**Neonatology**

# An Update on the Use of C-Reactive Protein in Early-Onset Neonatal Sepsis: Current Insights and New Tasks

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**Abstract**

C-reactive protein (CRP) is one of the most studied and most used laboratory tests for neonatal sepsis. As part of the acute-phase reaction to infection, it plays a central role in the humoral response to bacterial invasion. The delayed synthesis during the inflammatory response accounts for its low sensitivity during the early phases of the disease. Diagnostic accuracy clearly improves by the performance of serial determinations and by the combination with earlier markers such as interleukins or procalcitonin. CRP is as well particularly useful for monitoring the response to treatment and guiding antibiotic therapy, though nothing replaces the clinical impression and the gold standard (i.e. culture results). In spite of the large amount of research done on CRP in neonates, some topics are still not fully understood, such as the influence of noninfectious factors on CRP levels in healthy as well as in symptomatic neonates and the role of gestational age and birthweight on CRP kinetics. In this review, we aim to give an update on the current evidence on the use of CRP in neonates.

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## Introduction

Despite the advances in neonatal care, early-onset neonatal sepsis remains a serious and potentially life-threatening disease with a mortality rate ranging from 1.5% in term to almost 40% in very-low-birthweight infants [1–2]. The signs and symptoms of neonatal sepsis may be subtle and nonspecific being clinically indistinguishable from various noninfectious conditions such as respiratory distress syndrome or maladaptation. The current practice of starting empirical antibiotic therapy in all neonates showing infection-like symptoms results in their exposure to adverse drug effects, nosocomial complications, and in the emergence of resistant strains [3].

Laboratory sepsis markers complement the evaluation of clinical signs and risk factors in diagnosis of neonatal sepsis. No currently available test is able to provide perfect diagnostic accuracy, and false-negative as well as false-positive results may occur; the usefulness of a laboratory test therefore primarily depends on the clinical condition of the child. For example, in a critically ill newborn a negative test result will not give much additional information on its infectious status, and in an apparently well infant a positive result will not dramatically increase the probability that the child is infected. Diagnostic tests will be most useful in infants with clinically un-

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clear infectious status [4]. There is great interest in rapid diagnostic tests that are able to distinguish infected from uninfected newborns, especially in the early phase of the disease [5]. In fact, a delayed start of the antibiotic treatment may be no more able to stop the fulminant clinical course with development of septic shock and death within hours after the first clinical symptoms [6]. In the era of multi-resistant microorganisms, it is also important to avoid the unnecessary use of antibiotics in sepsis-negative infants.

There is an abundance of studies evaluating laboratory markers in the diagnosis of neonatal sepsis. Despite the promising results for some diagnostic markers, current evidence suggests that none of them can consistently diagnose 100% of infected cases. C-reactive protein (CRP) is the most extensively studied acute-phase reactant so far, and despite the ongoing rise (and fall) of new infection markers, its wide availability and its simple, fast, and cost-effective determination make it one of the preferred indices in many neonatal intensive care units (NICUs) [7].

### CRP Function and the Acute-Phase Response

CRP was first described in 1930 by Tillet and Francis at Rockefeller University [8]. They observed a precipitation reaction between serum from patients suffering acute pneumococcal pneumonia and the extracted polysaccharide fraction C from the pneumococcal cell wall. This reaction could not be observed when using serum of either healthy controls or the same pneumonia patients after they had recovered. In view of the fact that the polysaccharide fraction was a protein, the C-reactive component in the serum was named C-reactive protein [8]. By the 1950s, CRP had been detected in more than 70 disorders including acute bacterial, viral, and other infections, as well as noninfectious diseases such as acute myocardial infarction, rheumatic disorders, and malignancies [9]. All of these disorders of disparate etiology had in common the theme of inflammation and/or tissue injury [10].

The principal ligand to CRP is phosphocholine, which is found in lipopolysaccharide, bacterial cell walls, as well as in most biological membranes [11]. After binding, CRP is recognized by the complement system; CRP activates it, and promotes phagocytosis of the ligand by neutrophil granulocytes, macrophages, and other cells. CRP further activates monocytes and macrophages, and stimulates the production of proinflammatory cytokines [11, 12].

CRP is part of the acute-phase response, a physiological and metabolic reaction to an acute tissue injury of different etiologies (trauma, surgery, infection, acute inflammation, etc.) which aims to neutralize the inflammatory agent and to promote the healing of the injured tissue [10].

After a trauma or the invasion of microorganisms, an acute inflammatory reaction is initiated by activation of local resident cells which promote the recruitment and activation of further inflammatory cells, including fibroblasts, leukocytes, and endothelial cells. Once activated, they release proinflammatory cytokines including IL-1, TNF-$\alpha$, and IL-6. These cytokines induce the production of proteins of the acute-phase response in the liver. These include but are not limited to components of the complement system, coagulation factors, protease inhibitors, metal-binding proteins, and CRP [10, 12].

The production of CRP in the hepatocytes is mainly induced by IL-6, but can be further increased by synergy with IL-1 [13]. In 1981, Shine et al. [14] evaluated serum concentration of CRP determined by radioimmunoassay in 468 sera from normal adult volunteer blood donors, and reported on a median concentration of 0.8 mg/l with a 90th percentile of less than 3.0 mg/l. More recently, Rifai and Ridker [15] used three different high-sensitivity techniques to determine CRP distributions in their cohort consisting of 22,000 healthy adults from the United States. The median CRP values for men and women were 1.5 and 1.52 mg/l; the 90th percentiles were 6.05 and 6.61 mg/l, respectively. Similarly, Imhof et al. [16] examined CRP values from 13,000 apparently healthy men and women from different populations in Europe. The reported median concentration in the single cohorts ranged from 0.6 to 1.7 mg/l, the 90th percentiles from 3.2 to 8.0 mg/l.

During the acute-phase-response, CRP's hepatic synthesis rate increases within hours and can reach 1,000-fold levels [9, 11]. Levels remain high as long as the inflammation or tissue damage persists and then decrease rapidly. The half-life time has been reported by Vigushin et al. [17] to be 19 h in any of the diseases studied, being the fractional catabolic rate independent of the plasma CRP concentration. From this information, the synthesis rate of CRP therefore appears as the only significant determinant of its plasma level, supporting the clinical use of CRP measurements to monitor disease activity in all disorders characterized by a major acute-phase response.

## CRP in Neonatal Sepsis

Any elevation of serum CRP in the neonate always represents endogenous synthesis, since it passes the placenta in exceedingly low quantities [18]. De novo hepatic synthesis starts very rapidly after a single stimulus with serum concentrations rising above 5 mg/l by about 6 h and peaking at around 48 h [19].

For the diagnosis of early-onset sepsis in clinical practice, the sensitivity is more important compared to the specificity, as the consequences of unnecessarily treating an uninfected infant bear fewer complications than not treating an infected child.

In diagnosis of early-onset sepsis, previous studies reported on widely differing sensitivities and specificities of CRP ranging from 29 to 100% and from 6 to 100%, respectively [10, 20, 21]. These extreme variations are a result of different reference values, a posteriori-selected cutoff points, test methodologies, patient characteristics and inclusion criteria, as well as different definitions of sepsis, numbers of samples taken, and sampling times.

The sensitivity of CRP is known to be the lowest during the early stages of infection [22–24]. For a single CRP determination at the time of initial evaluation as well as for determinations from cord blood, the CRP diagnostic accuracy varies widely within an unacceptable range of sensitivity [22, 23, 25–32]. This may be related to the arbitrary choice of optimal cutoff points [27, 28, 30, 33] as well as the insensitive analytic methods with various limits of quantification used in the past [34] to detect the CRP pattern in the earliest course of infection, in particular in the very early neonatal period.

A raised CRP is not necessarily diagnostic for sepsis, as elevations may also occur due to the physiologic rise after birth or noninfection-associated conditions (see below). Therefore, concerns were raised about the reliability of CRP during the early stage of the disease being neither able to diagnose nor to rule out an infection with certainty [22].

Benitz et al. [22] found that the sensitivity in the diagnosis of culture-proven early-onset sepsis increased from 35% (95% confidence interval 30–41%) at the initial sepsis workup to 79% (72–86) after 8–24 h, and 89% (81–94) for the higher of two levels obtained after 8–48 h after the initial workup. Concurrently, they reported a decrease in specificity from 90% (88–92) to 78% (76–81) and 74% (71–77) for CRP levels performed as described above. Pourcyrous et al. [23] evaluated serial CRP levels in a large series of 689 investigations for neonatal sepsis (187 of them with positive blood culture results) in 489 neo-

nates, and determined CRP at the initial sepsis evaluation and 12 and 24 h later. The postnatal age at the time of the initial investigation ranged from less than one day (60%) to 191 days (infants were older than one month in 13%). They [23] reported a higher sensitivity for any of the three CRP values (obtained by three serial determinations at 12-hour intervals) compared to the first value (74 vs. 55%). In general, the sensitivity substantially increases with serial determinations 24–48 h after the onset of symptoms [10, 20]. Several studies reported on sensitivities and specificities ranging from 74 to 98% and from 71 to 94%, respectively, for either serial CRP determinations or a single determination at least 12 h after the onset of symptoms [22–26, 33]. However, by that point most newborns will be asymptomatic and will have confirmed negative culture results [35].

Philip [36] and later others [37–40] suggested that serial levels may also be useful for identification of infants who do not have a bacterial infection. A repeat CRP 24–48 h after the initiation of antibiotic therapy has been reported to carry a 99% negative predictive value in accurately identifying, in the early neonatal period, infants not infected [7, 20, 22, 41–43].

Serial CRP measurements can be helpful in monitoring the response to treatment in infected neonates, to determine the duration of antibiotic therapy, and to recognize possible complications [23, 24, 44]. In a cohort of 60 neonates with early-onset sepsis, Ehl et al. [45] demonstrated that after initiation of a successful antibiotic therapy, CRP values further increased, peaking and consecutively decreasing after 16 h. A CRP level that returned again to the normal range may indicate that the duration of antibiotic treatment has been sufficient, allowing discontinuation of antibiotics [41], provided the clinical condition of the child improved and culture results were negative.

Thus, CRP has been proposed as a key decision parameter for guiding the duration of antibiotic therapy [20, 22, 41–43]. However, CRP was not the single criterion evaluated in any infant in these studies. In fact, other criteria explicitly included in the decision of whether or not to discontinue antibiotics were clinical status, culture results, and results of other laboratory tests. Thus, the current literature does not sustain CRP as the single decision parameter to discontinue antibiotics.

The magnitude of the CRP response to sepsis was reported to depend also on the underlying pathogen. In 1974, Sabel and Hanson [46] reported that *Escherichia coli* infection increases CRP levels with impressive consistency. In 1993, Pourcyrous et al. [23] reported the same

phenomenon among 187 cases of positive blood culture in 691 investigations for sepsis in infants aged from birth to 191 days of life. They added evidence on a more distinct CRP increase in Gram-negative compared to Gram-positive strains with CRP levels above 10 mg/l in 92 and 64% of 174 single-organism blood cultures, respectively. Cultures with growth of *Escherichia coli*, group B streptococci, and *Staphylococcus aureus* were associated with abnormal CRP values in 100, 92, and 89% respectively. The percent incidence of abnormal CRP concentrations varied considerably among the organisms recovered with persistently normal CRP levels in 48 of 174 single-organism blood cultures. In 40 of them (36 with Gram-positive strains, mainly group D streptococci, *Streptococcus viridans*, and *Streptococcus epidermidis*), antibiotic therapy was not administered or inadequate. All of them had uneventful clinical courses, and thus these positive blood culture results may be caused by contamination [23]. Other reports on a pathogen-dependent CRP response in neonates include that of Rønnestad et al. [47] who evaluated CRP responses from day 1 to day 4 in 121 monomicrobial septic episodes in neonates. They reported significantly lower median values (day 1–4) in coagulase-negative staphylococci (23 mg/l) compared to *S. aureus*, group B streptococci, and *E. coli* (51–58 mg/l).

### Noninfection-Associated Elevations of CRP

Interpretation of CRP in diagnosis of early-onset sepsis may be hindered by several noninfectious conditions influencing values during the days after birth (see table 1).

In adults, CRP elevation is known to be associated with a large variety of disorders apart from bacterial, viral, and fungal infections including burns, surgery, rheumatic disorders, malignancies, and vasculitis [9].

However, the issue of noninfectious CRP elevations in the neonate is not undisputed; the available data are to some extent contradictory, and, despite the amount of studies on this issue, inconclusive. The earliest descriptions of noninfectious conditions influencing CRP derive from simple observations that elevated values in not infected infants might be connected to coincidental noninfectious conditions, though no statistical confirmation is given.

In 1982, Ainbender et al. [40] described CRP values >20 mg/l in 11 of 100 uninfected infants consecutively admitted to the special care nursery. The authors described that 8 of the 11 infants had, either singly or in combination, shock, meconium aspiration pneumonitis, fetal distress, maternal fever and PROM, and none was found to be infected. Forest et al. [48] reported elevated CRP values between 11 and 70 mg/l in 16/49 uninfected neonates admitted to the NICU with diagnoses of intraventricular hemorrhage, meconium aspiration pneumonia, anoxic encephalopathy, PROM, respiratory distress syndrome, chorioamnionitis, aspiration pneumonia, and transitory tachypnea. There exist further studies that were conducted similarly (see table 1), but rather small sample sizes and the lack of statistical confirmation impair their reliability.

Identifying independent variables that influence the interpretation of CRP in symptomatic neonates or in neonates at risk for infection may represent an important aid in the differential diagnosis of early-onset sepsis. In 2004, Mathai et al. [49] evaluated CRP values in 250 infected and uninfected neonates, and found maternal fever and prolonged labor being significantly associated with values >12 mg/l at 24 h of life. Chiesa et al. [31] aimed to identify independent factors influencing CRP in 134 critically ill infected and uninfected neonates by multiple linear regression analysis, and described nonsignificant association with both of the above described conditions and with other, mainly maternal and perinatal factors, at birth, at 24 and 48 h of life.

Few investigations were performed on the association of CRP with noninfectious conditions in healthy neonates. Chiesa et al. [50] evaluated conditions influencing what constitutes normal CRP values in healthy neonates. In their analysis on 148 healthy term or near-term neonates, they identified low 5-min Apgar score and premature rupture of membranes being significantly associated with CRP response at birth and pregnancy-induced hypertension with CRP response at 24 h of life. In a similarly selected cohort of 421 healthy neonates including 200 premature infants, they confirmed an association with the time of ruptured membranes and added duration of active labor, prenatal steroids, and intrapartum antimicrobial prophylaxis as variables that had a significant effect on CRP concentrations when adjusted for gestational age, gender, and sampling time [51].

Even though there exist numerous studies on noninfectious factors influencing CRP in neonates, the wide-ranging inclusion criteria and thus study populations (infected, uninfected, symptomatic, healthy, at risk, critically ill), differences in methodology, upper limits for CRP, influencing factors chosen to analyze and their definitions hinder the comparison of these studies and impair drawing generally applicable conclusions.

**Table 1.** Noninfectious conditions influencing CRP values during the first days of life

| | | p value | Population | Commentary |
|---|---|---|---|---|
| Maternal fever during labor | Ainbender et al. [40] | n.s.c. | 100 admissions to the special care nursery, plus 33 neonates with PROM or maternal fever during labor | 3/11 neonates with CRP >20 mg/l had maternal fever, 14/33 neonates with a history of PROM or maternal fever had CRP ≥10 mg/l |
| | Mathai et al. [49] | <0.05 | 250 uninfected and infected neonates with maternal risk factors | association with CRP >6 mg/l in cord blood and >12 mg/l in neonatal blood at 24 h of life |
| Prolonged rupture of membranes | Ainbender et al. [40] | n.s.c. | see above | 2/11 neonates with CRP >20 mg/l had PROM, 14/33 neonates with a history of PROM or maternal fever had CRP ≥10 mg/l |
| | Forest et al. [48] | n.s.c. | 49 uninfected symptomatic neonates | CRP values of 12, 18, and 19 mg/l in 3 cases of PROM |
| | Mathai et al. [49] | <0.05 | see above | association with CRP >6 mg/l in cord blood |
| | Chiesa et al. [51] | <0.05[a] | 421 healthy neonates | increase in CRP by 0.4% per hour of ruptured membranes |
| | Chiesa et al. [50] | 0.001[a] | 148 healthy neonates | 1.32-fold CRP increase at birth if the time of ruptured membranes was ≥18 h |
| Stressful delivery or fetal distress | Ainbender et al. [40] | n.s.c. | see above | 4/11 neonates with CRP >20 mg/l had fetal distress |
| | Forest et al. [48] | n.s.c. | see above | CRP value of 29 mg/l in 1 infant with in utero fetal distress |
| | Kääpä and Koistinen [18] | <0.01 | 267 uninfected neonates | vacuum extraction was associated with a CRP rise at 24 h after delivery |
| Prolonged labor | Ishibashi et al. [55] | 0.037 | 110 uninfected symptomatic neonates | significant association with length of active labor ≥20 h |
| | Mathai et al. [49] | <0.05 | see above | association with CRP >6 mg/l in cord blood |
| | Chiesa et al. [51] | <0.01[a] | see above | increase of CRP by 14.5% per hour of active labor |
| Perinatal asphyxia/ shock | Ainbender et al. [40] | n.s.c. | see above | 2/11 neonates with CRP >20 mg/l had shock |
| | Forest et al. [48] | n.s.c. | see above | CRP value of 45 mg/l in 1 infant who died from anoxic encephalopathy |
| | Berger et al. [33] | n.s.c. | 106 uninfected critically ill neonates | 4/14 neonates with CRP >20 mg/l had severe asphyxia |
| | Chiesa et al. [50] | 0.01[a] | see above | 1.5-fold CRP increase at birth if the Apgar score at 5 min was ≤8 |
| Meconium aspiration syndrome | Ainbender et al. [40] | n.s.c. | see above | 4/11 neonates with CRP >20 mg/l had MAS |
| | Dyck et al. [76] | n.s.c. (p not given for MAS alone) | 87 symptomatic uninfected and infected neonates | higher levels in 13 infants with pneumonia, aspiration, pneumothorax, sepsis, etc. compared to 55 with RDS and 19 with unstable cardiovascular status; no data for MAS alone |
| | Forest et al. [48] | n.s.c. | see above | CRP value of 47 mg/l in one infant with MAS |
| | Pourcyrous et al. [77] | n.s.c. | 114 symptomatic neonates with negative blood cultures | 6/6 neonates with MAS had CRP values ≥9 mg/l |
| | Pourcyrous et al. [23] | n.s.c. | 287 investigations in symptomatic neonates with possible infection | 8/10 neonates with MAS had CRP values >9 mg/l |
| | Berger et al. [33] | n.s.c. | see above | 2/14 neonates with CRP >20 mg/l had MAS |
| | Hofer et al. [54] | 0.009[a] | 499 uninfected neonates admitted to the NICU | significant association in term neonates |
| Clinically silent meconium aspiration | Mathai et al. [49] | <0.05 | see above | association with CRP >6 mg/l in neonatal blood at 24 h of life |
| Surfactant application | Kukkonen et al. [78] | 0.001 | 228 neonates with respiratory distress and need for surfactant application | association of administration of porcine surfactant with CRP >40 mg/l (though as well with leucopenia and sepsis) compared to synthetic surfactant |
| | Hofer et al. [54] | <0.001[a]; 0.025[a] | see above | significant association in preterm and term neonates |
| Intraventricular hemorrhage | Forest et al. [48] | n.s.c. | see above | CRP value of 70 mg/l in 1 infant with cerebral hemorrhage and RDS |
| | Berger et al. [33] | n.s.c. | see above | 3/14 neonates with CRP >20 mg/l had a severe intraventricular hemorrhage |
| Pneumothorax | Berger et al. [33] | n.s.c. | see above | 2/14 neonates with CRP >20 mg/l had a pneumothorax |
| Tissue injury | Pourcyrous et al. [23] | n.s.c. | see above | 6/19 neonates with tissue injury had CRP >9 mg/l |

n.s.c. = Not statistically confirmed. [a] Factors identified by multivariate analyses.

The current literature suggests that CRP may be elevated in some noninfectious conditions, of which some may per se clinically mimic a bacterial infection as well. Thus, the up-to-date available information lacks robust evidence to support a claim that withholding antibiotics may be justified in infants with raised CRP in the above-mentioned conditions.

## Effects of Development and Maturation on CRP Performance

Even though advances in neonatal intensive care have led to increasing preterm birth rates and survival rates, the influence of prematurity on laboratory test results have been poorly investigated, and have not been assessed systematically. This is also true for CRP, which is one of the most extensively studied infection markers in the neonatal period. Reports on the influence of gestational age and birthweight on kinetics of CRP in infected and uninfected infants are limited.

In a pilot study, Turner et al. [52] demonstrated an association of gestational age with the magnitude of clinically relevant CRP responses during the first 7 days after birth. In case of a clinically relevant CRP rise >10 mg/l, the proportion of a pronounced response >60 mg/l increased with gestational age from 8% in newborns from 24 to 27 weeks to 25% in newborns from 40 to 41 weeks. This pattern of results was also found when infants were grouped according to birthweight.

In a cohort of 348 infants, Kawamura and Nishida [24] reported a lower sensitivity of CRP in the diagnosis of neonatal sepsis in preterm compared to term newborns (61.5 vs. 75%).

Doellner et al. [53] described a significantly lower CRP increase induced by infection in NICU preterm compared to NICU term infants. In their cohort of 42 newborns with either culture-proven or probable sepsis, infants with a gestational age <35 weeks had lower median CRP values and lower CRP peak values compared to infants with a gestational age greater than 35 weeks (median CRP 0 vs. 18 mg/l, CRP peak 15 vs. 52 mg/l).

We have recently reported on a lower CRP response to infection in preterm compared to term newborns with a lower sensitivity (53 vs. 86%), lower median values (9 vs. 18.5 mg/l), and a lower area under the receiver operating characteristics curve (0.799 vs. 0.890) [54].

Some previous studies have addressed the correlation of CRP values with gestational age and/or birthweight in uninfected newborns.

In 2002, Ishibashi et al. [55] demonstrated that birthweight is independently associated with high sensitivity CRP values within 48 h after birth in a cohort of 110 uninfected symptomatic newborns. In 2011, in another cohort including 499 uninfected newborns hospitalized in a NICU, we have reported CRP values determined within the first 3 days of life being significantly lower in preterm compared to term newborns (0.5 vs. 2 mg/l) [54]. Finally, Chiesa et al. [51] have analyzed CRP values in 421 healthy term and preterm newborns from birth to 4 and 5 days of life (for term and preterm neonates, respectively). They found that the healthy preterm babies have a lower and shorter CRP response compared with that in healthy term babies, demonstrating the independent effects of prematurity on CRP values. Mean CRP values increased by 6.0% per week of gestational age at delivery and by 2.4% per 100 g increase in birthweight.

For neonates, assessment of laboratory tests occurs within a complex context of prenatal growth and neonatal development [56]. Though the current literature reveals some minor disagreement on the effect of gestational age on CRP, there is a growing body of evidence suggesting that the so far reported CRP performance may be different among neonates born preterm and term as well as among neonates with low and high birthweight [51, 56]. Prematurity of the organ systems and maturational changes in the immune system might result in a more distinct CRP response to delivery in uninfected newborns and to bacterial invasion in infected newborns. The few studies so far addressing this issue suggest that the diagnostic accuracy of CRP in preterm infants may benefit from a reevaluation of the reference intervals and, thus, upper limits of the normal range in this age group [51, 53].

## CRP Reference Values

Especially in the early neonatal period, many physiological and metabolic processes change and differ from every later moment in life. These changes affect several laboratory parameters as well, and many reference values and serum kinetics substantially differ from later time periods [57].

Reliable reference values are crucial for obtaining an adequate diagnostic accuracy. Upper limits for CRP during the first days of life have mainly been established from uninfected but symptomatic neonates. The few studies assessing upper limits in healthy neonates were mostly based on rather small sample sizes or did not

take into account their postnatal age. In 1984, Gutteberg et al. [39] reported on CRP levels of 16 apparently healthy neonates with 5 mg/l being the 97.5th percentile during the first month of life. Forest et al. [48] analyzed CRP values in a cohort of 69 newborns with a normal postnatal course, and reported 68 of them maintained CRP values <10 mg/l during the follow-up period of up to 18 weeks. In 1992, Schouten-Van Meeteren et al. [58] found that 95% of 38 apparently healthy neonates had CRP values ≤10 mg/l taken 12 and 24 h after birth. However, rather small sample sizes, significant error rates of the determination methods used and only partially given follow-up data on the initially apparently healthy neonates represent weaknesses of the above-mentioned studies [4].

Cutoff values reported in the literature range from 1.5 to 20 mg/l with wide-ranging sensitivities and specificities [4, 10]. The up-to-date most used upper limit for CRP during the first days of life of 10 mg/l has been established in 1987 by Mathers and Pohlandt [27]. One decade later, Benitz et al. [22] evaluated this cutoff value in 1,002 episodes of suspected early-onset sepsis and confirmed the value being an appropriate threshold above which results can be considered abnormal.

Use of CRP in the first few days after birth is complicated by a nonspecific rise primarily related to the stress of delivery [10, 50]. In 1993, Kääpä and Koistinen [18] described significantly higher CRP values in neonates after 24 h of life compared to values taken immediately after birth (p < 0.001). Thereafter, neonatal CRP values remained unchanged for the first 3 days of life. In 2001, Chiesa et al. [50] aimed to assess the 'normal' dynamics of CRP after birth. In their cohort of 148 neonates with an unremarkable clinical course from birth to the 4-week follow-up visit, the 95th percentiles of CRP values at birth, 24, and 48 h life were 5.0, 14.0, and 9.7 mg/l. In 2011, Chiesa et al. [51] examined the postnatal reference intervals separately for term and preterm neonates, and described a lower and shorter CRP rise in preterm compared to term infants with peak values of 11 mg/l and 13 mg/l, respectively.

These observations raise concern about the static cutoff value not reflecting the physiologic kinetics of CRP after birth. At the moment, there exist some studies that have aimed to determine age-specific reference values for CRP during the first days of life. [31, 55]. However, these values are still to be validated in an independent patient cohort.

## CRP in the Era of 'Old' and Other 'New' Infection Markers

An important limitation of CRP is the low sensitivity during the early phases of sepsis. CRP takes 10–12 h to significantly change after the onset of infection [7]. Earlier in the inflammatory cascade, activated macrophages release proinflammatory cytokines and growth factors. Their increase therefore precedes the changes in CRP. Of the many mediators studied, much attention has been focused on IL-6, IL-8, and TNF-$\alpha$.

IL-6 increases rapidly after the bacterial invasion, and was demonstrated to have a high sensitivity during the early stages of sepsis (80–100%) even when determined from umbilical cord blood (87–100%) [28, 59–61]. Chirico and Loda [7] report that the IL-6 short half-life results in a rapid normalization of its serum levels, even though the infection persists. However, Panero et al. [62] demonstrated that NICU neonates with clinical and/or microbiologic evidence of infection have persistently elevated IL-6 compared to neonates without clinical and microbiologic evidence of infection. Chiesa et al. [50] described significantly higher IL-6 concentrations in healthy near-term (35–36 weeks of gestation) compared to term neonates (≥37 weeks of gestation) at birth and at 24 and 48 h of life, thus suggesting a gestational age-dependent effect on IL-6 values over the first 48 h of life. IL-8 and TNF-$\alpha$ have very similar characteristics and kinetic properties to IL-6 [59]. While studies report on a reliable diagnostic accuracy of IL-8 with a sensitivity of 69–100%, the usefulness of TNF-$\alpha$ as a diagnostic marker has not been found to be as good as IL-6 or IL-8 [59, 60]. IL-8 was described to vary with gestational age, with preterm infants having a wide variety of associated comorbidities and thus higher IL-8 values compared to term infants as determined in cord blood [63] and serum collected on postnatal days 2–5 [64]. As such, the independent effect of gestational age on IL-8 neonatal response has yet to be assessed.

Procalcitonin (PCT) is an acute-phase reactant which has the advantage of increasing rapidly after contact to bacterial endotoxin with levels rising after 4 h and peaking at 6–8 h [59, 65]. In a recent meta-analysis, the sensitivity and specificity in the diagnosis of early-onset sepsis were 76% (range 68–82) and 76% (60–87) [66]. In a recent single-center, prospective, randomized intervention study, Stocker et al. [67] have analyzed the effect of PCT-guided decision-making on duration of antibiotic therapy in suspected neonatal early-onset sepsis and reported significant higher proportion of infants treated for less

than 72 h in the PCT-guided compared to the conventional group (45 vs. 18%).

Specific leukocyte cell surface antigens are known to be expressed in substantial quantities after inflammatory cells are activated by bacteria or their cellular products [68]. They have the advantage of requiring only a minimal volume of blood for determination (0.05 ml whole blood) [59]. From the amount of surface markers studied, neutrophil CD11b and CD64 appear most promising for diagnosis of neonatal sepsis. CD11b expression increases considerably within a few minutes after the inflammatory cells come into contact with bacteria and endotoxins [68, 69]. The sensitivity and specificity of CD11b for diagnosing early-onset neonatal sepsis are 86–100 and 100%, respectively [5, 59]. CD64 has a sensitivity ranging between 78 and 96% and a NPV between 89 and 97% [70, 71]. Though promising, estimation of cell surface markers is limited by the need for sophisticated equipment and the need to process blood samples rapidly before neutrophils die from apoptosis or the surface antigens are downregulated [72].

Despite the favorable claims by many studies, the high costs, the limited availability of specimens at the appropriate time, the complexity of the assay methods, the laboratory turnover time, and the test reliability all limit the clinical applicability of most diagnostic markers [59]. More importantly, the relatively small sample size in most studies and the lack of clear reference values for many markers still prohibit the use of many of them in clinical practice.

Currently, hematological indices including white blood cell count and absolute neutrophil count are frequently used to help assess the likelihood of infection in neonates that are symptomatic or at risk. The studies of both reference ranges and diagnostic accuracy report widely differing results [21, 73–75]. In a current analysis on almost 70,000 symptomatic or at-risk neonates, Newman et al. [73] demonstrated that during the first 72 h of life white blood cell count and absolute neutrophil count are associated with an increased likelihood of infection only when they are low, so that the informative value increases with postnatal age. The area under the receiver operating characteristics curve for the white blood cell count and absolute neutrophil count determined within the first hour of life was 0.52 and 0.55, respectively, but increased significantly when determined after at least 4 h of life (0.87 and 0.85, respectively) [73].

An elevated immature to total neutrophil ratio is well associated with neonatal sepsis, but its diagnostic accuracy for early-onset infections shows again wide-ranging values [21]. Furthermore, its determination is subject to observer bias and thus of poor reproducibility.

At the moment, none of the described current diagnostic markers are sensitive and specific enough to influence the decision whether or not to withhold antimicrobial treatment independent of the clinical findings. Efforts were made to improve diagnostic accuracy by combining multiple markers in order to further enhance the diagnostic accuracy of these mediators in identifying infected cases.

CRP has been investigated in combination with a variety of 'new' infection markers including cytokines, surface markers, and other acute-phase reactants with promising results. Especially the combination with an early sensitive marker such as PCT, IL-6, IL-8, CD11b, and CD64 increases the sensitivity to values between 90 and 100% in most studies (see table 2).

## Conclusion

### What Is Already Known?

- The delayed induction of the hepatic synthesis of CRP during the inflammatory response to infection lowers its sensitivity during the early phases of sepsis. The performance of serial determinations 24–48 h after the onset of symptoms is recommended, as it clearly improves diagnostic accuracy.
- CRP is particularly useful for monitoring the response to treatment and for ruling out an infection. A repeated determination of CRP 24–48 h after the initiation of antibiotic therapy has been reported to carry a 99% negative predictive value in accurately identifying uninfected neonates, though nothing replaces a clinical impression and the gold standard (i.e. culture results).
- CRP values undergo a physiological 3-day rise after birth. This physiologic dynamics as well as certain maternal and perinatal factors may affect interpretation of what constitutes 'normal' CRP values in healthy neonates. Furthermore, some reports suggest noninfectious confounders such as meconium aspiration syndrome and perinatal maternal risk conditions may significantly elevate CRP values in symptomatic or at-risk neonates and thus confound interpretation of CRP values in the diagnosis of sepsis.

### What Is New?

- A growing body of evidence suggests a link between gestational age and CRP kinetics with lower baseline CRP values and a lower CRP response to infection in preterm compared to term newborns.

**Table 2.** Diagnostic accuracy of CRP in combination with IL-6 (pg/ml), IL-8 (pg/ml), PCT (ng/ml), and CD64 (antibody-phycoerythrin molecules bound/cell)

| Source | Sepsis-positive infants/all infants | Sepsis definition | Diagnostic test | Sens. % | Spec. % | PPV % | NPV % |
|---|---|---|---|---|---|---|---|
| Laborada et al. [25] | 48/105 newborns with suspected sepsis | confirmed (clinical signs and positive blood, CSF and urine culture) and clinical sepsis (≥5 clinical signs) | CRP >10*<br>IL-6 >18*<br>IL-6 >18 and CRP >10* | 69<br>76<br>89 | 96<br>73<br>73 | 93<br>67<br>70 | 80<br>81<br>90 |
| Doellner et al. [53] | 24/241 neonates admitted to the NICU | clinical suspicion of sepsis with positive blood culture and clinical suspicion with elevated CRP and I/T ratio; pneumonia | IL-6 ≥50*<br>CRP ≥10*<br>IL-6 ≥50 and/or CRP ≥10* | 61<br>63<br>96 | 76<br>97<br>74 | 38<br>83<br>49 | 89<br>91<br>99 |
| Messer et al. [79] | 36/253 neonates from the obstetrics unit and NICU | infected (positive blood and/or CSF culture, abnormal WBC and CRP and clinical signs) and probably infected neonates (clinical signs, abnormal WBC and CRP) | IL-6 ≥10*<br>CRP ≥15*<br>IL-6 ≥100 and CRP ≥15* | 83<br>45<br>100 | 90<br>96<br> | 59<br><br> | 97<br><br> |
| Franz et al. [80] | 327/1,291 neonates with suspected sepsis or at risk | culture-proven (positive blood culture, clinical signs and CRP >10 mg/l within 12 − 60 h after the initial evaluation) and clinical infection (clinical signs and CRP >10 mg/l) | IL-8 ≥70*<br>CRP >10*<br>IL-8 ≥70 and/or CRP >10* | 44<br>54<br>80 | 90<br><br>87 | 58<br><br>68 | 83<br>86<br>93 |
| Laborada et al. [25] | 48/105 newborns with suspected sepsis | confirmed (clinical signs and positive blood, CSF and urine culture) and clinical sepsis (≥5 clinical signs) | CRP >10*<br>IL-8 >100*<br>IL-8 >100 and CRP >10* | 69<br>75<br>89 | 96<br>66<br>66 | 93<br>60<br>65 | 80<br>80<br>90 |
| Franz et al. [81] | 112/331 neonates with suspected sepsis[a] | culture-proven (positive blood or CSF culture, clinical signs or a maternal history) and clinical sepsis (clinical signs and CRP >10 mg/l 12–60 h after the initial evaluation) | IL-8 ≥70*<br>CRP >10*<br>IL-8 ≥70 and/or CRP >10* | 82<br>28<br>92 | 79<br>98<br>77 | 67<br>86<br>67 | 90<br>87<br>95 |
| Resch et al. [28] | 41/76 neonates with suspected sepsis | blood culture proven and clinical sepsis (clinical signs, laboratory sepsis markers including CRP and/or maternal risk factors, antibiotics ≥7 days) | PCT >6*<br>CRP >8*<br>PCT >6 and CRP >8* | 77<br>49<br>83 | 91<br>100<br> | 93<br>100<br> | 72<br>58<br> |
| Franz et al. [82] | 46/162 neonates with suspected sepsis | culture-proven and clinical sepsis (clinical signs and CRP >10 mg/l at 12–60 h after the first blood sampling) | PCT ≥0.5*<br>CRP >10*<br>PCT ≥0.5 and/or CRP >10* | 57<br>28<br>63 | 66<br>97<br>66 | 40<br>81<br>42 | 79<br>77<br>82 |
| Ng et al. [70] | 115/338 neonates with suspected sepsis | positive blood culture, other microbiology-confirmed bacterial infections (peritonitis, meningitis, necrotizing enterocolitis, pneumonia), clinical pneumonia | CD64 ≥7,060*<br>CRP ≥10.0*<br>CD64 ≥7,060 or CRP ≥10.0*<br>CD64 ≥7,060 or CRP ≥10.0** | 70<br>49<br>77<br>94 | 94<br>91<br>86<br>78 | 86<br>73<br>73<br>68 | 86<br>77<br>88<br>96 |

\* At the time of evaluation; \*\* at 24 h after onset.
[a] The study included two study periods, we list the results from the period with more sepsis cases (vs. 70/378 neonates).

- Still, data on noninfectious CRP elevations in otherwise healthy newborns as well as symptomatic and at-risk neonates demand further research in this topic before recommendations on the continuation or withdrawal of antibiotics in these infants can be given.
- Currently, the most used cutoff value is 10 mg/l irrespective of the gestational and postnatal age of the neonate. In view of the physiologic dynamics of CRP during the first days after birth and the influence of gestational age on its response to infection, it appears reasonable to reconsider this static cutoff value and evaluate the possible advantages of the introduction of dynamic reference values.
- CRP has the best diagnostic accuracy when combined with another infection marker that compensates for its diagnostic weakness and provides reliable sensitivity during the early phases of sepsis. Suitable markers include but are not limited to PCT, IL-6, and IL-8. Many further parameters may provide similar good results, but are not yet sufficiently examined to be applied in clinical practice.

CRP is one of the most widely available, most studied, and most used laboratory tests for neonatal bacterial infection, and despite the continuing emergence of new infection markers, it still plays a central role in the diagnosis of early-onset sepsis of the neonate. CRP has the advantage of being well characterized in numerous studies, and the extensive knowledge of its properties and limitations makes it safer compared to other, newer markers. Still, further research is needed on the topics of the influence of gestational age on CRP kinetics in infection, noninfectious confounders, and the evaluation of dynamic and gestational age-dependent reference values.

## References

1 Weston EJ, Pondo T, Lewis MM, Martell-Cleary P, Morin C, Jewell B, et al: The burden of invasive early-onset neonatal sepsis in the United States, 2005–2008. Pediatr Infect Dis J 2011;30:937–941.
2 Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR, et al: Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol 2007;196:147.e1–8.
3 Murray BE: Can antibiotic resistance be controlled? N Engl J Med 1994;330:1229–1230.
4 Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L: Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem 2004;50:279–287.
5 Mishra UK, Jacobs SE, Doyle LW, Garland SM: Newer approaches to the diagnosis of early onset neonatal sepsis. Arch Dis Child Fetal Neonatal Ed 2006;91:F208–F212.
6 Ng PC, Li K, Leung TF, Wong RPO, Li G, Chui KM, et al: Early prediction of sepsis-induced disseminated intravascular coagulation with interleukin-10, interleukin-6, and RANTES in preterm infants. Clin Chem 2006;52:1181–1189.
7 Chirico G, Loda C: Laboratory aid to the diagnosis and therapy of infection in the neonate. Pediatr Rep 2011;3:e1.
8 Tillett WS, Francis T: Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. J Exp Med 1930;52:561–571.
9 Pepys MB: C-reactive protein fifty years on. Lancet 1981;1:653–657.
10 Jaye DL, Waites KB: Clinical applications of C-reactive protein in pediatrics. Pediatr Infect Dis J 1997;16:735–746.

11 Volanakis JE: Human C-reactive protein: expression, structure, and function. Mol Immunol 2001;38:189–197.
12 Du Clos TW: Function of C-reactive protein. Ann Med 2000;32:274–278.
13 Weinhold B, Rüther U: Interleukin-6-dependent and -independent regulation of the human C-reactive protein gene. Biochem J 1997;327:425–429.
14 Shine B, de Beer FC, Pepys MB: Solid phase radioimmunoassays for human C-reactive protein. Clin Chim Acta 1981 25;117:13–23.
15 Rifai N, Ridker PM: Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. Clin Chem 2003;49:666–669.
16 Imhof A, Fröhlich M, Loewel H, Helbecque N, Woodward M, Amouyel P, et al: Distributions of C-reactive protein measured by high-sensitivity assays in apparently healthy men and women from different populations in Europe. Clin Chem 2003;49:669–672.
17 Vigushin DM, Pepys MB, Hawkins PN: Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease. J Clin Invest 1993;91:1351–1357.
18 Kääpä P, Koistinen E: Maternal and neonatal C-reactive protein after interventions during delivery. Acta Obstet Gynecol Scand 1993;72:543–546.
19 Pepys MB, Hirschfield GM: C-reactive protein: a critical update. J Clin Invest 2003;111:1805–1812.

20 Hengst JM: The role of C-reactive protein in the evaluation and management of infants with suspected sepsis. Adv Neonatal Care 2003;3:3–13.
21 Fowlie PW, Schmidt B: Diagnostic tests for bacterial infection from birth to 90 days – a systematic review. Arch Dis Child Fetal Neonatal Ed 1998;78:F92–F98.
22 Benitz WE, Han MY, Madan A, Ramachandra P: Serial serum C-reactive protein levels in the diagnosis of neonatal infection. Pediatrics 1998;102:E41.
23 Pourcyrous M, Bada HS, Korones SB, Baselski V, Wong SP: Significance of serial C-reactive protein responses in neonatal infection and other disorders. Pediatrics 1993;92:431–435.
24 Kawamura M, Nishida H: The usefulness of serial C-reactive protein measurement in managing neonatal infection. Acta Paediatr 1995;84:10–13.
25 Laborada G, Rego M, Jain A, Guliano M, Stavola J, Ballabh P, et al: Diagnostic value of cytokines and C-reactive protein in the first 24 h of neonatal sepsis. Am J Perinatol 2003;20:491–501.
26 Wagle S, Grauaug A, Kohan R, Evans SF: C-reactive protein as a diagnostic tool of sepsis in very immature babies. J Paediatr Child Health 1994;30:40–44.
27 Mathers NJ, Pohlandt F: Diagnostic audit of C-reactive protein in neonatal infection. Eur J Pediatr 1987;146:147–151.
28 Resch B, Gusenleitner W, Müller WD: Procalcitonin and interleukin-6 in the diagnosis of early-onset sepsis of the neonate. Acta Paediatr 2003;92:243–245.

29 Joram N, Boscher C, Denizot S, Loubersac V, Winer N, Roze JC, et al: Umbilical cord blood procalcitonin and C reactive protein concentrations as markers for early diagnosis of very early onset neonatal infection. Arch Dis Child Fetal Neonatal Ed 2006;91:F65–F66.

30 Kordek A, Giedrys-Kalemba S, Pawlus B, Podraza W, Czajka R: Umbilical cord blood serum procalcitonin concentration in the diagnosis of early neonatal infection. J Perinatol 2003;23:148–153.

31 Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma M, et al: C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. Clin Chem 2003;49:60–68.

32 Kordek A, Hałasa M, Podraza W: Early detection of an early onset infection in the neonate based on measurements of procalcitonin and C-reactive protein concentrations in cord blood. Clin Chem Lab Med 2008;46:1143–1148.

33 Berger C, Uehlinger J, Ghelfi D, Blau N, Fanconi S: Comparison of C-reactive protein and white blood cell count with differential in neonates at risk for septicaemia. Eur J Pediatr 1995;154:138–144.

34 Roberts WL, Moulton L, Law TC, Farrow G, Cooper-Anderson M, Savory J, et al: Evaluation of nine automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. 2. Clin Chem 2001;47:418–425.

35 Escobar GJ: Effect of the systemic inflammatory response on biochemical markers of neonatal bacterial infection: a fresh look at old confounders. Clin Chem 2003;49:21–22.

36 Philip AGS: Commentary; in Oski FA, Stockman JA (eds): Year Book of Pediatrics. Chicago, Year Book Medical Publishers, 1981, pp 17.

37 Squire EN Jr, Reich HM, Merenstein GB, Favara BE, Todd JK: Criteria for the discontinuation of antibiotic therapy during presumptive treatment of suspected neonatal infection. Pediatr Infect Dis 1982;1:85–90.

38 Speer C, Bruns A, Gahr M: Sequential determination of CRP, alpha 1-antitrypsin and haptoglobin in neonatal septicaemia. Acta Paediatr Scand 1983;72:679–683.

39 Gutteberg TJ, Haneberg B, Jørgensen T: Lactoferrin in relation to acute phase proteins in sera from newborn infants with severe infections. Eur J Pediatr 1984;142:37–39.

40 Ainbender E, Cabatu EE, Guzman DM, Sweet AY: Serum C-reactive protein and problems of newborn infants. J Pediatr 1982;101:438–440.

41 Ehl S, Gering B, Bartmann P, Högel J, Pohlandt F: C-reactive protein is a useful marker for guiding duration of antibiotic therapy in suspected neonatal bacterial infection. Pediatrics 1997;99:216–221.

42 Bomela HN, Ballot DE, Cory BJ, Cooper PA: Use of C-reactive protein to guide duration of empiric antibiotic therapy in suspected early neonatal sepsis. Pediatr Infect Dis J 2000;19:531–535.

43 Philip AG, Mills PC: Use of C-reactive protein in minimizing antibiotic exposure: experience with infants initially admitted to a well-baby nursery. Pediatrics 2000;106:E4.

44 Franz AR, Steinbach G, Kron M, Pohlandt F: Reduction of unnecessary antibiotic therapy in newborn infants using interleukin-8 and C-reactive protein as markers of bacterial infections. Pediatrics 1999;104:447–453.

45 Ehl S, Gehring B, Pohlandt F: A detailed analysis of changes in serum C-reactive protein levels in neonates treated for bacterial infection. Eur J Pediatr 1999;158:238–242.

46 Sabel KG, Hanson LA: The clinical usefulness of C-reactive protein (CRP) determinations in bacterial meningitis and septicemia in infancy. Acta Paediatr Scand 1974;63:381–388.

47 Rønnestad A, Abrahamsen TG, Gaustad P, Finne PH: C-reactive protein (CRP) response patterns in neonatal septicaemia. APMIS 1999;107:593–600.

48 Forest JC, Larivière F, Dolcé P, Masson M, Nadeau L: C-reactive protein as biochemical indicator of bacterial infection in neonates. Clin Biochem 1986;19:192–194.

49 Mathai E, Christopher U, Mathai M, Jana AK, Rose D, Bergstrom S: Is C-reactive protein level useful in differentiating infected from uninfected neonates among those at risk of infection? Indian Pediatr 2004;41:895–900.

50 Chiesa C, Signore F, Assumma M, Buffone E, Tramontozzi P, Osborn JF, et al: Serial measurements of C-reactive protein and interleukin-6 in the immediate postnatal period: reference intervals and analysis of maternal and perinatal confounders. Clin Chem 2001;47:1016–1022.

51 Chiesa C, Natale F, Pascone R, Osborn JF, Pacifico L, Bonci E, et al: C reactive protein and procalcitonin: reference intervals for preterm and term newborns during the early neonatal period. Clin Chim Acta 2011;412:1053–1059.

52 Turner MA, Power S, Emmerson AJB: Gestational age and the C reactive protein response. Arch Dis Child Fetal Neonatal Ed 2004;89:F272–F273.

53 Doellner H, Arntzen KJ, Haereid PE, Aag S, Austgulen R: Interleukin-6 concentrations in neonates evaluated for sepsis. J Pediatr 1998;132:295–299.

54 Hofer N, Müller W, Resch B: Non-infectious conditions and gestational age influence C-reactive protein values in newborns during the first 3 days of life. Clin Chem Lab Med 2011;49:297–302.

55 Ishibashi M, Takemura Y, Ishida H, Watanabe K, Kawai T: C-reactive protein kinetics in newborns: application of a high-sensitivity analytic method in its determination. Clin Chem 2002;48:1103–1106.

56 Chiesa C, Osborn JF, Pacifico L, Natale F, De Curtis M: Gestational- and age-specific CRP reference intervals in the newborn. Clin Chim Acta 2011;412:1889–1890.

57 Van Lente F, Pippenger CE: The pediatric acute care laboratory. Pediatr Clin North Am 1987;34:231–246.

58 Schouten-Van Meeteren NY, Rietveld A, Moolenaar AJ, Van Bel F: Influence of perinatal conditions on C-reactive protein production. J Pediatr 1992;120:621–624.

59 Ng PC: Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed 2004;89:F229–F235.

60 Malik A, Hui CPS, Pennie RA, Kirpalani H: Beyond the complete blood cell count and C-reactive protein: a systematic review of modern diagnostic tests for neonatal infection. Arch Pediatr Adolesc Med 2003;157:511–516.

61 Døllner H, Vatten L, Linnebo I, Zanussi GF, Laerdal A, Austgulen R: Inflammatory mediators in umbilical plasma from neonates who develop early-onset sepsis. Biol Neonate 2001;80:41–47.

62 Panero A, Pacifico L, Rossi N, Mancuso G, Stegagno M, Chiesa C: Interleukin 6 in neonates with early and late onset infection. Pediatr Infect Dis J 1997;16:370–375.

63 Matoba N, Yu Y, Mestan K, Pearson C, Ortiz K, Porta N, et al: Differential patterns of 27 cord blood immune biomarkers across gestational age. Pediatrics 2009;123:1320–1328.

64 Dammann O, Phillips TM, Allred EN, O'Shea TM, Paneth N, Van Marter LJ, et al: Mediators of fetal inflammation in extremely low gestational age newborns. Cytokine 2001;13:234–239.

65 Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, et al: Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 1994;79:1605–1608.

66 Vouloumanou EK, Plessa E, Karageorgopoulos DE, Mantadakis E, Falagas ME: Serum procalcitonin as a diagnostic marker for neonatal sepsis: a systematic review and meta-analysis. Intensive Care Med 2011;37:747–762.

67 Stocker M, Fontana M, El Helou S, Wegscheider K, Berger TM: Use of procalcitonin-guided decision-making to shorten antibiotic therapy in suspected neonatal early-onset sepsis: prospective randomized intervention trial. Neonatology 2010;97:165–174.

68 Simms HH, D'Amico R: Lipopolysaccharide induces intracytoplasmic migration of the polymorphonuclear leukocyte CD11b/CD18 receptor. Shock 1995;3:196–203.

69  Lehr HA, Krombach F, Münzing S, Bodlaj R, Glaubitt SI, Seiffge D, et al: In vitro effects of oxidized low density lipoprotein on CD11b/CD18 and L-selectin presentation on neutrophils and monocytes with relevance for the in vivo situation. Am J Pathol 1995;146:218–227.

70  Ng PC, Li G, Chui KM, Chu WCW, Li K, Wong RPO, et al: Neutrophil CD64 is a sensitive diagnostic marker for early-onset neonatal infection. Pediatr Res 2004;56:796–803.

71  Ng PC, Li G, Chui KM, Chu WCW, Li K, Wong RPO, et al: Quantitative measurement of monocyte HLA-DR expression in the identification of early-onset neonatal infection. Biol Neonate 2006;89:75–81.

72  Haque KN: Neonatal sepsis in the very low birth weight preterm infants. 2. Review of definition, diagnosis and management. J Med Sci 2010;1:11–17.

73  Newman TB, Puopolo KM, Wi S, Draper D, Escobar GJ: Interpreting complete blood counts soon after birth in newborns at risk for sepsis. Pediatrics 2010;126:903–909.

74  Escobar GJ, Li DK, Armstrong MA, Gardner MN, Folck BF, Verdi JE, et al: Neonatal sepsis workups in infants >/=2,000 g at birth: a population-based study. Pediatrics 2000;106:256–263.

75  Da Silva O, Ohlsson A, Kenyon C: Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: a critical review. Pediatr Infect Dis J 1995;14:362–366.

76  Dyck RF, Bingham W, Tan L, Rogers SL: Serum levels of C-reactive protein in neonatal respiratory distress syndrome. Clin Pediatr (Phila) 1984;23:381–383.

77  Pourcyrous M, Bada HS, Korones SB, Barrett FF, Jennings W, Lockey T: Acute phase reactants in neonatal bacterial infection. J Perinatol 1991;11:319–325.

78  Kukkonen AK, Virtanen M, Järvenpää AL, Pokela ML, Ikonen S, Fellman V: Randomized trial comparing natural and synthetic surfactant: increased infection rate after natural surfactant? Acta Paediatr 2000;89:556–561.

79  Messer J, Eyer D, Donato L, Gallati H, Matis J, Simeoni U: Evaluation of interleukin-6 and soluble receptors of tumor necrosis factor for early diagnosis of neonatal infection. J Pediatr 1996;129:574–580.

80  Franz AR, Bauer K, Schalk A, Garland SM, Bowman ED, Rex K, et al: Measurement of interleukin 8 in combination with C-reactive protein reduced unnecessary antibiotic therapy in newborn infants: a multicenter, randomized, controlled trial. Pediatrics 2004;114:1–8.

81  Franz AR, Steinbach G, Kron M, Pohlandt F: Interleukin-8: a valuable tool to restrict antibiotic therapy in newborn infants. Acta Paediatr 2001;90:1025–1032.

82  Franz AR, Kron M, Pohlandt F, Steinbach G: Comparison of procalcitonin with interleukin 8, C-reactive protein and differential white blood cell count for the early diagnosis of bacterial infections in newborn infants. Pediatr Infect Dis J 1999;18:666–671.