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Research Article

Assessing Current C-reactive Protein Sampling Practices within the Neonatal Intensive Care Unit for Neonates with Suspected Early Onset Sepsis

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Abstract. Background: C-reactive protein is synthesized in the liver as part of the acute phase response activated in reaction to acute injury. It has been well established that CRP levels can be used as an acute marker of inflammation making it a useful aid in the diagnosis and management of sepsis. However, its use within the immediate postnatal period presents unique challenges.
Aim: This study aimed to elucidate and standardise CRP blood sampling intervals in neonates with suspected early onset sepsis, and to describe the relationship between CRP results and final blood culture results, with the aim of implementing NICE recommendations within the local setting.
Results: 316 infants were included in the study. 26.2% of neonates had at least 1 positive CRP value (>10 mg/dl) during the first 72 hours of life, with 12.7% resulting in a detectable bacterial growth on blood cultures. The largest percentage of positive CRP levels was obtained when blood was sampled within 18 to 24 hours post birth (30.3%). 40.7% of CRP samples were repeated between 24-48 hours of life. For 27.7% of neonates, a first positive CRP level of more than 10 mg/dl was noted after 24 hours of life.
Conclusion: The results show the importance of maintaining adequate timing intervals between serial CRP levels, which should be taken as a baseline on admission and then repeated not before 12 hours of age, to achieve optimal sensitivity. Our current sampling practice might lead to falsely reassuring negative CRP values, affecting outcomes in sepsis management.

Abbreviations

CRP	C-reactive Protein
CSF	Cerebrospinal Fluid
EOS	Early Onset Sepsis
NICE	The National Institute for Health and Care Excellence
NICU	Neonatal Intensive Care Unit

1 Introduction

C-reactive protein (CRP) is a protein synthesized in the liver as part of the acute phase response activated in reaction to acute tissue injury including trauma, surgery, infection and inflammation. (Jaye et al., 1997) CRP levels start to rise 6 to 8 hours after activation of the acute phase response but may take up to 24 to 48 hours to peak. Levels remain high for at least 24 to 48 hours after commencing treatment, and then rapidly decline 5 days later (Chiesa et al., 1998). Given its functional role within the inflammatory process, it has now been well established that CRP levels can be used as an acute marker of inflammation making it a useful aid in the diagnosis and management of infection including sepsis. Despite this, use of CRP levels within the immediate postnatal period to guide management of neonatal sepsis presents challenges. Although a level of >10 mg/dl is considered abnormal and is the most widely reported reliable cut-off used to date to indicate possible sepsis (Benitz et al., 1998), CRP level taken on initial presentation is often negative, with initial CRP levels being within the normal range in 60% of cases of subsequently proven sepsis (Franz et al., 1999). Similar findings have been reported for 2 CRP levels taken within the first 24 hours of presentation (Krediet et al., 1992). In view of this, various efforts have been made to try devise a standardised practice for CRP sampling. Various studies have shown increased sensitivity of CRP levels

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when taken at least 24 hours post presentation (Gerdes et al., 1987; NICE Guideline 195, 2021). Moreover, likelihood ratios indicated that positive CRP levels at initial sampling pointed towards a moderate increase in probability of neonatal sepsis. However, a negative result taken 24 to 48 hours later indicated a moderate decrease in infection risk, thus increasing its reliability in guiding clinicians re further management. Given these findings, NICE guidelines recommend taking a CRP level at the time of initial blood culture sampling (baseline), with a subsequent level taken not before 18 to 24 hours later. Locally, there is no standardised practice for the timing of CRP sampling in neonates with suspected early onset sepsis, with sampling intervals varying for each neonate admitted to the unit. The role of this study was to assess such sampling intervals, together with the relationship of CRP results with final blood and CSF culture results, to elucidate whether the NICE recommendations can be implemented within the local setting.

2 Material and Methods

Aim and Objectives

The objectives of this study were:

1. To describe the population of neonates admitted to the NICU with the suspicion of possible early onset neonatal sepsis.
2. To evaluate time intervals and results of CRP sampling in relation to time post birth.
3. To assess CRP sampling time intervals and CRP level results in relation to final blood and CSF culture results.

Guidelines

The guideline referred to within this study against which parts of the results were analysed and recommendations made accordingly, was the NICE Guideline 195: Neonatal Infection: antibiotics for infection and treatment published April 2021.

Data Collection

Permission to perform this study was obtained from the hospital administration, data protection office and the chairperson for the Department of Child and Adolescent Health at Mater Dei Hospital.

Data was collected from intradepartmental censuses, handover sheets and via the online hospital clinical manager system. The study period spanned over 13 months from 1^{st} February 2019 to 29^{th} February 2020.

Inclusion Criteria

- All term and preterm neonates admitted to the NPICU within the first 72 hours of life with clinical signs of suspected sepsis.
- Signs of suspected sepsis were taken to include lethargy, irritability, poor feeding, vomiting, tachypnoea, apnoea, temperature instability, hypoglycaemia or hyperbilirubinemia.
- A level of >10 mg/dl was considered as a positive CRP reading.

Exclusion Criteria

- Neonates presenting with clinical signs of sepsis after 72 hours of age.
- Preterm and term neonates with no clinical signs of sepsis.

Data collected

- CRP levels taken on admission to the unit
- CRP levels taken within the following 48 to 72 hours after birth
- gestational age (GA)
- date and time of birth
- time post birth of first, second ± third CRP sample
- blood and CSF culture results
- reason for NICU admission—presenting signs of possible sepsis

3 Results

3.1 General Demographics

A total of 316 infants were originally identified with signs of possible sepsis and were included in the study. 144 infants (45.6%) were less or equal to 37 weeks of gestation at time of sampling whilst 172 infants (54.4%) were above 37 weeks of gestation. The commonest reason for admission and CRP sampling was that of respiratory distress (54.7%). 83 neonates (26.2%) had at least 1 positive CRP value (>10 mg/dl) during the first 72 hours of life. For these 83 neonates with positive CRP results, blood cultures were only taken in 95% (79 neonates). Subsequently out of these, only 10 blood cultures (12.7%) resulted in a detectable bacterial growth. There was only one case in our cohort with a positive CSF culture, in addition to a detectable growth on blood culture, hence why our results focus on relationship between CRP levels and final blood culture results.

3.2 CRP results vs time of sampling post delivery

Data on CRP level results in relation to hours post delivery for neonates presenting with possible EOS was plotted for better graphical understanding of data as shown in figures 1 and 2 below.

Table 1 below shows the total number of CRP samples taken together with the resultant total number of positive

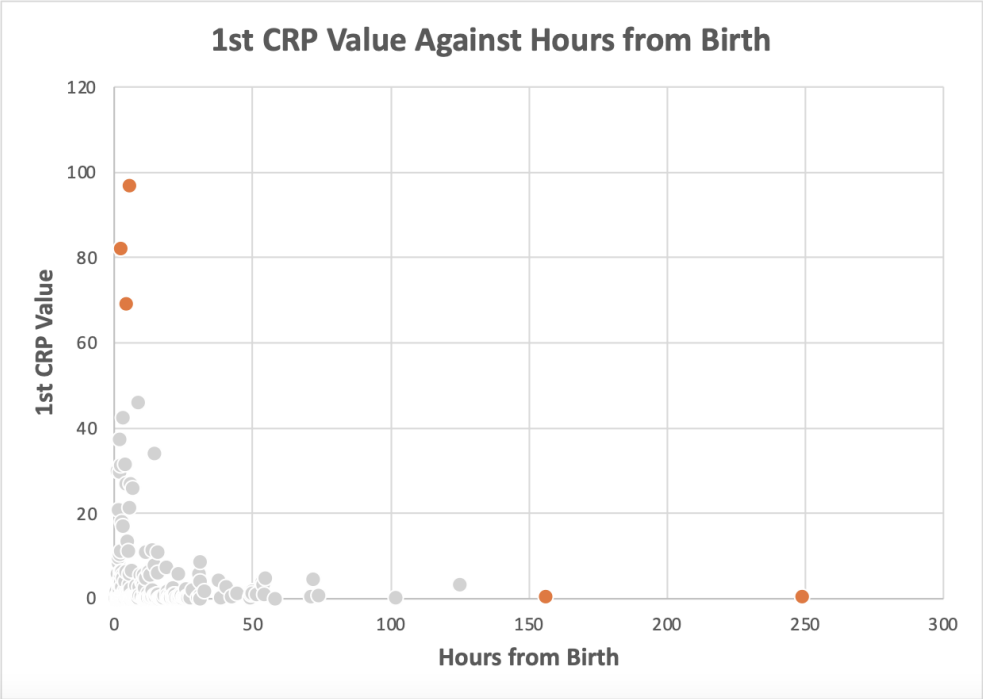

Figure 1: First CRP values obtained in relation to hours from birth.

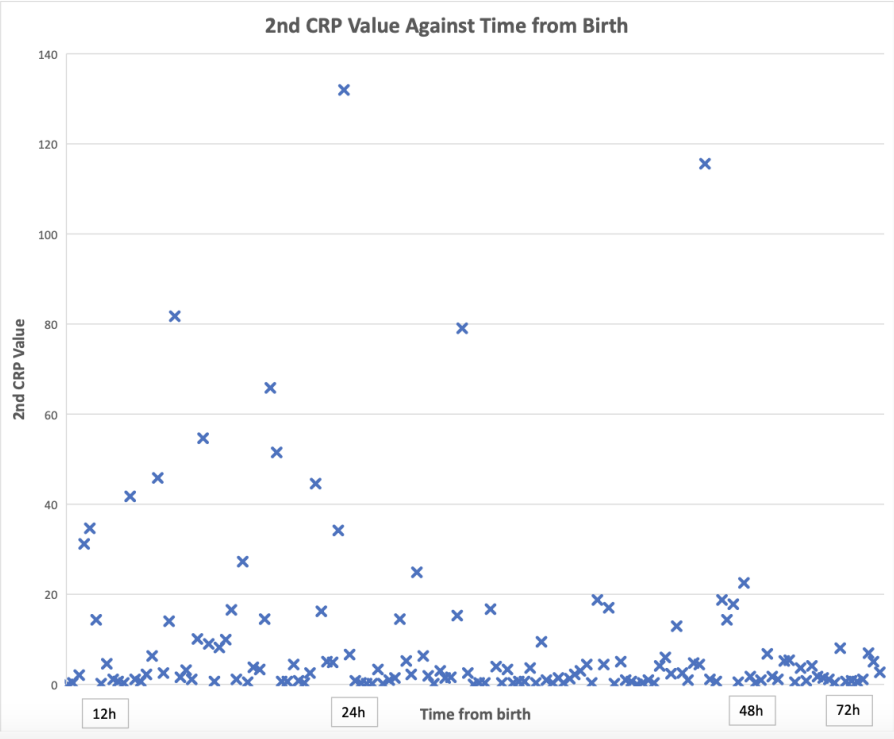

Figure 2: Results for second CRP level obtained in relation to time from birth.

CRP levels obtained at different time intervals after birth.

The data in table 1 shows that out of a total of 849 CRP samples, 270 samples were taken between 24–48 hours of age followed by 199 samples taken on admission. Despite this, the largest percentage of positive CRP levels was obtained when blood was sampled within 18 to 24 hours post birth (30.3%). This was followed by the 12-18 hour group (26.2%).

For neonates presenting with possible sepsis at less than 24 hours of life, a CRP taken within the first 6 hours resulted to be positive in only 11% of cases. Meanwhile a CRP level taken at 6–12 hours of age increased yield of a positive value to 23.5%.

A total of 200 samples were taken in the first 0–6 hours of life, with one sample being a repeat sample within 6 hours of admission. Of these, 6 (3%) were repeated within 12 hours of life, 42 (21.1%) within 12–18 hours, 42 (21.1%) between 18–24 hours of life, 81 (40.7%) between 24–48 hours and 20 (10%) beyond 48 hours of age.

Figure 3 below shows the number of first positive CRP levels recorded in relation to time post birth. Interesting to note is the fact that for the majority of neonates studied, a positive CRP level of more than 10 mg/dl was first noted after 24 hours of life (27.7%). This was followed closely by the less-than-6-hours and 18–24 hours categories (21.8% each respective category). A first positive CRP level was on the other hand least noted during the 6–18 hour time range (27.7%).

3.3 CRP sampling results vs blood culture results

Bacterial growth on blood cultures was detected in 11 cases. Out of these, only 4 samples were considered to be significant growth (Group B Streptococcus and Staphylococcus aureus), with the rest being considered as contaminants. A CRP rise was noted in 3 out of the 11 positive culture results. First positive CRP values were obtained between 12–18 hours of age in 1 case, and between 18–24 hours of age in 2 cases. Out of the 4 significant blood culture growths, 2 were associated with a rise in CRP levels.

4 Discussion

4.1 Neonatal sepsis

Despite advances in neonatal care, neonatal sepsis remains an important cause of morbidity and mortality. Neonatal sepsis is defined as early–onset sepsis (EOS) when it occurs at $\leq$72 hours after birth. In EOS, transmission of pathogens occurs vertically from the mother to the infant before or during delivery (Hornik et al., 2012). Although the gold standard for confirmation of neonatal sepsis remains positive blood cultures, many cases of suspected neonatal sepsis are managed empirically with antibiotics on admission to the neonatal intensive care unit (NICU) despite having no pathogen isolated from blood cultures.

The burden of sepsis on the NICU in terms of incidence and mortality, remains high, with a mortality up to 40% in EOS. The incidence of culture-proven neonatal sepsis is estimated between 0.77 and 1 per 1000 live births (Cohen-Wolkowiez et al., n.d.). Presenting signs and symptoms vary according to the gestational age and the severity of infection. These may include fever, hypothermia, lethargy, poor feeding, or vomiting (Lim et al., 2012). Most term neonates will develop respiratory distress as an early sign of sepsis as demonstrated in our study, and 80–90% of all cases of EOS will present in the first 24 to 48 hours of life (Polin et al., 2012). Although neonatal sepsis is potentially curable if diagnosed and treated early, it is important to realise that early clinical signs of sepsis are non-specific and may be easily missed.

4.2 Role of CRP in guiding sepsis management

All neonates admitted to the NICU with clinical signs of sepsis should have a thorough review of antenatal risk factors and a workup that includes a blood culture sampling. However, as demonstrated in the results section, even when taking the required blood volume using proper aseptic technique, a positive growth on blood culture was obtained in only a small percentage of cases of suspected sepsis, possibly due to a low colony count bacteraemia ($\leq$4 CFU/ml) in 12.7% of cases. In addition, a positive blood culture result with identification of the pathogen is usually not possible before 48 to 72 hours.

It is therefore necessary to have a rapid diagnostic test and adjuncts such as C-reactive protein that can aid clinical assessment in reliably confirming or excluding neonatal sepsis at its early stages (Mishra et al., 2006). Not only will this allow prompt treatment of infection but will also avoid judicious use of antibiotics in sepsis negative patients. CRP remains the most widely used infection marker due to it being a simple, fast, cost effective and widely available test (Chirico et al., 2011).

The positive predictive value of CRP increases with higher CRP levels. In addition, the magnitude of the CRP response varies according to the underlying pathogen with a greater CRP response seen in gram negative sepsis as opposed to gram positive sepsis (Rønnestad et al., 1999). CRP has a negative predictive value of 93% and is considered to be a useful tool to aid in the duration of antibiotic therapy and to assess response to treatment (Hofer et al., 2012).

The results of this study show that the largest percent-

Time after birth (hours)	CRP 1 (n)	CRP 2 (n)	CRP 3 (n)	Positive CRPs (>10mg/dl) n (%)	Total num. of CRPs taken (n)
0–6	199	1	0	22 (11)	**200**
6–12	55	13	0	16 (23.5)	**68**
12–18	33	47	0	21(26.2)	**80**
18–24	26	51	2	24 (30.3)	**79**
24–48	0	144	126	65 (24)	**270**
48–72	0	32	63	12 (12.6)	**95**
72+ (max 6 days of age)	0	20	37	3 (5.3)	**57**
Total	**313**	**308**	**228**	**163**	**849**

Table 1: Total number of CRP samples taken in relation to the total number of positive CRP levels obtained at different time intervals post birth.

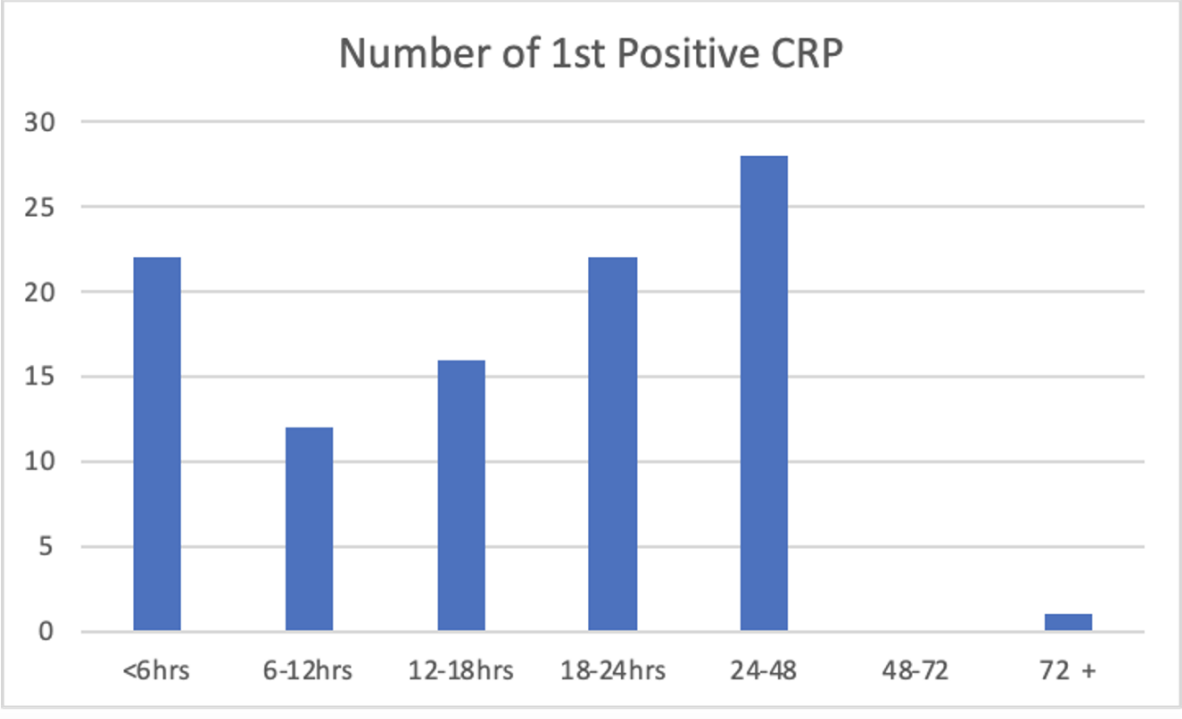

Figure 3: Yiming post birth of first positive CRP levels obtained.

age of positive CRP levels was obtained when neonates were sampled between 12 to 24 hours post birth. Meanwhile a CRP level taken within the first 6 hours of life was only positive in 11% of cases and for the majority of neonate studied, a positive CRP level was first noted after 24 hours of life. This highlights the importance of not solely using a single negative CRP value taken on admission to rule out sepsis. Serial CRP measurements, together with rate of rise, should always be used, ideally allowing at least 12 hours post admission before repeating samples.

Meanwhile bacterial growth on blood cultures was only detected in 11 cases, with only 4 growths being considered as being significant. A CRP rise (first noted within 12–24 hours of age) was associated with only 2 of these significant growths, further emphasising the fact that CRP levels cannot be reliably used to predict positivity or negativity of blood culture results.

In light of this, the timing of the sample, the presence of other sepsis related clinical symptoms, the presence of positive sepsis related blood results including use of early sensitive markers such as procalcitonin, should all be considered in the decision-making process. The combination of CRP with procalcitonin was shown to increase sensitivity to values between 90 and 100% in most studies (Hofer et al., 2012).

4.3 Developing alternative diagnostic techniques

Molecular testing such as PCR and DNA microarray-based methods are increasingly being used in the diagnosis of neonatal sepsis since they are able to detect bacterial DNA at much lower concentrations that would be required for bacterial culture (Peters et al., 2004). In addition, a result may be achieved in as quickly as 30 minutes. However, the lack of bacterial culture means no information can be given regarding antimicrobial resistance (Jordan et al., 2005; Jordan et al., 2006). Their main use is potentially in cases where antibiotic exposure has already occurred, with a low-density bacteraemia or nonviable pathogen resulting in culture-negative sepsis.

4.4 Study Limitations

The results presented within this study were based on absolute CRP values in relationship to a specific threshold (10 mg/dl). Trend and rate of change (rise/decline) in CRP levels over time which can also be used to guide infection risk and management of possible sepsis, were not assessed.

5 Conclusion

The fact that CRP takes 10–12 hours to significantly change is its most significant limitation. An initial CRP taken on admission in a neonate <12 hours of age should not influence the clinical decision on whether to start antibiotic treatment or not. This decision should be taken based on the presence or otherwise of risk factors for sepsis and on the clinical picture.

The results in this study show the importance of maintaining adequate timing intervals between serial CRP levels, which should be taken as a baseline on admission and then repeated not before 12 hours of age, to achieve optimal sensitivity. Our current practice of repeating CRPs too early after initial sampling might lead to early positive CRPs being missed apart from increased cost burden on the hospital labs.

It is therefore being recommended that timing of CRP sampling is standardised for all neonates admitted to the NICU with signs of suspected sepsis, especially given the low rate of culture proven sepsis within this population.

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