The Role of C-Reactive Protein and Implications to the Neonatal Intensive Care Unit

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Abstract

As neonatal infections are difficult to diagnose with accuracy and have a high prevalence with serious long-term consequences, biomarkers of infection have been widely studied to improve the diagnostic accuracy and reduce antibiotic exposure in patients who are not infected. C-reactive protein (CRP) is a serum protein that increases in the blood in response to inflammatory or infective triggers. The function of CRP is important in the humoral response to bacterial infection during the acute phase, and it has been widely used as a marker of severe bacterial infection in both adults and children; however, as a result of its delayed synthesis, the sensitivity during the earliest phases of infection is poor, and it may rise in response to other non-infectious triggers. Its accuracy and negative predictive value to rule out infection increase over time with serial investigations. In this review, we discuss the kinetics of CRP, its role in the diagnosis of infection in the neonate, factors that may affect its measurement, and its usefulness in monitoring the response to treatment in the infected neonate.

Keywords

C-reactive protein · Neonatal sepsis · Biomarkers · Neonate · NICU · Preterm · Term

Abbreviations

CRP	C-reactive protein
ELBW	Extreme low birth weight
EONS	Early-onset neonatal sepsis
hsCRP	High-sensitivity C-reactive protein
IL-1	Interleukin-1
IL-6	Interleukin-6
LONS	Late-onset neonatal sepsis
nCD64	Neutrophil CD64 percentage
NICU	Neonatal intensive care unit
PCT	Procalcitonin
POC	Point of care
RCT	Randomized controlled trial
ROC	Receiver operator characteristic curve
SNAP	Score for neonatal acute physiology
VLBW	Very low birth weight

Introduction

C-reactive protein (CRP) is an acute phase reactant protein measurable in serum that rises in concentration during states associated with inflammation or tissue necrosis (Gewurz et al. 1982). It is widely adopted as a marker of infection especially in the context of neonatal sepsis, which is any infection that occurs in the newborn in the first 28 days of life. C-reactive protein serum levels are often measured in serial specimens taken hours to days apart to improve the diagnostic likelihood of infection. Serial measurements may also provide a guide to clinicians to assess the response to antibiotic treatments and the necessary duration of treatments.

Many studies have evaluated neonatal sepsis and associated markers of infection with varying results (Hedegaard et al. 2015; Iroh Tam and Bendel 2017). These include blood cell counts and neutrophil ratios, acute phase reactants, and several cytokines and chemokines. Of these, CRP has been among the most extensively studied and widely used in practice (Hofer et al. 2012).

The plasma protein was discovered in 1930 by Tillet and Francis at Rockefeller University (Tillett and Francis 1930). While investigating the sera of patients suffering from acute pneumococcal infection, they observed a precipitation reaction between sera from patients suffering from acute pneumococcal pneumonia and the extracted polysaccharide fraction C from the pneumococcal cell wall. This reaction was not observed in healthy controls nor in patients that had recovered from pneumococcal pneumonia (Tillett and Francis 1930). CRP was thus named for its reaction with the capsular (C)-polysaccharide of pneumococcus. During the 20 years that followed, CRP was detected in more than 70 disorders including acute bacterial, viral, and other infections and non-infectious diseases such as acute myocardial infarction, rheumatic disorders, and malignancies (Pepys 1981). Though disorders of varying etiology, their commonality lies in an underlying process of inflammation and/or tissue injury.

C-reactive protein is homopentameric, composed of five identical subunits arranged in a cyclic pentameter shape (Fig. 1). The whole protein has a diameter of 102 Å (1 Ångström = 0.1 nanometer) and a molecular weight of 118,000 Daltons (Volanakis 2001). The major physiological role of serum CRP is to bind to microbial polysaccharides and immune complexes and activate the classical complement cascade.

In infants exposed to infectious inflammatory stimuli, serum CRP levels may rise by more than 100-fold, declining with a half-life of about 18 to 24 hours when the stimulus ceases (Ehl et al. 1999). However, many non-infectious inflammatory stimuli including chemical or physical irritation may also cause serum CRP levels to rise in infants (Hofer et al. 2012). Microbiological culture of a potentially pathogenic organism remains the gold standard for diagnosing early- and late-onset infection. However, a blood culture sample may take 24 to 48 hours to flag as positive (Cantey et al. 2016). Hence, CRP has been used as a biomarker to make an immediate assessment of the overall likelihood that an infant is truly infected.

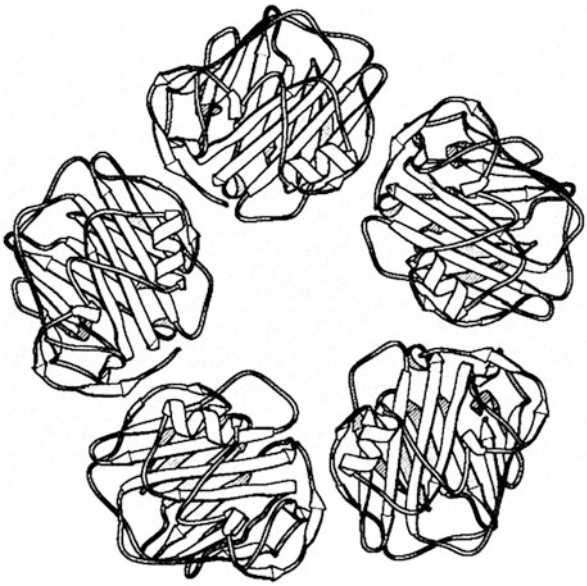

Fig. 1 Pentameric structure of CRP viewed down the fivefold symmetry axis. (From Volanakis (2001) with permission)

Neonatal Sepsis

The occurrence of sepsis in the neonate is a serious complication that is extremely common. Morbidity and mortality due to sepsis is high, particularly in preterm hospitalized infants. The incidence is estimated at three million cases annually worldwide live births with much higher incidences in developing countries reported (Fleischmann-Struzek et al. 2018). The reported mortality of neonatal sepsis accounts for up to 30% of infant deaths annually (Ershad et al. 2019).

The clinical diagnosis of neonatal sepsis is fraught with difficulties, some of which stem from the fact that neonates collectively are a heterogenous population with distinct subsets. They encompass the well neonate born at term with a normal birthweight who is discharged home within hours of birth and, on the other end of the spectrum, extremely preterm babies who have ineffective skin and mucosal barrier protection and limited humoral and immature cellular immune systems (Collins et al. 2018). The latter group are largely born with a very low birth weight (VLBW) of less than 1500 grams and remain in neonatal intensive care for several weeks to months. They have several risk factors which include invasive mechanical ventilation, nutritional, and feeding challenges. The presence of invasive indwelling vascular or other catheters and invasive procedures causes breaks in skin or mucosal integrity and places these patients at extreme risk of infection. A large study of extreme low birth weight (ELBW) infants born with birthweight below 1000 g found that 65% surviving infants had at least 1 infection during their hospital stay (Stoll et al. 2004).

Neonatal sepsis can be broadly categorized into early-onset neonatal sepsis (EONS) and late-onset sepsis and (LONS). Although definitions in the literature vary, EONS is typically described as infections that occur up to 72 hours of life and LONS is infections that occur after 72 hours of age up until the end of the neonatal period (Ershad et al. 2019; Walker et al. 2019). The distinction between EONS and LONS is useful for considering the different etiologies. Neonates with EONS are commonly infected by vertical transmission of pathogens from maternal sources, the commonest organisms being Group B *Streptococcus* and *Escherichia coli* (Stoll et al. 2011). Hospital-acquired infections account for the majority of LONS; predominant pathogens are coagulase-negative staphylococci, followed by Gram-negative bacilli and fungi (Dong and Speer 2015). Large multicenter studies in the United States have found that EONS occurs in 1.5 to 2% of VLBW infants and LONS in 21% of VLBW infants (Stoll et al. 2002; Stoll et al. 2011). Infants with LONS are significantly more likely to die than those who were uninfected, especially if they were infected with Gram-negative organisms or fungi (Stoll et al. 2002), and there is significant risk of long-term neurodevelopmental sequelae in survivors (Stoll et al. 2004).

Rational Antibiotic Use

Neonates who have infection may present insidiously with a constellation of non-specific symptoms, and prompt and reliable confirmation of infection remains challenging. Given the high risk of mortality of long-term morbidity in survivors, empirical antibiotic treatment is initiated on suspicion of infection. As the pathogens are variable and unknown, antibiotic therapy is generally broad spectrum; often unnecessary and frequently treatment is prolonged beyond what is needed (Dong and Speer 2015). A retrospective cohort study of more than 50,000 infants in 127 NICUs across a large US state demonstrated 40-fold variation of antibiotic usage, from 2.4% to 97.1% of patient days. At all levels of care, it was independent of proven infection, NEC, surgical volume, or mortality. Half of intermediate NICUs were in the upper quartile of antibiotic use despite most of the units reporting zero infections (Schulman et al. 2015), supporting the argument that antibiotics are overused. The serious and concerning impact of this is an increasing number of multidrug-resistant Gram-negative microorganisms in neonatal intensive care units (NICU) worldwide (Dong and Speer 2015). In addition, unnecessary antibiotic exposure may lead to an alteration in the preterm neonatal gut microbiome by diminishing microbial species alpha-diversity, reduced protective bacterial genera, and increased proportions of potentially pathogenic bacteria (Van Belkum et al. 2020). In the short term, there is concern that this dysbiosis will lead to gut illnesses like necrotizing enterocolitis in the preterm infant, but there are long-term concerns of immunologically mediated diseases like inflammatory bowel disease, wheezing, and eczema as well as obesity (Murgas Torrazza and Neu 2011; Turta and Rautava 2016) There is also emerging evidence of a microbiota-gut-brain axis in humans

during early life; exposure to gut-microbiome disruption may impact the neurodevelopment of infants (Hickey et al. 2021).

For the reason mentioned above, it would be desirable to limit the antibiotic exposure in these infants, using a structured antimicrobial stewardship program. This would include using local microbiological surveillance data to adapt empiric treatments that target the prevailing antimicrobial resistance patterns, to use narrow spectrum antibiotics wherever possible, and to only use antibiotics when significant infection is likely (Russell et al. 2012). However, the difficulty in diagnosing the infection or confirming with a reasonable certainty that infection is unlikely is where the challenge lies. Currently, diagnosis of suspected infection in the clinically ill neonate is confirmed by the isolation of the causative organism in cultures from blood, cerebrospinal fluid or other samples. In the absence of the confirmed bacteriological culture, the diagnosis of infection is often suspected and treated empirically by considering the clinical picture of the patient as well as the measurements of biomarkers of infection in patient blood samples. A host of biomarkers of infection have been identified and utilized in clinical practice, of which C-reactive protein is the most widely studied in the neonatal population (Iroh Tam and Bendel 2017).

Blood Cultures

Blood cultures are still considered the gold standard microbiological test in aiding the diagnosis of sepsis, although this method has several limitations, especially in relation to the neonate (Cantey and Baird 2017). The successful culture of a microorganism is dependent upon various factors including the number of blood cultures, volume of blood collected, technique, and antibiotic exposure.

In the neonate, the standard practice is for the collection of a single blood culture, often due to the small total blood volume of neonate, especially if in septic shock, the increased risk for the need of blood transfusions, and difficulty of venipuncture (Buttery 2002). A single blood culture is however considered of limited use; increasing the number of blood culture bottles from a single culture improves the diagnostic yield (Buttery 2002; Ntusi et al. 2010).

An adequate volume of blood is required to shorten the detection time by automated blood culture systems. At least 1–2 ml of blood per blood culture bottle is recommended (Schelonka et al. 1996). In the ELBW or infant with septic shock, these volumes might not be achievable. Contamination of the specimen due to poor technique complicates the patient management and prolong empiric antibiotic therapy (Cantey and Baird 2017). Arterial venipuncture is not considered superior to peripheral venous collection, although stringent skin preparation is recommended to reduce the risk of blood culture contamination (Buttery 2002). Prior antibiotic exposure via intrapartum antibiotic prophylaxis may reduce the bacterial concentrations, calculated as colony forming units, to below the detection limit of the automated systems. Ideally at such low bacterial concentrations, the neonate's innate immune system should be able to overcome the infection (Cantey and Baird 2017). Cantey et al. (2016) highlighted neonates with negative blood cultures, due to low

bacterial concentrations, and appropriate empirical antibiotic treatment of up to 48 hours did not require repeated antibiotic treatment. They questioned the relevance of low bacterial concentrations in the neonate with at least 48 hours of empiric antibiotic treatment (Cantey et al. 2016).

These various factors contribute to the low positivity rate of blood cultures, with only 30–50% of blood cultures collected from suspected septic patients reported as positive (Gupta et al. 2016). In many developing countries, blood culture negative sepsis accounts for most of the reported cases (Zea-Vera and Ochoa 2015). This sensitivity and the increased understanding of the systemic inflammatory response and the role of endo- and exotoxins in sepsis are indicative that bacteremia is not always present in patients with sepsis (Zrodlowski et al. 2020). With consideration for the prolonged period required to detect bacterial growth in automated systems, the diagnosis of sepsis cannot solely depend on a positive blood culture but requires the consideration of both clinical and additional laboratory biomarkers.(Zea-Vera and Ochoa 2015; Zrodlowski et al. 2020) Although newer molecular techniques could improve the sensitivity and specificity of microbiological testing for sepsis, these are more expensive and not readily available at most hospital laboratories. (Zea-Vera and Ochoa 2015).

CRP Kinetics and Clinical Utility

CRP production is triggered by inflammatory cytokine induction of CRP gene transcription. This predominantly occurs in the liver, but other tissues can also express the CRP gene. The strongest induction is through interleukin-6 (IL-6), a response often enhanced by interleukin-1 (IL-1), although IL-6 is not capable of triggering CRP gene expression on its own accord (Sproston and Ashworth 2018). Once released into circulation, CRP recognizes, binds, and aggregates various cellular structures. This includes plasma lipoproteins, phospholipids, damaged and apoptotic cells, as well as extrinsic components of various microorganisms (Pepys and Hirschfield 2003). The strongest binding affinity is towards phosphocholine, expressed on membranes of various microorganisms, as well as most eukaryotic cells. This partially explains the limited specificity of CRP in diagnostic testing (Sproston and Ashworth 2018). Once aggregated, CRP interacts with the complement pathway of the innate immune system. CRP is recognized by C1q, leading to formation of the terminal membrane attack complex and activation of the classical complement pathway. Binding to factor H through secondary binding sites, CRP also activates the alternative complement pathway with production of C5 convertases. The activated complement system is then able to facilitate opsonization and phagocytosis of microorganisms and partake in the proinflammatory response to infection (Pepys and Hirschfield 2003). Binding of CRP to the Fc receptors on IgG antibodies triggers the production of additional proinflammatory cytokines, and its interaction with neutrophils, natural killer cells, and platelets promotes antibody-dependent cellular cytotoxicity (Povoa 2002; Sproston and Ashworth 2018). Within 4 to 6 hours after cytokine stimulation, CRP will appear in circulation,

Table 1 Non-infectious conditions that have been associated with elevated C-reactive protein concentrations in infants categorized by occurrence in mother or infant

Non-infectious conditions that have been associated with elevated C-reactive protein concentrations in infants
Maternal and intrapartum:
Maternal intrapartum fever
Prolonged rupture of membranes
Prolonged labour
Fetal distress
Ventouse assisted delivery
Perinatal asphyxia
Infants:
Meconium aspiration syndrome
Surfactant administration for respiratory distress
Intraventricular hemorrhage
Pneumothorax
Tissue injury
Immunization

Adapted from (Hofer et al. 2012)

doubling every 8 hours and reaching a peak value in 36 to 50 hours. Termination of the cytokine stimulus will decrease the concentration of CRP, with an average half-life of 19 hours (Pereira et al. 2019).

The CRP concentration is independent of the causative pathology, and changes will only reflect interventions directed at reducing or removing the cytokine inflammatory stimulus that triggered the acute phase response. In essence, CRP value is only dependent on the degree of inflammation, with production rate and the concentration increasing with any inflammatory process, except when associated with hepatic failure (Povoa 2002). Marked CRP elevations are associated with most systemic bacterial and fungal infections, with only mild increases noted in acute viral infections, although some viral pathogens (adenovirus, measles/mumps, and influenza) can trigger high CRP concentrations during uncomplicated infections. When associated with systemic infections, cytomegalovirus and herpes simplex virus also cause severe increases in CRP (Povoa 2002). Non-infectious conditions associated with increases in CRP include malignancies, trauma, recent surgery, auto-immune diseases, and acute myocardial infarction (Table 1). It is widely accepted that CRP values are greater in infectious conditions than non-infectious conditions, and in adult patients with fever, a level of 87 mg/L or more is suggestive of infection. With severe infections, the value can be more than 1000 times the upper range of normal but will not correlate with the possible focus of the infection (Pereira et al. 2019).

Factors Affecting CRP Measurement

In healthy neonates, CRP increases physiologically over the first 24–48 hours, with concentrations affected (increased) by gestational age and birth weight, but not by gender (Chiesa et al. 2011a, b). Concentration peaks between 27–36 hours (to as

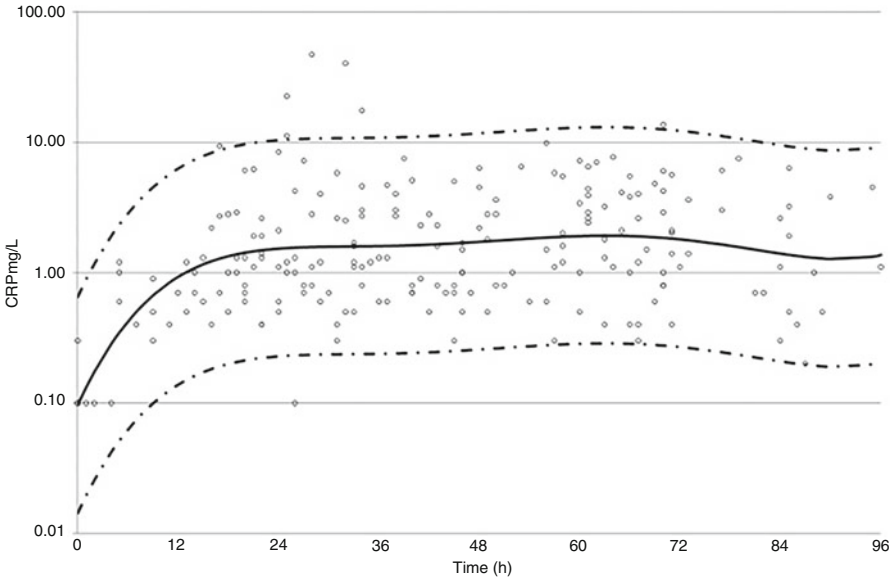

Fig. 2 Age-specific 95% reference intervals for C-reactive protein (CRP) in healthy-term neonates from birth to 96 h of life. The circles represent single values; the dotted lines represent lower and upper limits: the bold line represents the predicted geometric mean. Note the logarithmic scale of CRP. (From (Chiesa et al. 2011a, b) with permission)

high as 13 mg/L), declining by about 90 hours (to a maximum of 4.7 mg/L). Figure 2 shows age-specific 95% reference intervals for C-reactive protein (CRP) in healthy-term neonates from birth to 96 h of life (Chiesa et al. 2011a, b).

Very little CRP crosses the placenta, implying that any elevation represents endogenous synthesis (Hofer et al. 2012). Non-infective stimuli associated with CRP synthesis in the early neonatal period include shock, meconium aspiration pneumonitis, fetal distress, intraventricular hemorrhage, anoxic encephalopathy, respiratory distress syndrome, low 5 min APGAR, maternal fever, premature rupture of membranes, prolonged labor, pregnancy-induced hypertension, and vacuum extraction (Hofer et al. 2012). Early-onset sepsis may present with similar clinical signs to the conditions above, making the diagnosis of sepsis difficult to exclude or confirm using CRP. Furthermore, several studies have suggested that gestational age may play a role in CRP kinetics with lower baseline CRP values and lower sensitivities to infection in preterm newborns compared to term newborns (Hofer et al. 2012). In a study of 1010 episode of LONS confirmed by positive blood stream infections in 793 neonates, Lai et al. found patients with a low CRP ($\leq$ 10 mg/L) had a lower birth body weight and gestational age and an earlier onset of infection than patients with intermediate (11–100 mg/L) and high CRP (>100 mg/L) measurements (Lai et al. 2015).

Biological variation may be an additional consideration. In children, CRP has been shown to have a biological variation of 19.3% intra-individually and 125.4%

between individuals (Bailey et al. 2014). However, there does not appear to be a significant diurnal (Meier-Ewert et al. 2001) or seasonal rhythm (Sproston and Ashworth 2018).

In terms of preanalytical considerations of measurement, CRP is an ideal analyte as it is stable in serum. It is stable for 11 days at room temperature and 60 days in the fridge and remains unchanged for months to years at −70 degrees Celsius (Wilkins et al. 1998), adding to its practical utility. CRP levels are unaltered by enteral nutrition (Ledue and Rifai 2001) and display little interference by drugs that do not alter the inflammatory process. However, certain assays may be affected by lipemia (Knezevic et al. 2020) and the high-dose hook effect (antigen excess).

CRP is measured by immunoassay (competitive or sandwich), usually by immunoturbidimetry or nephelometry on automated analyzers. These methods are accurate, freely available, affordable, and rapid, with acceptable turnaround times, usually around 1–2 hours depending on the distance from the laboratory. There are many instrument platforms currently available for the measurement of C-reactive protein, demonstrating different performance characteristics (area under curve ROC, analytical sensitivity, measuring range, precision) and employing different methodologies for detection, antibodies, incubation periods, and wash steps. These differences result in variability in results obtained on different instruments, despite most being traceable to a single reference material (ERM-DA470) (Merlini et al. 2010, Päivi Ranta et al. 2017).

Point-of-care (POC) testing devices (usually employing lateral flow sandwich immunoassay methods) have been shown to be clinically viable in low-income settings where laboratory-based testing is not readily available or turnaround time is compromised (Prince et al. 2019); however, care must be exercised when employing published medical decision limits, as significant negative (Matheeussen et al. 2018) and positive biases may be present compared with automated laboratory-based methodologies. This variability is due to the different analytical methodologies employed, but also the different sample matrices being used. Point-of-care instruments use whole blood samples, which have been shown to demonstrate a negative proportional bias compared with serum samples, on which most medical decision limits have been derived (Roberts et al. 2001; Phommasone et al. 2016; Escadafal et al. 2020).

Clinical Applications of C-Reactive Protein in Sepsis

Features of infection, such as raised white cell counts and fever, are influenced by various factors and are of limited reliability as sepsis biomarkers (Povoa 2002; Hedegaard et al. 2015). The clinical applications of CRP have been studied extensively in both adult and neonatal patient populations for the diagnosis of infection, and the evidence favors the value of serial CRP measurements over a single reading, as CRP levels are only dependent on the rate of production (Povoa 2002).

Although several studies indicate that a single CRP measurement between 50 and 100 mg/L could be considered as a useful marker of sepsis, a true cut-off value for

sepsis is poorly defined and may differ in various types of infections and in various patient populations (Povoa 2002). Ugarte et al. reported that at a value of 50 mg/L, CRP had a sensitivity of 98.5% and specificity of 75% for the diagnosis of sepsis (Ugarte et al. 1999). Numerous studies agree that when serial CRP measurements show a steady increase in value over 48–72 hours, infection should always be considered. Limited data is available regarding the kinetics of CRP prior to the onset of sepsis, although an earlier report by Matson et al. showed that in critically ill patients, increases of 25% or more within 24 hours were a good indicator of sepsis (Matson et al. 1991).

In neonates, Benitz et al. (1998) found that the sensitivity of CRP to diagnose EONS increased from 39% at the initial sepsis workup to 84% by 24 hours to 89% for the higher of two levels obtained between 8 and 48 hours after initial workup. The corresponding specificities, however, declined from 90% at initial workup to 78% and 74%, respectively. They described the optimal cut-off value to be 10 mg/L (Benitz et al. 1998). Chiesa et al. (2003) found the cut-off value that maximized the sum of the sensitivity and specificity for CRP was 4 mg/L at birth, whereas at both 24 and 48 h of life, it was 10 mg/L in the diagnosis of culture-positive EONS (Chiesa et al. 2003). They found the sensitivity at those cut-off values to increase from 73% at birth to 91% at 24 and 48 hours at those cut-off values, while the sensitivity remained similar at 83%, 87%, and 84%, respectively. The low sensitivity at birth or initial sepsis workup in suspected EONS does not add value to clinical decision-making, as those patients who are ill or at risk of sepsis would not be spared from antibiotic exposure.

A recent systematic review and meta-analysis of 22 cohort studies with a total of 2255 infants included reported a pooled sensitivity of 62% (95% CI 50 to 72%) at a median specificity of 74% for CRP diagnosing LONS in newborn infants (Brown et al. 2020). The studies included mostly preterm or VLBW infants and used a prespecified CRP cut-off of 5–10 mg/L. Six studies calculated the CRP threshold level retrospectively by modeling the area under the ROC curve. In five of the studies, the threshold ranged from 2.2 mg/L to 18 mg/L, and in the sixth study, the threshold serum CRP level was 111 mg/L (Brown et al. 2020). Most studies used positive culture of a pathogenic organism from blood as a reference standard. The median prevalence sepsis rate in all the included studies was 40% (interquartile range 27–61%). If CRP determination was applied to a hypothetical cohort of 1000 newborn infants investigated for possible late-onset infection, the authors estimated that, if the prevalence of true infection was 40%, on average 152 cases of infection would be missed (false-negative) and 156 non-infected infants would be wrongly diagnosed (false-positive) (Brown et al. 2020). The review however was limited as it solely focused on the accuracy of CRP to determine the likelihood of infection in infants where there is a clinical suspicion of infection. Most of the studies in the meta-analysis were performed in high- or middle-income settings, limiting its generalizability.

The poor sensitivity and specificity of CRP to diagnose LONS in neonates from the meta-analysis by Brown et al. makes its utility questionable at best, especially in units with a high prevalence of infection. The result of the CRP would not

change the management of the patient with suspected infection when taken at initial presentation, as it would not prevent empirical antimicrobial treatment in an infant that appears unwell. Additionally, the positive predictive value worsens as the prevalence rate of LONS declines, leading to an increasing occurrence of treatment of "culture negative" sepsis (Cantey and Baird 2017; Cantey and Bultmann 2020).

Disease Severity and Outcome Prediction

Most agree that although serial measurements for trend analysis are of more clinical relevance than a single measurement, higher CRP levels correlate with more severe inflammatory responses and accordingly more serious or complicated infections (Chalmers et al. 2008). The CRP concentration may therefore reflect both the presence and severity of infection (Povoa 2002). Furthermore, CRP levels that fail to decrease or increase after initial decreases should raise the suspicion of the development of infection complications (Povoa 2002).

In newborn patients, CRP concentrations correlate with severity of illness as determined by the Score for Neonatal Acute Physiology (SNAP) and SNAP perinatal extension scores (Chiesa et al. 2003). The extent of response is further complicated by causative organisms. Much higher CRP concentrations are seen in neonates infected with Gram-negative organisms as well as *Staphylococcus aureus* and group B streptococci compared to other Gram-positive organisms (Hofer et al. 2012). Pourcyrous et al. reported normal CRP in patients with positive cultures with predominantly Gram-positive strains such as group D streptococci, *Streptococcus viridans*, and *Streptococcus epidermidis* who had uneventful clinical courses despite being inadequately or completely untreated, questioning the possibility of these organisms as contaminants rather than pathogens (Pourcyrous et al. 1993).

CRP kinetics has also been assessed as a prognostic marker, with several studies reporting on its value as a predictor of mortality, and is considered one of the more accurate inflammatory markers for the prediction of clinical outcomes in patients with sepsis (Povoa 2002; Pereira et al. 2019). In their meta-analysis, Zhang et al. (Zhang and Ni 2011) concluded that the mean difference between initial baseline CRP and measurements after 48 hours was lower in survivors when compared with non-survivors, suggesting serial CRP measurement over 48 hours can aid in the outcome prediction of patients with critical illness. Similarly, higher CRP levels on admission and higher peaks during the hospitalization have been reported in non-surviving patients than surviving patients presenting with infections (Povoa 2002). There is however sparse evidence in the literature regarding the utility of CRP to predict mortality in neonates. Singh et al. showed that infants with suspected serious bacterial infection and with a CRP raised above 40 mg/L showed a 4.1-fold increased risk of mortality in a low-income setting (Singh et al. 2018).

CRP Response to Guide Antibiotic Therapy

CRP kinetics have been described in terms of response to antibiotic treatment as "fast," "slow," "unresponsive," and "biphasic" patterns and associated with prognostic outcomes. Cases identified as either "fast" or "slow" responders had better clinical outcomes to antibiotic treatment in adult patients (Povoa et al. 2005). A single CRP value may not alter decisions about the initiation of antibiotic treatment; however, serial measurements can aid in identifying patients for safe discontinuation of empiric antibiotic treatment for neonatal sepsis, shortening the antibiotic exposure and hospital stay (Stocker et al. 2021). This makes CRP measurement an ideal tool in antibiotic stewardship programs with the aim of reducing antibiotic resistance and inappropriate antibiotic use (Povoa 2002).

In their study, Ehl et al. (1997) used a cut-off value of 10 mg/L to identify 99% of the neonates in the study group that did not have sepsis and could discontinue antibiotic treatment. Similarly, using a cut-off value of 10 mg/L, Philip and Mills (2000) were also able to discontinue treatment in almost 40% of neonates, with none of them representing within 30 days with features of recurrent or persistent infections.

A meta-analysis of studies assessing the use of CRP to tailor antibiotics found CRP-based algorithms reduced antibiotic treatment duration by −1.45 (95% CI -2.61 to 0.28) days in 2 RCTs and by −1.15 (95% CI -2.06 to −0.24) days with no differences in mortality or infection relapse (Petel et al. 2018). The authors caution about the relatively small sample sizes in the RCTs in the meta-analysis.

The timing of CRP testing to exclude infection has been looked at in several studies (Hofer et al. 2012). A repeat CRP taken 24 to 48 hours after initiation of therapy has been reported to have a 99% negative predictive value in ruling out EONS (Ehl et al. 1997). In a secondary analysis of the Neonatal Procalcitonin Intervention study, the authors found that normal serial CRP and procalcitonin measurements within 36 hours after the start of empiric antibiotic therapy can exclude the presence of EONS with a high probability. The negative predictive values of CRP and procalcitonin did not increase after 36 hours (Stocker et al. 2021).

CRP Combined with Other Biomarkers

CRP on its own is unreliable as a diagnostic marker of infection; however, several studies have investigated the utility of CRP combined with other biomarkers of infection to improve the accuracy of diagnosis (Deleon et al. 2015). Several combinations have been looked at, with an attempt to combine the earlier rise of some of the markers with CRP which rise more slowly but remain elevated for longer. The most extensively studied in combination with CRP are neutrophil CD64% (nCD64), PCT, IL-1β, IL-6, IL-8, and TNF-alpha.(Deleon et al. 2015) PCT may be a more sensitive marker of infection in adult and childhood infection; however, its utility in the NICU is reduced due to its physiological rise after birth (Chiesa et al. 2011a, b).

A meta-analysis of 28 studies found the pooled sensitivity and specificity increased from 71% (95% CI 63–78%) and 88% (95% CI 80–93%) for CRP alone to 91% (95% CI 84–95%) and 89% (95% CI 81–93%) when CRP was combined with PCT. There was however significant heterogeneity observed in the analysis (Ruan et al. 2018). The membrane glycoprotein nCD64, involved in the mediation of endocytosis, phagocytosis, and cytokine release, is expressed at low concentration on non-activated neutrophils but can be markedly upregulated at the onset of the sepsis process (Hashem et al. 2020). Song et al. (2019) found in their meta-analysis of 8 studies that the combined application of nCD64 and CRP produced a sensitivity of 95% (95%CI 86–98%) and specificity of 86% (95%CI 74–93%) (Song et al. 2019). However once again, the authors noted significant heterogeneity in the study analysis, due in part to different study designs and patient profiles so generalization of the findings was cautioned. Several studies have reported better diagnostic accuracy when combining IL-6 and CRP (Deleon et al. 2015). Combining multiple tests may greatly improve the sensitivity and negative predictive value of the test panel to 100% but reduce its specificity and its practical clinical application (Dilli et al. 2010). The discussion of the individual biomarkers' performance is beyond the scope of this chapter.

Conclusion

The literature on biomarkers and neonatal sepsis is challenged by the complexity and heterogeneity of the condition being studied. There is no consensus definition of sepsis and how it is diagnosed, with not all studies using positive cultures as the gold standard, and variability in what is considered pathogenic organisms. Even the timeframe after birth is variable for what is considered EONS or LONS (Shane et al. 2017). The ideal sepsis biomarker has been described as measurable with near-perfect sensitivity and specificity and would have a rapid turnaround time. Such a biomarker would facilitate a reasonable delay to the initiation of antibiotics for infants with a negative biomarker test (Cantey and Lee 2021). While rapid turnaround is possible in a cost-effective manner with existing POC tests, CRP lacks the sensitivity and specificity to reliably diagnose or exclude sepsis at the time of clinical assessment of the patient. At present, there is no single test for neonatal sepsis that meets the criteria of an ideal biomarker, and even when CRP is combined with other biomarkers, the accuracy of infection diagnosis is limited. Despite this, the use of CRP in the NICU continues to be widespread mostly with proponents of CRP citing its negative predictive value. There has been a recent shift in the literature away from CRP to rule out sepsis with some authors considering this negative predictive value only slightly better than flipping a fair coin in populations with a low prevalence of sepsis (Cantey and Bultmann 2020).

The accurate diagnosis of sepsis in patients with clinical suspicion of sepsis remains a challenge, and the investigation into diagnostic aids is widening to the fields of molecular diagnostics, proteomics, metabolomics, and gene expression offering a promise for potentially better diagnostic markers in the future (Iroh Tam and Bendel 2017). Currently, microbiological diagnosis for neonatal sepsis remains the cornerstone of diagnosis of infection. The overall approach to the management of neonatal sepsis in the NICU should start from before birth with adequate antenatal

and perinatal care to reduce the impact of maternal risk factors, followed by adherence to infection control practices in the NICU, gentle handling and meticulous skin care, early initiation of enteral feeding with human milk, limited use of invasive devices and catheters, and standardized bundles related to the care of catheters to reduce the burden of hospital-acquired LONS.

Applications to Other Diseases or Conditions

In this chapter, we reviewed the role of CRP in the diagnosis and management of neonatal sepsis. Studies suggest a single CRP test done at initial presentation has a low sensitivity to diagnose infection and would not effectively alter the decision to administer or withhold antibiotic therapy (Benitz et al. 1998; Brown et al. 2020; Stocker et al. 2021). Serial CRP testing did however improve diagnostic accuracy (Benitz et al. 1998; Stocker et al. 2021), and CRP-based algorithms can reduce the antibiotic treatment duration in newborn patients (Petel et al. 2018). There may however be a role for the use of point-of-care testing of CRP in adult outpatients. A meta-analysis of randomized controlled trials demonstrated reduced antibiotic treatment in adult patients who presented with acute fever or respiratory symptoms when a CRP-based algorithm for antibiotic initiation had been implemented (Petel et al. 2018), although the studies further demonstrated no differences in hospitalization or mortality when the algorithms were utilized. While CRP was used in these studies as a marker of infection, it is also elevated in states of inflammation and cardiac dysfunction (Pepys 1981). An association between high-sensitivity C-reactive protein (hsCRP) with risk for cardiovascular disease in adults has been well described. Patients with a higher baseline hsCRP have been shown to have an increased risk of cardiovascular and coronary events (Pepys and Hirschfield 2003). Baseline hsCRP greater than 3 mg/L can predict an approximately 50% increase in risk compared to levels below 1 mg/L. In addition, testing hsCRP has demonstrated value in predicting the risk of death or recurrent major cardiovascular events in patients with previous myocardial infarction when tested a month after initial recovery (Carrero et al. 2019). Since CRP may rise due to intercurrent pathologies, it is important to differentiate between true baseline values and temporarily elevated CRP with serial testing when being used to predict cardiovascular disease.

Mini-Dictionary of Terms

Acute Phase Reactant

Acute phase reactants are inflammation markers that exhibit significant changes in serum concentration during inflammation. Acute phase reactants can be classified as positive or negative, depending on their serum concentrations during inflammation. Positive acute phase reactants are upregulated, and their concentrations increase during inflammation. Negative acute phase reactants are downregulated, and their concentrations decrease during inflammation.

Antimicrobial Stewardship

Antimicrobial stewardship is a coordinated program that promotes the appropriate use of antimicrobials (including antibiotics), improves patient outcomes, reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms.

Biomarker

A naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, etc. can be identified.

Immunoassay

Immunoassay is a bioanalytical method of measuring the presence of substances which range from small molecules to macromolecules in a solution by the use of an antibody or an antigen to recognize it.

Immunoturbidimetry

A method that measures the absorbance of light from a sample which is used for quantifying an amount of analyte based on the level of turbidity produced by the formation and precipitation of an immune complex containing the analyte.

Microbiome

A community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment and especially the collection of microorganisms living in or on the human body.

Nephelometry

Technique used to determine levels of antibodies or antigens in a suspension based on its light-scattering properties.

Negative Predictive Value

The ratio of subjects truly diagnosed as negative to all those who had negative test results. The characteristic predicts how likely it is for a patient to truly be disease

free, in case of a negative test result. Negative predictive value = True negative/(true negative + false negative).

Point-of-Care Test

Point-of-care testing is defined as medical diagnostic testing at the time and place of patient care.

Positive Predictive Value

The ratio of patients truly diagnosed as positive to all those who had positive test results (including healthy subjects who were incorrectly diagnosed as positive). Positive predictive value = True positives/(true positives + false positives).

Sensitivity

Also known as true positive rate, refers to the proportion of those who received a positive result on this test out of those who have the condition when judged by the Gold Standard.

Specificity

Specificity or true negative rate refers to the proportion of those who received a negative result on this test out of those who do not actually have the condition when judged by the Gold Standard.

Vertical Transmission

A vertically transmitted infection is an infection caused by pathogens (such as bacteria and viruses) where transmission is directly from the mother to an embryo, fetus, or baby during pregnancy or childbirth.

Hospital-Acquired Infection

Healthcare-associated infections which are nosocomially acquired infections and are typically not present or might be incubating at the time of admission. These infections are usually acquired after hospitalization and manifest 48–72 hours after admission to the hospital.

Key Facts of Neonatal Sepsis

- Neonatal sepsis is a diagnosis made in infants less than 28 days of life.
- The clinical syndrome includes systemic signs of infection, circulatory shock, multisystem organ failure, and progress to death rapidly.
- An estimated 15% of all neonatal deaths globally are due to sepsis.
- Neonates are disproportionately affected in low-income and middle-income countries with a high prevalence rate of infectious disease and restricted access to care.
- In Sub-Saharan Africa, neonatal sepsis resulted in an estimated loss of 5.3–8.7 million disability-adjusted life-years and an estimated economic burden of up to US$469 billion (2014 data).
- Early-onset sepsis is disproportionally more prevalent in preterm infants.
- In countries with widespread use of intrapartum antibiotics and screening for Group B streptococcus infection in mothers, *Escherichia coli* is emerging as the predominant pathogen causing early-onset sepsis.

Summary Points

- C-reactive protein (CRP) is a nonspecific acute phase reactant protein that rises in states of inflammation and infection.
- CRP production is triggered by inflammatory cytokines, predominantly IL-6 and IL-1, whereafter it binds to various cellular structures to facilitate in opsonization and phagocytosis of microorganisms and assists in activation of complement system and the proinflammatory response to infection.
- The rise in CRP starts 4 to 6 hours after cytokine stimulation, doubling every 8 hours to reach a peak between 36 and 50 hours.
- The removal of the cytokine stimulus results in a decline of CRP concentration with an average half-life of 19 hours.
- In healthy newborns, serum CRP increases physiologically at birth reaching a peak as high as 13 mg/L between 27 and 36 hours after birth.
- Neonatal sepsis is a severe condition that presents with nonspecific clinical signs and has a high morbidity and mortality especially in developing countries.
- The diagnosis of neonatal sepsis is confirmed by positive microbiological cultures; however, these may only be reported positive 36 to 48 hours after the specimens have been taken.
- Current evidence suggests CRP, at a cut-off value of 5–10 mg/L, has a low sensitivity of 62% and median specificity of 74% for diagnosing neonatal sepsis.
- Serial normal CRP measurements taken 24 to 36 hours after initiation of empiric antibiotic can exclude the presence of early-onset infection with a high probability.
- Despite its limitations, CRP testing is still widely used in newborns to assist in the diagnosis of neonatal sepsis and in the safe discontinuation of antibiotics.

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