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Original Article

Paediatrics Section

Role of Procalcitonin and C-Reactive Protein in the Early Diagnosis of Neonatal Sepsis

SUMEDHA YADAV[1], MUMTAZ SHARIF[2], AMIT SAXENA[3], SHITAL KOLHE[4], DIVYANI DHOLE[5]

ABSTRACT

Introduction: Neonatal Sepsis (NS) is responsible for 30-50% of total neonatal deaths in developing countries. Blood culture is gold standard diagnostic test but has a low yield and is the time consuming. C-Reactive Protein (CRP) and Procalcitonin (PCT) are commonly used for diagnosis of sepsis.

Aim: To study the role of CRP and PCT in the screening of NS and to compare PCT and CRP in relation to sensitivity, specificity and accuracy.

Materials and Methods: A cross-sectional study was conducted in a tertiary care Neonatal Intensive Care Unit (NICU) from February 2018 and November 2019. Neonates with signs and symptoms of sepsis or born to mothers with risk factors for sepsis were included. Those who received antibiotic before admission or had co-morbidities such as meconium aspiration, birth asphyxia, etc., were excluded. Investigations for sepsis including Complete Blood Count (CBC), CRP, PCT and Blood Culture were done. They were classified into three groups as group 1 clinical sepsis, group 2 suspected sepsis and group 3 confirmed sepsis. The statistical test used was Analysis of Variance (ANOVA) test and tests for sensitivity, specificity, positive predictive value, negative predictive value were also used.

Results: The sensitivity, specificity, PPV, and NPV of CRP and PCT versus culture report were evaluated. The sensitivity was 61.90% and 90.47%, specificity was 72.45% and 82.75%, PPV was 61.90% and 79.16% and NPV was 72.45% and 92.30% for CRP and PCT, respectively. The accuracy of the test was 68.00% and 86.00% for CRP and PCT, respectively.

Conclusion: PCT is a better septic marker than CRP in relation to sensitivity, specificity and accuracy and correlates positively with blood culture.

Keywords: Blood culture, Infection, Inflammatory markers, Newborn, Septic screen

INTRODUCTION

Neonatal Sepsis is a common cause of mortality and morbidity throughout the world. In the developing countries alone, it is responsible for about 30-50% of the total neonatal deaths [1,2]. NS is a clinical syndrome which is characterised by signs and symptoms of infection with or without accompanying bacteremia within the first 28 days of life [3]. Signs of sepsis are generally non-specific and subtle like decreased activity, refusal to feed, temperature instability, etc., and do not distinguish among aetiological organisms, whether bacterial and viral. In conjunction to clinical signs and symptoms, the investigations which can be used for diagnosis of NS includes blood culture, total leucocyte count and differential count; an immature-to-total neutrophil ratio, neutropenia; thrombocytopenia; acute phase reactants like levels of CRP, PCT, haptoglobulin, fibrinogen, and cytokines [4,5].

Early diagnosis of sepsis is required for timely initiation of treatment and reducing the mortality. Blood culture, though the gold standard test, is time-consuming. Unnecessary antibiotic administration may cause problems of antibiotic resistance and its related complications. Hence, other markers of sepsis with the optimum sensitivity, reliability, and positive predictability, are required for early diagnosis and management of NS. Among these markers, CRP and PCT are most commonly used for diagnosis and follow-up. CRP level begins to rise after six hours of infection and peaks at 24 to 48 hours and then, decline as the inflammation decreases and PCT which is also an acute phase reactant rises as early as four hours after infection or exposure to bacterial toxins, peaks at 6-8 hours and correlates to the severity of illness. But CRP though cost-effective can also get elevated in non-infectious condition such as meconium aspiration syndrome/birth asphyxia and they can affect the specificity of the test. PCT values are not affected by such non-clinical conditions but can get affected by viral infection [6].

There are various studies in western literature but only few studies are available in Indian literature that compares CRP

and PCT with exclusion of confounding factors [7,8]. With this background, this study was conducted with an objective to assess the role of CRP and PCT in the screening of NS and to compare PCT and CRP in relation to sensitivity, specificity and accuracy after exclusion of confounding factors.

MATERIALS AND METHODS

This cross-sectional study was conducted in the neonatology unit in the tertiary teaching institute in DY Patil Hospital, Navi Mumbai, Maharashtra, India between February 2018 and November 2019. Ethics committee approval was taken prior to commencing the study (Ref No. PDDYPMC/Ethics/PG Dissert/2018). Informed consent of the parents was taken before recruiting into the study.

Inclusion criteria

- Newborn infants <28 days of life with signs and symptoms of NS (breathing problems, respiratory distress, apnea, tachycardia, bradycardia, hypotension, delayed Capillary Refilling Time (CRT), feeding intolerance, abdominal distension, Gastrointestinal (GI) bleed, diarrhoea, vomiting, jaundice, reduced movements, reduced sucking, seizures, hypothermia, hyperthermia, hypoglycaemia, hyperglycaemia, petechial rash, purpura, mottling, sclerema).
- Any neonate born to with maternal risk factors for sepsis, e.g., Prolonged Preterm Rupture of Membrane (PROM) >18 hours, maternal intrapartum pyrexia, urinary tract infection, prolonged labour, or chorioamnionitis were included.

Exclusion criteria

- Neonates who were on antibiotics prior to admission and those who developed the signs of sepsis within 72 hours of discontinuation of the antibiotics.
- Neonates with other co-morbidities such as meconium aspiration, birth asphyxia, hyaline membrane disease, laboratory findings which were suggestive of or confirmed for the inborn errors of metabolism and congenital anomalies and infants of diabetic mothers were excluded.

Newborns meeting inclusion criteria were then divided into three groups based on the following criteria. Criteria Employed for defining sepsis group [7-9].

Group 1 (Clinical sepsis)

- Clinical signs suggestive of sepsis are present.
- Atleast one parameter for sepsis screen positive. Total Leukocyte Count (TLC) >24,000/cubic millimetre (cumm) or less than 5,000/cumm, Absolute Neutrophil Count (ANC) <1800/mm, Platelets <1,50,000 mcL, CRP more than 6 mg/L, PCT level of ≥0.5 ng/mL).
- Negative Blood culture.

Group 2 (Suspected sepsis)

- Clinical signs suggestive of sepsis are present.
- At least two parameters for sepsis screen positive.
- Blood culture negative.

Group 3 (Confirmed sepsis)

- Clinical signs suggestive of sepsis are present.
- At least two parameters for sepsis screen positive.
- Blood culture positive.

The blood specimen was obtained from each neonate before the commencement of antibiotics. The septic workup included haematological parameters like TLC, the ANC, platelet count, blood culture and antibiotic sensitivity, PCT and CRP estimation. Serum CRP level was measured by using the A-15 CRP Kit. The quantitative measurement of CRP from the serum was done by a nephelometric method in the laboratory according to the manufacturer's instructions. The serum PCT level was measured by using the Chemiluminescence Immunoassay (CLIA) method on Maglumi1000. A PCT level of ≥0.5 ng/mL and CRP more than 6 mg/L is considered as pathological.

STATISTICAL ANALYSIS

The statistical analysis was done using ANOVA test and diagnostic tests (for sensitivity, specificity, PPV, NPV) and the software used was Statistical Package for Social Sciences (SPSS) Version 24.0 (IBM Corporation, Chicago, USA).

RESULTS

Out of 50 neonates, Group 1 had 21 cases (42%), Group 2 had 8 cases (16%), and group 3 had 21cases (42%). The mean age was 6.76 days, gestational age was 36.22 weeks, and birth weight was 1995.24 grams. There was a slight male preponderance (58%) in the study population. Overall, prematurity was seen in 66% (33 cases). [Table/Fig-1] shows demographic distribution. The mean gestational age and mean birth weight was significantly low in group 3.

[Table/Fig-2] shows comparison of maternal risk factor in various groups and PROM was seen more in group 3 but statistically not significant. [Table/Fig-3] shows distribution of signs and symptoms of NS. Most common symptom was feeding intolerance. [Table/Fig-4] shows distribution of hemoglobin, leucocytes and platelets. Anemia was seen more in group 3 but statistically not significant. Thrombocytopenia was more in group 3 and was statistically significant.

The [Table/Fig-5] shows distribution of organism isolated from blood culture. Most common organisms isolated was *Klebsiella*

Variables	Group 1 (n=21)	Group 2 (n=8)	Group 3 (n=21)	p-value (ANOVA test)
Mean birth weight (grams)	2001±449	2056±281	1826±450	0.080
Mean Gestational age (weeks)	36.85±1.66	37.00±1.41	35.36±2.19	0.023
Preterm (n)	12	4	17	0.289
Term (n)	9	4	4	0.108
Gender (M,F)	14,7	5,3	10,11	0.439

[Table/Fig-1]: Demographic details of the study.

Risk factors	Group 1 (n) (%)	Group 2 (n) (%)	Group 3 (n) (%)	p-value (ANOVA test)
PROM	7 (14)	1 (2)	11 (22)	0.162
Maternal UTI	0	0	4 (8)	-
Leaking PV	1 (2)	0	2 (4)	-
Chorioamnionitis	0	0	1 (2)	-

[Table/Fig-2]: Maternal risk factors.

Neonatal signs and symptoms	n	%
Hypothermia	12	24.0
Fever	10	20.0
Lethargy	7	14.0
Irritability	2	4.0
Bulging anterior fontanelle	2	4.0
Seizures	13	26.0
Increased respiratory rate	17	34.0
Respiratory distress	5	10.0
Tachycardia	17	34.0
Feed intolerance	34	68.0
Diarrhea	6	12.0
Abdominal distension	21	42.0
Ryle's tube bleed	18	36.0
Pustules	2	4.0
Abscess	1	2.0
Mottling	14	28.0
Sclerema	6	12.0

[Table/Fig-3]: Neonatal signs and symptoms.

pneumoniae. [Table/Fig-6] shows distribution of mean CRP and PCT in various groups. [Table/Fig-7] shows comparison of PCT and CRP in terms of sensitivity, specificity, PPV, NPV and accuracy. There were eight deaths in total, all of which were in Group 3. It was also seen that the mean PCT level was high in these cases [Table/Fig-8].

Groups	Hb (g/dL) (Mean±SD)	TLC (Mean±SD)	Platelet (Mean±SD)
Group 1	15.98±2.46	13.21±6.72	207.38±121.33
Group 2	16.21±2.09	11.63±5.64	150.37±55.50
Group 3	12.56±2.81	8.34±7.68	144.09±159.59
p-value (ANOVA test)	0.213	0.12	0.001

[Table/Fig-4]: Group-wise distribution of haemoglobin, total leucocyte count and platelet.

Blood culture report	n	%
E.coli	3	6.0
Enterococcus	3	6.0
Klebsiella pneumoniae	11	22.0
Staphylococcus aureus	3	6.0
Streptococcus	1	2.0
No growth	29	58.0
Total	50	100

[Table/Fig-5]: Blood culture report of the study population.

Variables	Group 1 (n)	Group 2 (n)	Group 3 (n)	p-value (ANOVA test)
CRP				
Positive	3	5	13	0.003
Negative	18	3	8	
Mean (mg/L)±SD	9.74±14.83	17.26±17.23	30.73±25.40	0.015
PCT				
Positive	2	3	19	<0.001
Negative	19	5	2	
Mean (ng/mL)±SD	0.57±0.92	0.29±0.17	12.38±12.57	<0.001

[Table/Fig-6]: Distribution of CRP and PCT levels.
p-value<0.001 is considered to be statistically significant

Diagnostic tests	CRP (%)	95% CI (CRP)	PCT (%)	95% CI (PCT)
Sensitivity	61.90%	38.69% to 81.04%	90.47%	68.17% to 99.33%
Specificity	72.45%	52.51% to 86.55%	82.75%	63.51% to 93.47%
PPV	61.90%	38.69% to 81.04%	79.16%	57.29% to 92.06%
NPV	72.45%	52.51% to 86.55%	92.30%	73.40% to 98.65%
Accuracy	68.00%	73.26% to 94.18%	86.00%	86.29% to 99.51%

[Table/Fig-7]: Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of CRP and PCT v/s culture.

	Group 1	Group 2	Group 3
Number	0	0	8
Mean CRP	-	-	49.46
Mean PCT	-	-	17.8

[Table/Fig-8]: Distribution of mortality.

DISCUSSION

In this study, the two most commonly used markers of sepsis, i.e., CRP and PCT, which are used alone or in combination were compared. Clinical profile and risk factor for NS were also analysed.

Fifty neonates were divided on the basis of clinical features and laboratory parameters into three groups. It was observed that most of them (90%) were Low Birth Weight (LBW) babies, and 66% were preterms suggesting prematurity and LBW as important risk factors for NS. Similar results are seen by Bala Y et al., where 84.6% were LBW and 79.3% were preterms. A study by Ghosh P et al., reported 75% LBW [10,11]. Premature newborns have immature skin and mucous membrane and underdeveloped defense mechanism against infection; hence they have a greater risk of developing sepsis. There was slight male preponderance though statistically insignificant. Similar study by Park IH et al., highlights male preponderance [12].

Amongst the maternal risk factors, majority (38%) had PROM and the maximum of these babies were culture positive though statistically not significant. Maternal Urinary Tract Infection (UTI) was seen in 8% and one case of chorioamnionitis. Studies done by Jafar H et al., and by Kumar R et al., identified PROM >24 hours, prolonged labour and maternal fever as the predominant risk factor [13,14].

Amongst the clinical features, 68% had feeding intolerance almost half had features suggestive of shock. Other clinical features like tachycardia, hyperthermia, seizures, lethargy, irritability, sclerema were also noted. Various studies have depicted the similar clinical spectrum [7,8,15] which are the established clinical features of sepsis. Anaemia was more in the confirmed sepsis group though statistically insignificant. Thrombocytopenia was significantly more in the confirmed sepsis group, especially with *Klebsiella* sepsis. Most common bacterial organisms isolated were *Klebsiella* followed by *E.coli*, *Staphylococcus* and *Enterobacter*. Culture positivity was found to be inversely related to birth weight and gestational age.

The mean CRP of subjects with confirmed sepsis was higher compared to clinical and suspected sepsis group and the difference was statistically significant. The sensitivity of CRP for proven sepsis was 61.90%, its specificity was 72.45%, PPV was 61.90%, and its NPV was 72.45% sepsis. The accuracy of this test was 68%.

According to various studies, the sensitivity of CRP ranged from as low as 28% to as high as 100% while specificity ranged from 69.7% to 100% [9,16]. These wide ranges can be due to different sample sizes, cut-off values, analytical tests and methodologies, characteristics of cases, the difference in exclusion and inclusion criteria, and different sampling timing. In a study by Thyai S and Ramesh TV, the sensitivity, specificity, PPV, NPV and CRP accuracy were 66.66%, 76.19%, 54.54%, 84.21% and 62.3%, respectively which were quite similar to present study [7]. While Jaffar H et al., reported the sensitivity, specificity, PPV, NPV and accuracy as 79.7%, 50.7%, 41.6%, 85%, respectively [13]. These two studies had similar inclusion and exclusion criteria as this study. CRP level begins to rise after six hours of infection and peaks at 24 to 48 hours and then decline as the inflammation decreases. However, it can rise in a non-infectious condition also and does not differentiate between bacterial and viral infection [17].

In this study, PCT was positive in almost half the cases. There was a difference in PCT levels between confirmed sepsis cases and suspected and clinical cases which were statistically significant. The sensitivity, specificity, PPV, NPV and accuracy were 90.47%, 82.75%, 79.16, 92.30 and 86.00%, respectively. In various studies, PCT sensitivity in the early diagnosis of NS was 80-100% while the specificity was 70-100% [7,18]. These studies stated that PCT levels were also high in non infected neonates with conditions like perinatal asphyxia, intracranial haemorrhage and resuscitation measures which affected the specificity of PCT. However, the protocol of this study implemented exclusive criteria that omit the influence of non-infectious confounders. PCT which is also an acute phase reactant rises as early as four hours after infection or exposure to bacterial toxins, peaks at 6-8 hours and the levels correlate with the level of severity of infection [19].

In this study, it was also noted that high mortality was seen among confirmed sepsis group. All babies who died also had high PCT level (more than 2ng/mL). Similar studies by Brunkhorst FM et al., and Jensen JU et al., have demonstrated higher PCT values related to septic shock and death [20,21]. The present study confirmed the findings of other investigators that PCT was more sensitive than CRP in detecting NS, as the PCT level rose earlier than the CRP level during sepsis.

Limitation(s)

The limitation of the study is that sampling time did not consider timing of onset of sepsis and PCT and CRP was not repeated due to financial constrain.

CONCLUSION(S)

Based on the present study results, PCT is a better septic marker than CRP in relation to sensitivity, specificity and accuracy. It

correlates well with the gold standard test, i.e., blood culture. CRP can be used as a good marker for diagnosis of sepsis, especially in poor resources settings as it is easily measurable and more affordable.

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PARTICULARS OF CONTRIBUTORS:

1. Resident, Department of Paediatrics, D Y Patil University, Navi Mumbai, Maharashtra, India.
2. Professor, Department of Paediatrics, D Y Patil University, Navi Mumbai, Maharashtra, India.
3. Associate Professor, Department of Paediatrics, D Y Patil University, Navi Mumbai, Maharashtra, India.
4. Associate Professor, Department of Paediatrics, D Y Patil University, Navi Mumbai, Maharashtra, India.
5. Resident, Department of Paediatrics, D Y Patil University, Navi Mumbai, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shital Kolhe,
601, Mangal Prabha, Sec 9, Nerul, Navi Mumbai-400706, Maharashtra, India.
E-mail: kolhe.nelson@gmail.com

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