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Original Article

Utility of serial serum C-reactive protein in the diagnosis of neonatal infection

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ABSTRACT

Introduction: C-reactive protein (CRP) has been used as a biomarker to diagnose neonatal sepsis. **Aim:** The aim of the study was to study the utility of serial CRP levels in the neonatal infection/sepsis diagnosis. **Materials and Methods:** Neonates admitted to a tertiary care teaching hospital were classified into three groups: proven sepsis (culture positive from any body fluid), probable sepsis (culture negative but clinical and laboratory parameters suggestive of sepsis), and no sepsis (not suggestive of sepsis). CRP was measured as follows: CRP 1 at the time of clinical presentation and CRP 2 and 3 at 24 and 48 h, respectively. Sensitivity, specificity, predictive values, and likelihood ratios were calculated. **Results:** Suspected infection/sepsis was evaluated as early-onset (≤72 h) in 300 neonates and late-onset (LONS) (>72 h) on 150 occasions in 100 neonates. There was a strong correlation between the diagnoses of proven or probable sepsis and elevated CRP levels (≥1.0 mg/dl), for both early- and LONS episodes, supporting the diagnostic utility of CRP. The sensitivity of CRP 2 was > CRP 1, but maximum sensitivity was achieved by CRP 3 level only. A CRP level has a much higher predictive value in ruling out than ruling in neonatal infection/sepsis (reaching almost 99-100% for both sepsis types). **Conclusions:** Serial CRP measurements are useful in the diagnosis of neonatal infection/sepsis. The predictive value of CRP 1 level being normal at initial evaluation cannot justify discontinuing antibiotic therapy. CRP 3 level may virtually rule out or rule in the diagnosis of neonatal sepsis.

Key words: *Bacteremia, Biomarker, Inflammation, Infection, Neonate, Validity*

Neonatal infection refers to any infection occurring in the first 4 weeks of life, whereas neonatal sepsis refers to invasive bacterial infection during this period [1]. The incidence is high in developing countries with a figure of around 10-15 per 1000 live births [1], and in India, it is around 11-25 per 1000 live births [2]. Neonatal sepsis is an important cause of morbidity and mortality during neonatal period and mimics many non-infectious neonatal conditions because of lack of characteristic features [1]. Inability to identify neonatal sepsis early leads to increased mortality. The pressing situation of early identification coupled with non-specific clinical pictures has resulted in the widespread use of antibiotics posing the threat of antibiotic resistance [3].

Of all the diagnostic tests employed to identify or isolate the causative organism, culture of body fluid is the gold standard. However, this method takes a longer time (more than 24-48 h) which makes this test less or not useful in deciding the initiation of antibiotic therapy [3]. There is no single test or a combination of tests that are more useful in this scenario and may be more costly (e.g. serum procalcitonin), particularly, in a developing country setting. C-reactive protein (CRP) is an acute-phase reactant whose level increases gradually during any inflammatory stimulus. Its level parallels the activity of inflammatory process and decreases rapidly than any other acute phase parameter making it useful in monitoring response to antibiotic treatment [4].

The Same principle applies to neonatal sepsis, and the value is reliable in the first 24-48 h [5]. If the serial CRP level is normal, then the probability of sepsis is probably much less likely [5]. It is also cost effective as a single test in the diagnosis and monitoring treatment response in neonatal sepsis. Hence, the present study was planned to evaluate the utility of serial CRP measurements in the diagnosis of neonatal infection/sepsis in the Indian scenario.

METHODS

This prospective observational study was carried out in the neonatal unit, department of pediatrics at a tertiary care teaching hospital of South India over a 2-year period. Neonates having birth weight >1500 g with infection/sepsis constituted the study population. Neonatal infection/sepsis was diagnosed through clinical features coupled with the results of investigations (described below). Neonates with severe birth asphyxia, meconium aspiration syndrome, birth weight <1500 g, birth injuries, prior antibiotic administration, and any underlying surgical conditions were excluded from the study. A standard treatment protocol was followed for the treatment of neonatal infection/sepsis in the unit.

The initial investigations before starting of antibiotics (empirical antibiotic of choice was ampicillin and gentamicin initially, which was changed later if needed according to sensitivity

pattern in culture) included sepsis screen (total leukocyte count, I: T ratio, toxic granules, and micro-erythrocyte sedimentation rate), complete blood counts (CBC) including platelets, and blood cultures. Abnormal CBC was defined as described previously [6,7]. Additional tests that were done at the decision of the treating neonatologist included examination of cerebrospinal fluid (CSF), urine, and/or chest X-ray. The CRP 1 level was measured at the time of presentation and CRP 2 and CRP 3 were measured at 24 and 48 h, respectively. A CRP value of ≥1 mg/dl was taken as positive (as per the manufacturer instructions).

A pre-designed pro forma was made to record the birth weight, gestational age, postnatal age, discharge diagnoses, and final outcome for each neonate. For each episode of suspected infection/sepsis, the above-mentioned workup including the CRP levels was recorded. A-15 CRP kit Biosystem (Costa Brava, Barcelona, Spain) was used for the quantitative measurement of CRP from the serum by turbidimetric immunoassay. The study was approved by the Institute Ethical Committee and written informed consents were taken from the parents/legal guardian before enrollment of neonates in the study.

Categorization of neonatal sepsis:

a. Proven sepsis: If pathogenic bacteria were isolated from any body fluid such as blood, CSF, or urine.
b. Probable sepsis: If clinical along with laboratory findings consistent with bacterial infection, but cultures were negative, and the situation could not explain by any other diagnosis or resolve completely within 8 h.
c. No sepsis: Nothing (clinical, radiological, and investigations) suggestive of sepsis.

Culture positive was taken as significant unless the recovered organisms were normal skin or upper respiratory flora, all other investigations were normal, and either there were no signs of infection or such signs resolved without antibiotics. All the neonates were followed up daily till discharge.

Statistical Analysis

All the data were entered into the Microsoft Excel sheet. The data were analyzed using SPSS software (version 20.0 Chicago, IL, USA). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for serial CRP values were calculated [8]. Differences in the prevalence of proven or probable sepsis between groups were analyzed using the G-test of independence [9]. Confidence intervals for sensitivity, specificity, PPV, and NPV were also calculated. $p<0.05$ was considered statistically significant.

RESULTS

Classification of Neonatal Infection/Sepsis

Suspected infection was evaluated as early-onset (EONS, ≤72 h) in 300 neonates and late-onset (LONS, >72 h) on 150 occasions in 100 neonates. The demographic characteristics of these populations are described in Table 1. There were 38 episodes of proven sepsis (EONS=18, LONS=20), 62 of probable sepsis (EONS=44 and LONS=18), and 350 of no sepsis. Organisms were isolated from 25 blood cultures (EONS=11 and LONS=14), 6 urine cultures (3 each in EONS and LONS), and no CSF cultures (Table 2). Blood and urine culture isolated the same organism in two cases each of EONS and LONS. *Staphylococcus epidermidis* (coagulase-negative staphylococci) was isolated from blood culture in two cases of EONS and three cases of LONS. Of these five cultures, sepsis screen was abnormal in one case. *Klebsiella pneumoniae* was the most frequently isolated organism in EONS

Table 1: Demography of the study population (neonatal infection/sepsis)

Characteristics	EONS	LONS
Total cases	300	100
Total episodes	300	150
Birth weight (g) (mean±SD)	1964±586 (range, 1576-3014 g)	2245±652 (range, 1860-3624 g)
Gestational age (weeks) (mean±SD)	35±4.2 (range, 34-40 weeks)	36±4.5 (range, 34-41 weeks)
Age at evaluation (days) (mean±SD)	0.3±0.5 (range, 0-3 days)	8.4±5.3 (range, 4-28 days)
Male:female	54:46	31:19
Proven sepsis	18	20
Probable sepsis	44	18
No sepsis	238	112

EONS: Early-onset, LONS: Late-onset, SD: Standard deviation

Table 2: Culture positivity in different types of neonatal sepsis/infection

Types of sepsis/infection	Blood	CSF	Urine
EONS			
Organisms			
E. coli	2	-	1
K. pneumoniae	4	-	-
S. aureus	3	-	-
S. epidermidis (CoNS)	2	-	1
E. fecalis	-	-	1
Total positive	11	0	3
Total samples taken	300	105	24
LONS			
Organisms			
E. coli	4	-	2
K. pneumoniae	3	-	-
S. aureus	4	-	-
S. epidermidis (CoNS)	3	-	-
E. fecalis	-	-	1
Total positive	14	0	3
Total samples taken	150	135	36

***E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus aureus, E. fecalis: Enterococcus fecalis,* EONS: Early-onset, LONS: Late-onset, CoNS: Coagulase-negative staphylococci, CSF: Cerebrospinal fluid**

and *Escherichia coli* and *Staphylococcus aureus* in LONS. In EONS, proven sepsis was less common and probable sepsis was more common, *vice versa* was observed in LONS. Although CSF culture was negative in all the cases, the cell counts, glucose, and protein levels were abnormal in one case of EONS and four cases of LONS suggesting the fact that meningitis is probably a common occurrence in LONS.

Serum CRP Levels and Correlation with Sepsis Diagnoses

As mentioned above, CRP levels were obtained in 300 episodes of suspected EONS and 150 of suspected LONS. The relationship between serum CRP levels (CRP 1, CRP 2, and CRP 3) and correlation with sepsis diagnoses has been shown in Table 3. For each CRP, the sensitivity was highest at a cutoff of 1 mg/dl, and this cutoff was used in all the measurements. There was a strong correlation between the diagnoses of proven or probable sepsis and elevated CRP levels (≥1.0 mg/dl), for both EONS and LONS episodes ($p<10^{-5}$ by 2×3 G-tests and $<10^{-6}$ by 2×2 G-tests for no sepsis *vs.* either proven or probable sepsis), supporting the diagnostic utility of CRP. The sensitivity, specificity, PPV, and NPV were calculated for each of the CRP levels and has been shown in Table 4. The sensitivity of CRP 1 for EONS was low, both for proven sepsis (32%) and for probable sepsis (37%). Although the sensitivity of CRP 1 was higher for LONS, both for proven sepsis (60%) and for probable sepsis (65%), initial CRP levels were normal in >one-third of all sepsis episodes. The sensitivity of CRP 2 was >CRP 1, but maximum sensitivity was achieved either by a combination of CRP 2 and 3 or CRP 3 level only.

Regarding the predictive value of different CRP levels to either rule in (PPV) or rule out (NPV), the sepsis diagnosis among the included neonates, as it can be seen from Table 4 that CRP levels have a much higher predictive value in ruling out than ruling in the diagnosis of neonatal infection/sepsis (reaching almost 99-100% for EONS and LONS).

Limitations of CRP Levels

Elevated CRP levels were observed in 23 episodes (EONS=12 and LONS=11), in which sepsis was excluded (no sepsis) either because culture was negative or there was other parameters suggestive of sepsis/infection. Elevated CRP levels were not observed in two episodes, in which the culture grown a pathogenic organism. In one of these episodes, CRP level was obtained only initially, and further levels were obtained only in the first 16 h after presentation in another episode. Subsequent levels were not measured as culture reports were already positive.

Discontinuation of Antibiotics

The CRP levels were normal in 238 of the 300 neonates evaluated for EONS in whom antibiotics could be safely discontinued. The CRP levels were also normal for 112 episodes evaluated for LONS. Of them, in 110 episodes, the antibiotics could be safely discontinued, and in rest two episodes, CRP levels were falsely negative leading to a continuation of antibiotics.

Table 3: CRP in suspected neonatal infection/sepsis

CRP 1	CRP 2	CRP 3	Probable sepsis	Proven sepsis	No sepsis
EONS					
Negative	N/A	N/A	0	1	7
	Negative	N/A	0	0	23
		Negative	1	0	188
		Positive	2	2	2
	Positive	N/A	6	1	1
		Negative	4	1	2
		Positive	9	4	1
Positive	N/A	N/A	7	2	5
	Negative	N/A	0	0	2
		Negative	0	1	1
		Positive	3	3	1
	Positive	N/A	5	0	0
		Negative	1	2	3
		Positive	6	1	2
		Total	44	18	238
LONS					
Negative	N/A	N/A	0	1	4
	Negative	N/A	1	0	7
		Negative	1	0	84
		Positive	1	0	0
	Positive	N/A	0	2	3
		Negative	2	0	1
		Positive	3	3	0
Positive	N/A	N/A	3	4	6
	Negative	N/A	1	2	4
		Negative	0	1	1
		Positive	2	2	0
	Positive	N/A	0	3	1
		Negative	3	1	0
		Positive	1	2	1
		Total	18	20	112

CRP negative: <1 mg/dl. EONS: Early-onset, LONS: Late-onset, CRP: C-reactive protein

DISCUSSION

The present study evaluated the diagnostic utility of serial CRP levels in suspected neonatal infection/sepsis in 400 neonates and found a strong correlation between the diagnoses of proven or probable sepsis and elevated CRP levels (≥1.0 mg/dl), for both early- and LONS episodes, supporting the diagnostic utility. The initial CRP levels were normal in >one-third of all sepsis episodes suggest the fact that serial measurements have a much diagnostic value than single measurements as reported earlier [10,11].

There have been studies that report that serial normal levels of CRP may be useful for identification of neonates who do not have

Table 4: Performance of individual CRP levels in various neonatal sepsis diagnoses

Types of sepsis	CRP 1	CRP 2	CRP 3
EONS	N=300	N=275	N=237
Proven sepsis			
Sensitivity	32% (27-36)	81% (74-86)	86% (78-91)
Specificity	94% (89-97)	72% (66-79)	62% (54-67)
Positive predictive value	15% (9-21)	21% (14-25)	23% (17-29)
Negative predictive value	98% (96-99)	99% (97-100)	99% (98-100)
Probable sepsis			
Sensitivity	37% (30-42)	87% (79-93)	91% (87-95)
Specificity	96% (91-98)	84% (78– 90)	79% (70-88)
Positive predictive value	30% (24-36)	34% (29-40)	36% (31-43)
Negative predictive value	95% (92-97)	99% (96-100)	99% (97-100)
LONS	N=150	N=132	N=108
Proven sepsis			
Sensitivity	60% (54-67)	83% (77-89)	93% (85-95)
Specificity	71% (64-82)	74% (71-81)	71% (89-79)
Positive predictive value	41% (37-46)	43% (37-49)	45% (41-53)
Negative predictive value	87% (83-93)	94% (89-97)	98% (97-100)
Probable sepsis			
Sensitivity	65% (59-69)	84% (78-93)	94% (88-97)
Specificity	73% (64-79)	78% (71-83)	75% (70-80)
Positive predictive value	50% (44-55)	55% (47-60)	52% (47-59)
Negative predictive value	85% (80-91)	94% (87-96)	97% (95-99)

Values in parentheses indicate 95% confidence interval. EONS: Early-onset, LONS: Late-onset, CRP: C-reactive protein

infection/sepsis [10-15]. In the largest series published to date on this subject emphasizes the fact that obtaining CRP levels initially and then at 12 hr intervals increases the sensitivity and NPV either in detecting or ruling out infection/sepsis [14]. Previous studies have also reported that three normal serial CRP levels can safely lead to discontinuation of antibiotics without causing an early recurrence of infection/sepsis [10,16].

The utility of any diagnostic test should always be referenced in the background of a gold standard test. For the diagnosis of sepsis, culture of an organism from the body fluid is the gold standard. While interpreting the culture, it should be kept in mind that there may be contaminant, particularly in cases of blood and urine culture and they may pose both false-positive or false-negative risk while interpretation leading to decrease or increase in the validity of the test under evaluation. The same principle also applies in the case of CRP level measurement [10,14]. To minimize this, in the present study, stringent methods were adopted to collect the samples from aforesaid body fluids. We could not find any contaminant in any of our culture report. However, it is also not always possible to get culture positive in all the cases of sepsis because of various factors that is not under control (e.g., small sample of the body fluid, either low-grade or intermittent bacteremia, and intrapartum antibiotics leading to suppressed growth of the organism). Keeping this in mind, a separate sepsis group was created as "probable sepsis." This later group is probably the most valuable group to evaluate the specificity and NPV of either single CRP or serial CRP levels.

The prevalence of probable sepsis in EONS group was around 2.5 times more than that in the LONS group, and the prevalence of proven sepsis was more in the LONS group. This is in accordance with previously published studies [10,17-19]. This could be because of some factors leading to early evaluation of neonates (prolonged rupture of membrane, foul-smelling liquor, vaginal examination >3 in labor, intrapartum fever, and urinary tract infection in the last trimester) and selecting neonates for LONS evaluations only if they had signs suggestive of infection/sepsis.

Few differences in the performance of CRP between EONS and LONS groups were noted groups. The sensitivity of early CRP levels for EONS was low (both proven and probable sepsis) and higher for LONS (both proven and probable sepsis). The PPV of early CRP levels similarly also increased for LONS than EONS groups. This could be because for a higher chance of infection, longer duration of infection, and development of clinical signs at the time of assessment in the LONS group [10].

The present study findings are in accordance with the findings of previously published studies which show the poor sensitivity of single/initial CRP levels on the clinical presentation of a neonate with possible infection/sepsis [10,11,14]. Considering an isolated normal CRP value would rarely lead to discontinuation of antibiotics in a suspected case of neonatal sepsis; however, serial normal measurements or normal CRP 3 level can safely lead to discontinuation of antibiotics in almost all the cases.

CONCLUSION

Serial CRP measurements are useful in the diagnosis of neonatal infection/sepsis. The predictive value of initial CRP level being normal at initial evaluation cannot justify discontinuing antibiotic therapy. CRP 3 level may virtually rule out or rule in the diagnosis of neonatal sepsis.

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