



Role of serum inflammatory biomarkers in diagnosis and therapeutic follow-up of neonatal sepsis: a longitudinal study from eastern India

Bushra Aslam, Haimanti Bhattacharya, Soumyabrata Bagchi, Kaustav Nayek, Debkumar Ray

ABSTRACT

Objectives: The study aimed to compare levels of serum inflammatory biomarkers (CRP, PCT and IL-6) on Day 0, 1 and 7 of intravenous antibiotic administration. It also measured their sensitivity, specificity, and predictive values in relation to blood culture in clinically suspected patients of neonatal sepsis.

Methods: An institution based, descriptive study with longitudinal study design was conducted over a period of 12 months at a tertiary care institute in eastern India. Neonates presenting with clinically suspected neonatal sepsis, fulfilling the inclusion criteria were included after informed written consent from parents. The study population was divided into 4 categories according to sepsis score and median levels of serum biomarkers were compared across the groups on day 0, 1 and 7. Diagnostic accuracy: The data was tabulated in Microsoft excel and analysed with SPSS V.24 software. Sensitivity, specificity, positive predictive value, negative predictive value, and Receiver operating characteristic (ROC) curve analysis were done. A p-value of <0.05 was considered statistically significant for all tests performed. Therapeutic follow-up: The data was tabulated in Microsoft excel spreadsheets and analysed with Jamovi 2.7.3. The normality of data was tested by Shapiro-Wilk test. As the data was not normally distributed, Friedman Rank by Analysis of variance (ANOVA) was applied for statistical analysis of biomarkers (on Day 0, Day 1 and Day 7). Chi-square/ Fischer exact test was done for statistical analysis of categorical variables. A p value of <0.05 was considered statistically significant.

Results: IL-6 was found to have higher sensitivity and specificity than PCT and CRP. ROC curve analysis showed statistically significant association (p value <0.001). The median score of each biomarker was decreased with administration of antibiotics. Friedman Rank by ANOVA test showed significant statistical association (p value

<0.05) of biomarkers (IL-6, PCT, CRP) at Day 0, Day 1 and Day 7 with antibiotic administration. Significantly higher median values of biomarkers (p value <0.001) on day 7 were recorded in patients requiring any intervention- antibiotic upgradation, inotropes, mechanical ventilation, NICU transfer.

Conclusion: Procalcitonin and Interleukin-6 help in early diagnosis of neonatal sepsis and facilitate timely initiation of antibiotics. C-reactive protein should be used in conjunction with PCT/IL-6 in cases with clinical symptoms of sepsis but negative CRP to prove sepsis. IL-6 and PCT levels help in monitoring response to therapy, thus they can be used to avoid prolonged use of unnecessary antibiotics and reduce hospital stay.

Key-words: Biomarkers, C-reactive protein, Interleukin-6, Neonatal Sepsis, Procalcitonin

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INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without bacteraemia in the first 28 days of life. It is the third most common cause for Neonatal Mortality, accounting for 2,25,000 deaths globally every year.¹ It can be classified into two categories depending upon duration of symptom onset²- Early onset neonatal sepsis (EONS) occurs within 72 hours of birth while Late onset neonatal sepsis (LONS) presents beyond 72 hours of life. Despite the huge global burden of Neonatal Sepsis, early accurate diagnosis remains a difficult challenge due to lack of specific clinical features, low incidence of culture proven sepsis (15.8 per 1000 live births in South Asia)³, non-specific rise of conventional markers and prolonged time delay of blood Culture.⁴ Thus, reliable diagnostic biomarkers are needed to promptly diagnose neonatal sepsis, provide specific antimicrobials, and guide therapy during recovery. Although cytokines and acute phase proteins have been evaluated as early inflammatory markers, very few studies comparing their diagnostic properties have been conducted in the developing countries where neonatal sepsis is a large, looming concern. Data regarding the same is lacking in the Indian scenario and no longitudinal studies have been done in the Eastern part of India comparing the diagnostic and prognostic values and measures of validity

for different serum inflammatory biomarkers (Interleukin-6, C-Reactive Protein, Procalcitonin) in relation to blood culture. Given the limited region-specific evidence of role of above-mentioned biomarkers in diagnosis and therapeutic follow-up of sepsis, this study was designed to compare their levels before and after antibiotic administration and establish their role in early diagnosis of neonatal sepsis, early initiation and discontinuation of intravenous antibiotic therapy thus decreasing adverse impact on patient's health, hospital stay and costs.

METHODS

Study Design and Setting

This descriptive study with longitudinal study design was conducted over 12 months (January 2023– December 2023) at the Sick Newborn Care Unit (SNCU) and Newborn Intensive Care Unit (NICU) of a tertiary care teaching hospital in Eastern India. Admitted neonates satisfying any of the three criteria (maternal risk factors for sepsis, three or more clinical signs of sepsis, positive peripheral blood culture) [Table 1] were eligible for inclusion. Exclusion criteria were as follows: Neonates with congenital malformations, history of birth asphyxia, prior antibiotic administration, surgical intervention and infants of diabetic mothers. A total of fifty neonates were enrolled after obtaining written informed consent from parents or legal guardians.



Table 1: Inclusion Criteria employed for selecting study population-

Maternal Risk Factors	Prolonged Labour PROM Maternal Intrapartum fever Urinary tract infection Chorioamnionitis AND/OR
Presence of $\geq$ three categories of clinical signs	Skin colour (pallor/ jaundice/ cyanosis) Respiratory function (apnea/ tachypnea/ grunting/ nasal flaring/ retractions/ need for oxygen or ventilation) Cardiovascular function (Bradycardia/ Tachycardia/ Poor peripheral perfusion/ Hypotension) Neurological Findings (Hypotonia/ Irritability/ Lethargy/ Convulsions) Gastrointestinal Function (Abdominal distension/ vomiting/ bloody or green residuals) AND/ OR
Positive Peripheral Blood Culture	

Sample size calculation

This was an institution based descriptive study with longitudinal design. Thus, the following formula $n=(z1-\alpha/2/\epsilon)^2$ was used to derive the sample size of 50 participants.⁵

Data collection and Interpretation

Patients were clinically examined after thorough history taking. Tests were done accordingly to establish the presence of sepsis screen and blood culture positive cases. Measurements of the serum inflammatory biomarkers were done at repeated intervals (day 0, 1 and 7) prior to and after intravenous antibiotic administration. The study population was divided into 4 categories according to sepsis score⁶ [Table 2] and median levels of serum biomarkers were compared across the groups on day 0,1 and 7. All information were recorded in the case record forms and after completion of data collection, statistical analysis was done to arrive at a conclusion.

Table 2: Criteria employed for defining the sepsis score-

GROUP 1: HIGHLY PROBABLE SEPSIS	a) At least 3 sepsis related clinical signs b) CRP > 6 mg/dl c) At least 2 other altered blood parameters* in addition to CRP d) Blood Culture: Positive or Negative
GROUP 2: PROBABLE SEPSIS	a) Less than 3 sepsis related signs b) CRP > 6 mg/dl c) At least 2 other altered blood parameters* in addition to CRP d) Blood Culture: Negative
GROUP 3: POSSIBLE SEPSIS	a) At least 3 sepsis related signs b) CRP < 6 mg/dl c) Less than 2 other altered blood parameters* in addition to CRP d) Blood Culture: Negative
GROUP 4: NO SEPSIS	a) No sepsis related signs b) CRP < 6 mg/dl c) No altered blood parameters* d) Blood Culture: Negative

**Blood parameters other than CRP: -White blood cell count (< 5 x 10⁹/L or > 21 x 10⁹/L) -Absolute neutrophil count (< 7.8 x 10⁹/L in first 60 hours, < 1.75 x 10⁹/L after 60 hours of life) -platelet count (< 150 X 10⁹/L)*

(a) Sample Collection: Blood samples were collected under aseptic measures from each participant. 1 ml was inoculated immediately into the blood culture bottle; one blood culture bottle was routinely drawn from each patient. 2 ml was collected in clot vial to separate serum, stored immediately at -20°C for further CRP, PCT, and IL-6 measurement.

(b) CRP Measurement. CRP analysis was done using immunoturbidimetry (CRP-Turbilatex) method. It was analysed with the help of Thermo Konelab™ Prime 60i system [Figure 1] which has analytical sensitivity of 1 to 2 mg/L, a diagnostic sensitivity of 95.6% and diagnostic specificity of 96.2% according to the manufacturer's guidelines.



Figure 1: Figure showing the Thermo Konelab™ Prime 60i system which was used to measure CRP in our study by CRP Turbidometry method.

(c) PCT Measurement: Serum PCT was measured using ADVIA Centaur Brahms PCT 100T Kit and analysed with Siemens ADVIA Centaur® XP System [Figure 2] using CLIA (Chemiluminescent immunoassay). It has a measuring range of

0.02 to 75 ng/ml, a functional sensitivity of < 0.05 ng/ml and an analytical sensitivity of < 0.02 ng/ml. Chosen cutoff thresholds (0.5 ng/ml or 2 ng/ml) yield a diagnostic sensitivity of 65% to 85% and specificity of 70% to 80%.

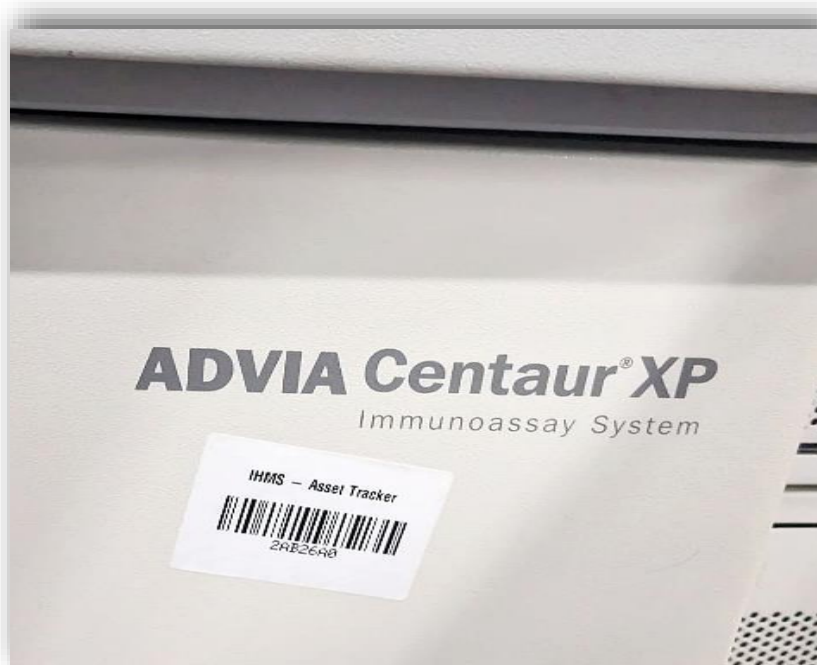


Figure 2: Figure showing the Siemens ADVIA Centaur® XP System which was used to measure IL-6 and PCT by Chemiluminescent Immunoassay (CLIA) method

(d) IL-6 Measurement: Serum IL-6 was measured using ADVIA Centaur IL6 assay and analysed using Siemens ADVIA Centaur® XP System using CLIA. According to manufacturer guidelines, it has analytical sensitivity of 2.7 pg/ml, diagnostic sensitivity of roughly 85.7%, specificity of 64.4%, PPV of ~45.5% and NPV of ~92.9%.

Statistical Analysis

(a) Diagnostic accuracy: The data was tabulated in Microsoft excel and analysed with SPSS V.24 software. The continuous variables were presented with mean and standard deviation. The categorical variables were presented with frequency and percentage. Sensitivity, specificity, positive predictive value (PPV), negative

predictive value (NPV), and ROC curve analysis were done. A p-value of <0.05 was considered statistically significant for all tests performed.

(b) Therapeutic follow-up: The data was tabulated in Microsoft excel spreadsheets and analysed with Jamovi 2.7.3. The normality of data was tested by Shapiro-Wilk test. As the data was not normally distributed, Friedman Rank by ANOVA was applied for statistical analysis of biomarkers (at baseline, on Day 1 and Day 7). Chi-square/ Fischer exact test was done for statistical analysis of categorical variables. A p value of <0.05 was considered statistically significant.

Table 3: Distribution of study population according to clinico-social profile characteristics (n=50)-

Variable	Category	n (%)
Sex	Male	34 (68%)
	Female	16 (32%)
Weight	>2500g	15 (30%)
	<2500g	35 (70%)
Age of presentation	≤3 days	32 (64%)
	>3 days	18 (36%)
Gestational age	Term (≥37 weeks)	12 (24%)
	Preterm (< 37 weeks)	38 (76%)
Mode of Delivery	Normal	23 (46%)
	LUCS	27 (54%)
Religion	Hindu	26 (52%)
	Muslim	22 (44%)
	Others	02 (4%)
Residence	Rural	32 (64%)
	Urban	18 (36%)
Socioeconomic status	Upper middle class	05 (10%)

	Lower middle class	14 (28%)
	Upper lower class	23 (46%)
	Lower class	08 (16%)
Sepsis Screen	Positive	35 (70%)
	Negative	15 (30%)
Blood Culture	Positive	26 (52%)
	Negative	24 (48%)

RESULTS

A total of 50 neonates with clinically suspected neonatal sepsis were included in the study. The mean age of participants was 5.12 ± 2.06 days with a male predominance (68% male vs 32% female). Majority of participants (64%) presented before or at 3 days of age with EONS. The study population had a mean weight of 2100.04 ± 611.48 g with 70% of neonates weighing less than 2500 g (52%- Low birth weight, 18%- Very low birth weight). Majority of neonates (54%) were born by Lower Uterine Caesarean Section. Most participants were from rural areas (64%), reflecting the predominantly rural catchment population of the tertiary care centre. In terms of religious distribution, 52% were Hindu and 44% Muslim, with a small proportion of 4% belonging to other religions. Most families belonged to the lower middle socioeconomic class (46%). [Table 3].

Sepsis screen was positive in 35 participants (70%) and blood culture showed positivity in 26 participants (52%). The most common isolated organisms in decreasing order of frequency: *Klebsiella* sp. (38.5%), *Acinetobacter baumannii* (15.4%), *Staphylococcus aureus* (15.4%), *Candida* sp. (15.4%), *Enterobacter* sp. (11.5%), *Staphylococcus hemolyticus* (3.8%). [Figure 3]

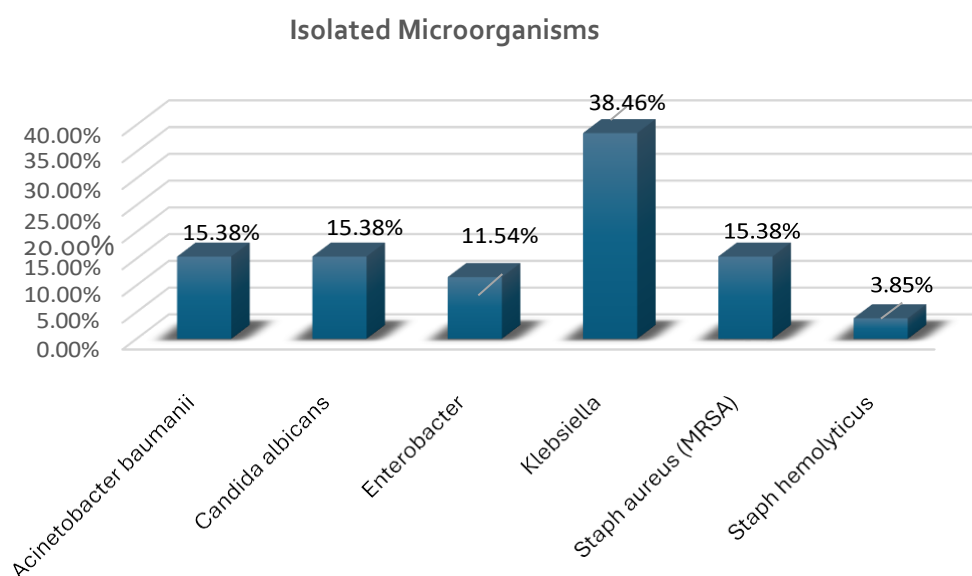


Figure 3: Bar diagram showing isolated microorganisms: *Acinetobacter baumannii* (15.4%), *Candida* sp. (15.4%), *Enterobacter* sp. (11.5%), *Klebsiella* sp. (38.5%), *Staphylococcus aureus* (15.4%), *Staphylococcus hemolyticus* (3.8%)

The study population was divided into 4 subgroups based on sepsis score- Group 1 (Highly probable sepsis), Group 2 (Probable sepsis) and Group 3 (Possible sepsis), Group 4 (No sepsis). 29 neonates (58%) belonged to Group 1, 13 neonates (26%) belonged to Group 2 and 8 neonates (16%) belonged to Group 3. Culture positive neonatal sepsis was seen in 24 neonates (82.7%) from Group 1. Higher median values of CRP, IL-6, and PCT (26mg/dl, 96.75 pg/ml, 13.4 ng/ml) were observed in Group 1 neonates as compared to Group 2 (21.5 mg/dl, 22.1

pg/ml, and 7.3 pg/ml) and Group 3 neonates (6.5 mg/dl, 19.6 pg/ml, and 6.1 ng/ml).

Measures of Diagnostic accuracy of serum inflammatory biomarkers

The sensitivity of IL-6 (91.4%) was more than that of PCT (87%) and CRP (78.3%). The specificity of IL-6 (88.9%) was more than that of PCT (77.8%) and CRP (77.8%). PPV of CRP, IL-6 and PCT was 75%, 87.5% and 76.9% respectively. NPV of CRP, IL-6 and PCT was 80.8%, 92.3% and 87.5% respectively [Table 4].

Table 4: Comparison of diagnostic measures of biomarkers in detecting neonatal sepsis as compared to reference standard-

Biomarker	Sensitivity	Specificity	PPV	NPV	AUC	P value
CRP	78.3%	77.8%	75%	80.8%	.829	<0.001
IL-6	91.4%	88.9%	87.5%	92.3%	.957	<0.001
PCT	87%	77.8%	76.9%	87.5%	.826	<0.001

The p value of Area under Curve (AUC) in a ROC curve analysis of biomarkers showed

statistically significant association (p value <0.001) [Figure 4].

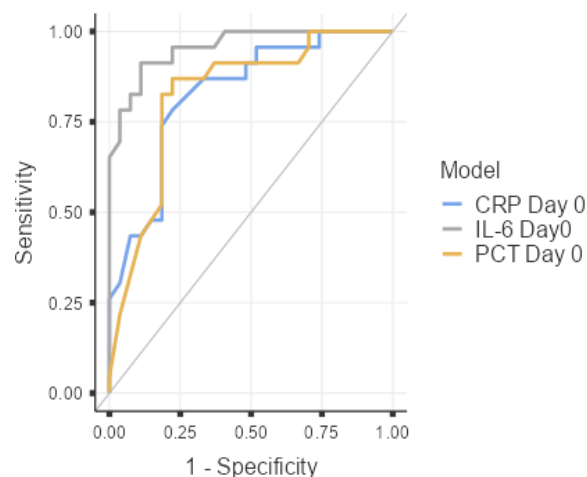


Figure 4: ROC analysis to estimate the sensitivity, specificity and AUC of biomarkers CRP, IL-6 and PCT at baseline (Day 0)

Therapeutic Follow-Up

The median score of each biomarker decreased with administration of antibiotics. The Friedman's ANOVA Rank test revealed that administration of

antibiotics had statistically significant association with CRP (p value <0.001), IL-6 (p value <0.001) and Procalcitonin (p value <0.001). [Table 5]

Table 5: Comparison of biomarkers among neonatal sepsis patients following administration of antibiotics (n=50)-

Biomarkers	Median (Interquartile Range)	Friedman's test (χ^2)	P value
CRP			
Day 0	24 (23.3)	68.2(2)	<0.001
Day 1	15.5 (22.8)		
Day 7	6 (7)		
IL-6			
Day 0	66.6 (118)	98 (2)	<0.001
Day 1	34.7 (72)		
Day 7	11 (17.9)		
PCT			
Day 0	11.6 (12.7)	70(2)	<0.001
Day 1	3.2 (1.9)		
Day 7	1.9 (2.7)		

During our study, certain neonates required interventions apart from routine and supportive care- 24% required inotropes, 36% needed antibiotic upgradation, 24% were transferred to NICU and 16% had to be mechanically ventilated. Significantly higher mean values of CRP, IL-6 and PCT were recorded on day 7 in neonates requiring any of the above interventions in comparison to neonates not needing any intervention and the difference was statistically significant (p< 0.001). [Figure 5]

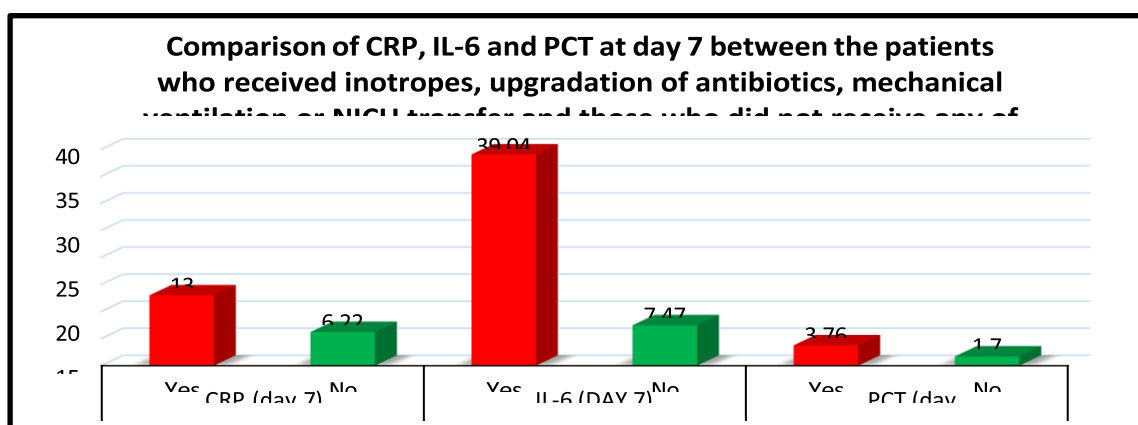


Figure 5: Bar diagram showing significantly higher values of CRP (p value < 0.001), IL-6 (p value < 0.001) and PCT (p value < 0.001) on day 7 in patients who required intervention (inotropes/ upgradation of antibiotics/ mechanical ventilation/ NICU transfer) as compared to those who did not require any intervention-

DISCUSSION

In our study comprising 50 neonates with clinically suspected sepsis, higher blood culture positivity of 61.5% was observed in males as compared to 38.5% in females. Piyush Gupta⁸ et al and Khair et al⁹ found a high male predilection for neonatal sepsis probably due to absence of X-linked immunoregulatory genes in males. This finding can also be attributed to the parental attitude in this region of seeking medical services more for their male babies than female babies.

Higher rates of caesarean section (51%) could be due to admission of many high-risk pregnancy cases in our tertiary care hospital. Tuuli and Odibo¹⁰ and Afsharpaiman et al¹¹ have similarly reported a higher occurrence of sepsis in neonates delivered via caesarean section due to its association with several adverse neonatal events requiring NICU admission.

Out of 50 included newborns, 12 were term and 38 were preterm. Khatua et al.¹², Anand et al.¹³ and Vamshi et al.¹⁴ in their studies documented that preterm babies are more affected by sepsis. Higher rate of culture positive sepsis was observed in low-birth-weight (LBW) newborns (57.1%) as compared to newborns with normal birth weight (40%). Buch et al.¹⁵, Piyush Gupta et al.¹⁶, Agarwal et al.¹⁷, Khatua et al.¹² observed that LBW babies are more prone to develop septicemia. Culture positivity was found to be higher in EONS group (56.3% EONS v/s 33.3% LONS).

Serum inflammatory biomarkers (CRP, IL-6, PCT) were measured at repeated intervals (Day 0, Day 1, and Day 7) prior to and after antimicrobial administration. CRP levels above 10mg/L, PCT levels above 1.1 ng/ml were considered positive. IL-6 had cut-off values of 80 pg/ml on Day of life (DOL) 1, 40pg/ml on DOL 2–7 and 30pg/ml after DOL

7. The cut-off levels were in accordance with various studies which have decided similar exploratory cut-offs.^{18,19}

CRP (Day 0) was found to be positive in 24 neonates (48%). The most used upper limit of 10 mg/L for CRP during neonatal period has

been established in 1987 by Mathers and Pohlandt²⁰ which was later confirmed as an appropriate threshold by Benitz et al.²¹ CRP (day 0) positivity of 77% was lower than CRP (day 1) positivity of 88.5% in blood culture positive cases. This finding aligns with the fact that CRP has lowest sensitivity during early stages of infection²¹ as it significantly rises 10 to 12 hours after onset of infection.²² CRP (day 0) was positive in 4 out of 26 culture negative cases (16.7%). Median values of CRP recorded on Day 0, Day 1 and Day 7 were 24 mg/L, 15.50 mg/L and 6.00 mg/L respectively. Reduction in CRP levels from day 0 to day 7 was statistically significant (p value < 0.001). Ehl et al²³ in their study demonstrated a similar decrease in CRP levels after 16 hours of initiation of successful antibiotic therapy. CRP level returning to normal range may indicate sufficient duration of treatment permitting discontinuation of antimicrobials.²⁴

Among the study population, IL-6 levels (day 0) were found to be positive in 32 neonates (64%). IL-6 (day 0) was elevated in 24 cases of culture positive neonatal sepsis (92.3%) and in 8 cases of culture negative neonatal sepsis (33%). This affirms the fact that IL-6 is a pro-inflammatory cytokine and rises rapidly before PCT or CRP in neonatal sepsis²⁵. Median values of IL-6 recorded on Day 0, Day 1 and Day 7 were 66.55 pg/ml, 34.65 pg/ml, and 11 pg/ml. High values of IL-6 in culture positive sepsis were recorded on DOL-1 in our study as compared to CRP which takes around 48 hours to reach its peak. Thus, Kung et al¹⁸ suggested determination of serum IL-6 on DOL-1 for diagnosis of neonatal sepsis due to its high accuracy. The reduction of IL-6 levels from day 0 to day 7 was statistically significant (p value < 0.001). This agrees with the observation from many studies that IL-6 falls rapidly after initiation of effective antimicrobials facilitating fast monitoring, early escalation, or de-escalation of treatment.^{26,27}

Khoshdel et al,²⁸ Minoo Adib et al,²⁹ Aydemir C et al³⁰ found moderate accuracy of PCT at a cut-

off level of 1.1 ng/ml. PCT (day 0) levels were found positive in 22 cases of culture positive neonatal sepsis (84%) and 5 cases of culture negative sepsis (21%). Median values of PCT recorded on Day 0, day 1 and day 7 were 12.35 ng/ml, 5.30 ng/ml, and 2.20 ng/ml. The reduction of PCT levels from day 0 to day 7 was statistically significant (p value < 0.001). The study of Zahedpasha et al in Iran showed remarkably high PCT levels in proven sepsis and the significant drop in levels following antimicrobial therapy.³¹ Serial PCT measurements may help to differentiate between sepsis and non-infectious conditions reducing unwarranted use of antibiotics.³²

Gram negative bacterial sepsis is more common than gram positive bacterial sepsis in developing countries like India.²² Bhagat R et al. and Chhabra S G et al. found MRSA,

E. coli, and *Klebsiella* as the predominant bacteria isolated from culture positive cases of neonatal sepsis. Pal A C et al. and Gupta K et al. demonstrated *Klebsiella*

pneumoniae and *Escherichia coli* as the most common causative organisms. Our study similarly found *Klebsiella* sp. (38.5%), *Acinetobacter* sp. (15.4%), *Staphylococcus aureus* (15.4%), *Candida albicans* (15.4%) as the most isolated organisms on blood culture. The mean levels (day 7) of CRP, IL-6 and PCT in blood culture positive cases recorded as 11.06 ± 7.28 mg/L, 27.94 ± 29.44 pg/ml, and 3.30 ± 1.55 ng/ml were significantly higher (p value < 0.001) than mean levels of 4.74 ± 1.79 mg/L, 3.98 ± 1.93 pg/ml, and 1.04 ± 0.55 ng/ml recorded in blood culture negative cases respectively. Morad et al, Abdollahi et al, reported that patients with confirmed sepsis had significantly higher levels of CRP, IL-6 and PCT when compared with those with uncertain sepsis.^{33,34}

Studies in older paediatric patients and adults show that higher IL-6 levels at onset of sepsis are associated with a poorer outcome.^{35,36,37} PCT (day 7) mean level in neonates requiring above interventions was recorded as 3.76 ± 1.76 ng/ml in contrast to 1.70 ± 1.06 ng/ml in neonates not needing any intervention and the difference was statistically significant (p <

0.000). A large cohort study conducted by Kurul et al³⁸ concluded that IL-6 and PCT offer valuable information about disease severity and mortality risk in suspected preterm LONS. It also suggested that both IL-6 and PCT are more informative than CRP and could help in increasing awareness for imminent clinical deterioration and timely intervention. IL-6, PCT and CRP levels correlated with clinical measures of sepsis severity, such as need for inotropic support, antibiotic upgradation, and mechanical ventilation. Among these, IL-6 and PCT demonstrated the strongest associations and greater discriminative value compared to CRP.³⁸

In our study, CRP (day 0) was found to have a sensitivity of 78.3%, specificity of 77.8% with PPV of 75%, NPV of 80.8% and AUC of 0.829. The slow rise of CRP during the first 24-48 hours of infection decreases the sensitivity. Increase in CRP in non-infective conditions like Meconium aspiration, PROM is thought to affect the specificity.³⁹ Morad et al.³³ demonstrated sensitivity 89.5%, specificity 66.7%, PPV 92.5%, NPV 60% for a cutoff value of CRP ≥ 10 mg/dl. Other studies declared that CRP sensitivity ranged from 70% to 93%, specificity 41%–98%, PPV 6%–83%, and NPV 97%–99%.^{40,41}

IL-6 (day 0) was documented to have the highest sensitivity of 91.4%, specificity of 88.9% with high PPV (87.5%), high NPV (92.3%) and AUC of 0.957. Tam et al.⁴² reported that IL-6 has a sensitivity of 54–84%, a specificity of 70–86%, PPV of 38–100% and NPV of 59–97% for EONS; a sensitivity of 44–100%, a specificity of 74–93%, a PPV of 40–86% and a NPV of 74–100% for LONS. In our study, no such division was made into EONS and LONS due to physiological changes in postnatal IL-6 values, still our results are in accordance with published values. Ganesan P et al⁴³ similarly concluded that IL-6 is a highly sensitive diagnostic marker with a sensitivity of 100% and specificity of 62.86% for cut-off level of 51.29 pg/ml.

PCT (day 0) was found to have intermediate sensitivity (87%), specificity (78.9%), PPV (76.9%), NPV (87.5%) and AUC of 0.826. Rise of

PCT in non- infected neonates with perinatal asphyxia, intracranial hemorrhage or pneumothorax decreased the specificity.^{44,45} Kurul et al.³⁷ reported a sensitivity of 80.0% and specificity of 73% with an exploratory cut-off of 1.06 ng/ml which are consistent with our study findings. PCT is considered a promising diagnostic marker with sensitivity of 83-100% and specificity of 70-100%.^{46,47,48,49}

In this study, 2 neonates (4%) succumbed to death during course of the disease. Both neonates demonstrated Klebsiella growth on blood culture with intermediate sensitivity to Colistin. They had markedly elevated levels of biomarkers on day 7 despite upgradation of antibiotics and timely intervention. CRP (day 7) was 38mg/L and 14mg/L, IL-6 (Day 7) was 160.6 and 91.2 pg/ml and PCT (day 7) was 9.6 ng/ml and 4.5 ng/ml. Boskabadi et al⁵⁰ in his study demonstrated IL-6 as having greatest value for predicting infection and possible mortality. Kurul et al³⁸ concluded that serum IL-6 and PCT, but not CRP, are associated with 7-day mortality in preterm infants suspected of LONS.

Strengths and Limitations

A major strength of this study was its longitudinal design - evaluation of biomarkers at repeated intervals instead of a single time measurement of their levels. Despite every possible effort, our study has certain lacunae. The study was done on a limited number of participants at a single centre, without a control group. It was carried out in a tertiary care hospital, so hospital bias maybe possible. These factors limit generalisability of our findings to the larger population.

Conclusion and recommendation

PCT and IL-6 help in early diagnosis of Neonatal Sepsis and facilitate timely initiation of antibiotics. CRP should be used in conjunction with PCT or IL-6 in patients with clinical symptoms of Sepsis and CRP (CRP ≤ 10 mg/L) maybe negative in sepsis at this stage. High sensitivity- CRP (hs-CRP) is more sensitive than conventional CRP with levels below 1 mg/L providing increased sensitivity for neonatal infection.

IL-6 is superior to other biomarkers in rapid diagnosis of Late-onset neonatal sepsis (LONS) especially in preterm neonates. Serum IL-6 and PCT can predict 7-day mortality in preterm infants with LONS due to their strong association with measures of sepsis severity, such as need for inotropic support and mechanical ventilation. Gram-positive neonatal sepsis in demonstrates a less pronounced inflammatory response which can be detected by IL-6 and PCT, but not by CRP alone. To differentiate between infection and non-infective conditions, PCT is more reliable than IL-6 or CRP. IL-6 and PCT levels help to monitor response to therapy and reduce hospital stay. CRP is cost-effective while IL-6 and PCT are expensive. Thus, their combined use in routine practice is limited in developing countries. Antibiotic policy should be revised from time to time and rational use of antibiotics should be followed.

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