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Creactive protein in healthy term newborns during the first 48 hours of life

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ABSTRACT

Background Early-onset neonatal sepsis (EOS) is a serious and potentially life-threatening disease in newborns. C reactive protein (CRP) is the most used laboratory biomarker for the detection of EOS. Little is known about normal reference values of CRP during the perinatal period as several factors are able to influence it.

Objectives To identify an appropriate range of CRP values in healthy term newborns during the first 48 hours of life.

Design CRP determination was performed in 859 term newborns at 12, 24 and 48 hours of life. Mode of delivery, maternal vaginal culture results, intrapartum antimicrobial prophylaxis (IAP) and other perinatal variables were recorded.

Results CRP mean values were significantly higher at 48 hours (4.10 mg/L) than at both 24 (2.30 mg/L) and 12 hours of life (0.80 mg/L). CRP levels were affected by a number of perinatal proinflammatory variables. In particular, CRP mean values were significantly higher in babies born by vaginal delivery (3.80 mg/L) and emergency caesarean section (3.60 mg/L) than in babies born by elective caesarean section (2.10 mg/L). Completed course of IAP led to lower CRP mean values (2.90 mg/L) than IAP not completed (3.80 mg/L) or not performed (4.70 mg/L).

Conclusions Postnatal age and mode of delivery significantly influence CRP values. Reliable reference values are crucial in order to obtain an adequate diagnostic accuracy.

What is already known on this topic?

- C reactive protein (CRP) is the most used marker in neonatal care all over the world.
- CRP reference values during the first hours of life have mainly been established in uninfected but symptomatic newborns and/or on relatively small populations.
- The association between CRP and non-infectious conditions is not completely clear as the majority of studies were performed on populations with wide-ranging inclusion criteria.

What this study adds?

- It is normal for CRP values to rise after delivery, up to 20 mg/L at 48 hours.
- CRP values are higher after normal delivery and emergency caesarean section compared with elective caesarean section.
- Guidelines for the interpretation of CRP values in apparently low-risk neonates may need to be reconsidered in the light of these data.

INTRODUCTION

C reactive protein (CRP) is an acute phase reactant protein and a marker of systemic inflammation produced by the liver. High CRP levels are known to be associated with early-onset neonatal sepsis (EOS), which is a serious and potentially life-threatening disease in newborns. EOS has a mortality rate around 1.5% in term newborns and up to 40% in very-low-birth-weight infants.[1] The aetiology of EOS is various, but group B *Streptococcus* (GBS) (also known as *Streptococcus agalactiae*) has a central role.[2] Early recognition of EOS in newborns still remains challenging due to the high variability of the clinical spectrum and the subtle and non-specific signs and symptoms of neonatal sepsis that are clinically indistinguishable from other non-infectious conditions, such as respiratory distress syndrome or difficult adaptation to extrauterine life.[3 4]

Numerous biomarkers have been proposed to distinguish septic babies from healthy ones.[5–9] The determination of CRP, in consideration of its huge availability, simplicity, speed and low cost, plays a central role in the diagnosis of EOS, therefore representing the most used marker in neonatal intensive care units (NICUs) all over the world. Although a huge body of literature is available on CRP in newborns, some issues have not yet been fully dealt with, such as the influence of non-infectious factors on CRP levels.

CRP levels in fact increase in all conditions able to activate the inflammatory cascade and/or tissue damage[1]: many prenatal, perinatal and neonatal factors might have an influence on CRP concentrations, so that an increased CRP is not necessarily linked to a septic status. The identification of independent variables influencing the interpretation of CRP may be useful in the differential diagnosis of EOS. Moreover, CRP levels physiologically increase in newborns within the first days of life. It is therefore mandatory to consider the normal CRP kinetics, as well as its behaviour in response to potential confounders, when evaluating a newborn

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Table 1 Clinical characteristics of study population

		Mean (±SD) or frequencies (%) (total n=859)
Gestational age (weeks)		**39.3 (±1.8)**
Birth weight (g)		3322.4 (±443.7)
Gender	M	407 (47.4%)
	F	452 (52.6%)
Mode of delivery	Vaginal delivery	636 (74.04%)
	Elective caesarean section	148 (17.23%)
	Emergency caesarean section	75 (8.73%)
Apgar score	1 min	9.1 (±0.8)
	5 min	9.5 (±0.4)
Maternal vaginal culture for GBS	Negative	240 (27.94%)
	Positive	208 (24.21%)
	Not done or remote (done >4 weeks before the delivery)	411 (47.85%)
IAP to the mothers	Complete IAP (two doses)	179 (20.84%)
	Incomplete IAP (only one dose)	236 (27.47%)
	IAP not done	444 (51.69%)
Meconium-stained amniotic fluid		72 (8.38%)
PROM >18 hours		107 (12.45%)

F, female; GBS, group B Streptococcus; IAP, intrapartum antimicrobial prophylaxis; M, male; PROM, premature rupture of membranes.

in the first hours of life in order to avoid inaccurate diagnosis and inappropriate treatment.

The aim of our study is to assess trend and normal range values of CRP in a large and homogeneous cohort of term newborns during the first 48 hours of life and the potential influence exerted by perinatal variables.

METHODS

Patients

A prospective study was conducted in the department of molecular and developmental medicine at the Siena University Hospital, Italy, between January 2014 and December 2015. The local ethics board approved the study protocol. A total of 859 consecutively born healthy term newborns were enrolled. For clinical details, see table 1.

Informed consent was obtained from the babies' parents before the enrolment.

Inclusion criteria were as follows: (A) gestational age 37–42 weeks; (B) at least one CRP determination in the first 48 hours of life; (C) continuous uncomplicated postnatal hospital stay and discharge as healthy from the hospital at 48 hours of life (if born by vaginal delivery) or at 72 hours of life (if born by caesarean section); (D) informed consent signed by a parent/legal guardian.

Exclusion criteria were considered as follows: (A) antibiotic therapy in the first 48 hours of life; (B) babies with congenital heart malformation, major congenital malformations and/or chromosomal anomalies; babies who received anti-hepatitis B vaccine; (C) clinical signs of chorioamnionitis (maternal fever >38°C; maternal leucocytosis; maternal tachycardia >100 beats per minute (bpm); fetal tachycardia >160 bpm); (d) babies who needed intensive assistance and were hospitalised in NICU; (E) acute intrapartum events such as cord prolapse, uterine rupture, sudden and sustained fetal bradycardia, shoulder dystocia and complicated breech extraction; and (F) clinical signs of prenatal/perinatal asphyxia (metabolic or mixed acidaemia (pH <7.00) in umbilical artery blood sample, if obtained; persistence of an Apgar score of 0–3 for longer than 5 min; neonatal neurological sequelae and multiple organ involvement).

None of the enrolled newborns developed sepsis; none of them was readmitted to the hospital within the first month of life.

All the antepartum and intrapartum data were collected, including mode of delivery, duration of active labour, interval between rupture of membranes and delivery, presence of meconium-stained or fetid amniotic fluid, maternal vaginal culture for GBS and administration of intrapartum antibiotic prophylaxis (IAP) to the mothers.

In our hospital, the revised guidelines from the Centers for Disease Control and Prevention (CDC)[10] for prevention of perinatal group B streptococcal disease are in force: each woman with a positive or unknown swab for GBS at the 35th–37th weeks of gestation is a candidate for the administration of antibiotic therapy during labour. The same treatment is performed in cases of unknown swab for GBS if either or both of the following risk factors are present: maternal fever or premature rupture of membranes (PROM) longer than 18 hours.[10]

In all babies, CRP was determined in the first 48 hours of life, according to an internal protocol. In particular, CRP evaluations were performed at 12, 24 and/or 48 hours of life with venipuncture together with routine blood tests or compulsory samples for screening of metabolic diseases. The adequate amount of blood required for the analysis was around 0.5 mL.

For the quantitative determination of CRP in human serum and plasma, we used Roche/Hitachi cobas c systems (CPLRX: ACN 019). It is a particle-enhanced immunoturbidimetric assay. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

Statistics

Descriptive analysis of the variables of the study population was performed. CRP levels at 12, 24 and 48 hours of life were comparatively analysed using an unpaired t-test, and the significance level was set at 0.05. When the assumption of uniform variance was violated, we used the Satterthwaite approximation based on t distribution.[11] In order to compare distributional aspects, the median and 5th–95th centiles were computed. Then CRP levels for neonates born by vaginal delivery (VD), emergency caesarean section (EMCS) and elective caesarean section (ELCS) at 48 hours of life were comparatively analysed using a unpaired t-test. Also, in this last case to compare distributional aspects, the median and 5th–95th centiles were computed. Power analysis was not performed because the difference was observed at prearranged significance level sets. The data analysis was conducted using Statgraphics V.17.2.02.

RESULTS

Perinatal characteristics of the newborns are described in table 1.

CRP mean and median values were higher at 48 hours than at both 24 and 12 hours of life (table 2 and figure 1).

Each pair of medians (CRP at 12 hours vs CRP at 24 hours, CRP at 12 hours vs CRP at 48 hours and CRP at 24 hours vs CRP at 48 hours) was statistically different (figure 2).

Neonates born by VD and EMCS showed significantly higher CRP mean and median values at 48 hours than those born by ELCS. No significant differences were found in CRP values between neonates born by VD and those born by EMCS (table 3 and figure 3).

Table 2 Mean, median and 5th–95th centiles for C reactive protein (CRP) at 12, 24 and 48 hours

	Mean (mg/L)	Median (mg/L)	Fifth centile (mg/L)	95th centile (mg/L)
CRP 12 hours	0.80	0.40	0.10	2.60
CRP 24 hours	2.30	1.50	0.30	6.90
CRP 48 hours	4.10	2.70	0.50	13.3

Neonates born to mothers who completed the course of IAP for the presence of a positive vaginal swab for GBS had significantly lower mean values of CRP (2.90 mg/L, 95% CI 2.40 to 3.30) than neonates whose mothers did not complete IAP (3.80 mg/L, 95% CI 3.10 to 4.40) or did not performed IAP (4.70 mg/L, 95% CI 4.00 to 5.30), with $p<0.05$.

Among the antenatal and perinatal variables, PROM, prolonged labour and presence of meconium-stained amniotic fluid showed a significant effect on CRP levels: newborns with at least one of the considered variables had significantly higher CRP mean values than babies who had an essentially uneventful perinatal and postnatal course (6 mg/L, 95% CI 4.30 to 7.70 vs 4.10 mg/L, 95% CI 3.70 to 4.40).

DISCUSSION

The main purpose of this study was to examine neonatal CRP values in the first hours of life in healthy term newborns and CRP modifications in relation to different perinatal conditions. When evaluating CRP in a newborn with suspected EOS, it is recommendable to consider its normal dynamics and trend in the healthy newborn: the selection of a particular CRP value as 'normal' or 'abnormal' regardless of potential influencing factors may lead to administration of antibiotics to newborns in whom the diagnosis of EOS will be ruled out 48–72 hours later with the normalisation of diagnostic biomarkers.[12]

Upper CRP cut-off values during the first days of life have mainly been established from studies on uninfected but symptomatic newborns. There are few studies assessing CRP reference values in healthy neonates, but those observations were based on rather small populations.[1] In our study, the analysis of a large population of healthy term newborns shows that CRP values undergo a non-specific rise after birth, consistently with the results of previous studies.[13] In a research by Chiesa *et al*,[13] the authors demonstrated that in addition to postnatal age, also gestational age has a positive effect on CRP: in fact, preterm

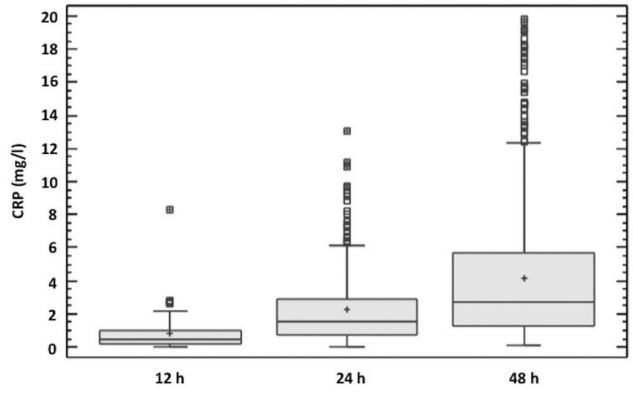

Figure 1 Box and whisker plot for C reactive protein (CRP) at 12, 24 and 48 hours.

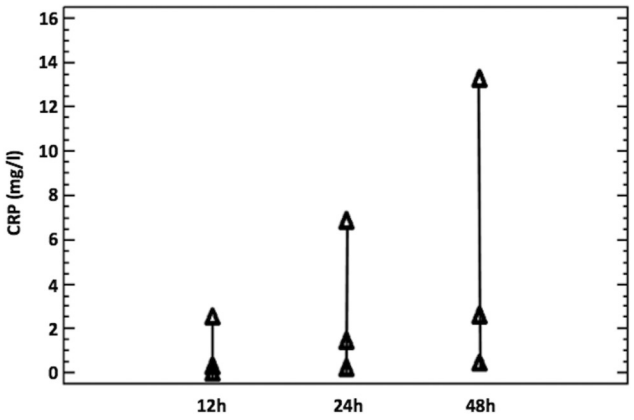

Figure 2 Median and 5th–95th centiles for C reactive protein (CRP) at 12, 24 and 48 hours.

babies showed a lower and shorter CRP response compared with full-term babies.[13]

It is likely that the stress of delivery plays a central role in the physiological dynamics of CRP during the first days of life.[14 15] Actually, we found that the mode of delivery considerably influences the values of CRP, which were significantly higher in babies born by VD and EMCS than in those born by ELCS. This could be explained by the fact that VD is associated with a larger production of stress hormones such as cortisol and catecholamines, which in turn are able to affect neutrophil function and cytokine production.[16] As the current literature suggests, delivery stress is related to CRP increase as a consequence of the rise of interleukin (IL) 6 and IL-10.[17] In fact, VD and EMCS share the same pathways of activation of inflammatory response in newborn, as previously demonstrated.[18 19]

To date, numerous studies were performed on the association between CRP values and non-infectious conditions in newborns; however, data were obtained from populations with wide-ranging inclusion criteria (infected, uninfected, symptomatic, healthy, at risk, critically ill).[20–22] Our study, performed on a homogeneous population of healthy term babies, shows that CRP reference intervals may be affected by several proinflammatory factors, such as PROM, presence of meconium-stained amniotic fluid and prolonged labour. An interesting finding is related to IAP. There are lots of studies in literature that stress the importance of IAP against potential neonatal sepsis.[10 23–27] It seems that its role is maximum only when the prophylaxis is done completely. CDC guidelines define as candidates for antibiotic prophylaxis the pregnant women who deliver vaginally with positive vaginal swab or uncertain/not performed swab in the presence of EOS risk factors. If the offspring is not protected by maternal prophylaxis, the EOS risk is greater than that of a neonatal population without any infectious risk. In consideration of this, the infants with a greater infectious risk are candidate to a routine CRP assessment at 48th hour of

Table 3 Mean, median, 5th–95th centiles for neonates born by vaginal delivery (VD), elective caesarean section (ELCS) and emergency caesarean section (EMCS) at 48 hours

	Mean (mg/L)	Median (mg/L)	5th centile (mg/L)	95th centile (mg/L)
VD	3.80	2.50	0.50	12.1
ELCS	2.10	1.30	0.30	6.00
EMCS	3.60	2.00	0.50	11.3

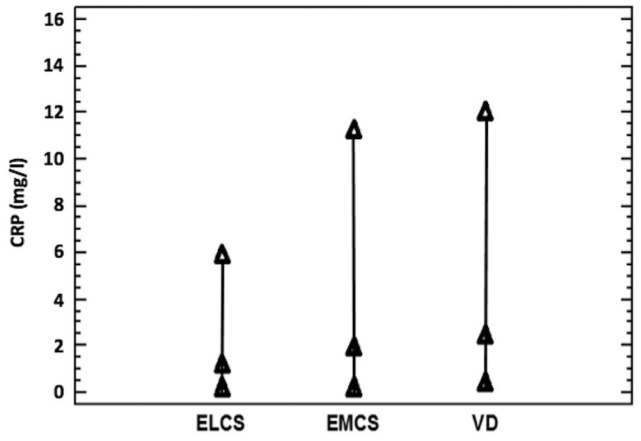

Figure 3 Median and 5th–95th centiles for neonates born by VD, ELCS and EMCS at 48 hours. CRP, C reactive protein; ELCS, elective caesarean section; EMCS, emergency caesarean section; VD, vaginal delivery.

life with a more careful attention to identify early signs and/or symptoms of sepsis. Certainly, IAP has an influence on CRP values trend in the perinatal period: in this study, we found a statistically significant difference in CRP values of babies born to mothers who had completed the course of IAP, compared with newborns whose mothers did not complete the prophylaxis.

CONCLUSIONS

CRP values undergo a physiological rise after birth. Moreover, there are several perinatal factors that may affect the interpretation of CRP values in healthy newborns. In particular, we found that mode of delivery has an evident influence on CRP. Currently, the most used cut-off value is 10 mg/L. In consideration of the variability of CRP values in healthy term newborns related to several factors, it would be appropriate to use normal CRP reference values differentiated for gestational age, postnatal age and mode of delivery. The use of a static cut-off value, which does not reflect the physiological kinetics of CRP, should not ensure an adequate diagnostic accuracy.

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Competing interests None declared.

Patient consent Guardian consent obtained.

Ethics approval Local ethics board of the University Hospital Santa Maria alle Scotte, Siena.

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Notes