidin and mannose-binding lectin seems to be useful as a part of diagnostic work up for neonatal sepsis, but also in following the effectiveness of treatment and determining the prognosis of the disease.

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