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Original Research Article

Procalcitonin vs C-reactive protein as diagnostic biomarkers for sepsis in preterm very low birth weight neonates

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ABSTRACT

Background: Few studies have compared use of procalcitonin (PCT) and C-reactive protein (CRP) in diagnosis of sepsis in preterm very low birth weight neonates (VLBW). This study aimed to compare sensitivity and specificity of PCT and CRP in predicting sepsis in preterm VLBW neonates and also to compare their levels in gram positive and negative sepsis, as well as in early and late onset sepsis.
Methods: This prospective comparative study included 50 preterm very low birth weight neonates (gestational age <37 weeks and birth weight <1500 grams) with suspected neonatal sepsis. After enrollment, 1 ml of blood was collected for blood culture in Brain Heart Infusion (BHI) broth medium, and another 1 ml was collected for PCT and CRP quantitative determination with Boditech Ichroma™ kits in Biochemistry lab using fluorescence Immunoassay. Other sepsis screen parameters, including Total Leucocyte Count (TLC), Absolute Neutrophil count (ANC), Immature-to-Total (I:T) Neutrophil ratio were also assessed. Cut-off values for CRP and PCT was taken as 10 mg/l and 2 ng/ml respectively. We considered blood culture as gold standard.
Results: Sensitivity and specificity of PCT was 68.40% and 3.20 % respectively whereas for CRP it was 57.89% and 64.52% respectively. Positive predictive value and negative predictive value of PCT was only 30.2% and 14.30% respectively as compared to 50% and 71.43% with CRP. Median value of PCT and CRP was higher in gram negative sepsis [4.00(1.51-23.25) ng/ml] and [12.70(4.60-49.10) mg/l] respectively as compared to gram positive sepsis [2.25(1.38-3.98) and 5.60(1.70-20.30) ng/ml respectively], but it was not statistically significant. Median of PCT was higher in LONS group as compared to EONS group [15.00(7.3-6.67) vs 4.60(2.01-8.50) ng/ml], but the difference was not statistically significant (p=0.178).
Conclusions: PCT is not a better marker as compared to CRP for diagnosis of sepsis in preterm VLBW neonates.

Keywords: Neonatal Sepsis, C-reactive protein, Blood culture, Procalcitonin

INTRODUCTION

Neonatal sepsis is the non-specific systemic inflammatory response syndrome caused by the production of toxins in blood stream during first 28 days of life.[1] In a cohort study done by Delhi neonatal infection study collaboration, the overall incidence of neonatal sepsis was found to be 14.3% with culture positive sepsis contributing to 6.2% of the population cohort.[2] Sepsis is responsible for 26% of the neonatal deaths worldwide, especially in preterm, very low birth weight (VLBW) neonates.[3] VLBW preterm population is at higher risk of mortality due to their immature immune system, low weight and other risk factors. Diagnosing infection in neonates is challenging because of its initial subtle and nonspecific clinical features, which can be confused with other non-infectious diseases.[4] Blood culture is the gold standard test for definitive diagnosis, but it has a low positivity rate even in the presence of clinical features of sepsis. So, given the uncertainly in

ruling out sepsis in sick neonates with negative blood culture, the current trend is overuse of antibiotics, thus leading to increased antibiotic resistance and health care cost.[5] Therefore, there is a need for a sensitive and specific laboratory test to help in early diagnosis. Certain sepsis markers, such as total leucocyte count (TLC), absolute neutrophil count (ANC), immature to total neutrophil ratio, micro ESR and C-reactive protein (CRP) are used as a sepsis screen for early diagnosis but have low negative predictive value and variable sensitivity and specificity.[6]

CRP, a routine sepsis screen marker, is used for the diagnosing as well as further progression of neonatal sepsis. However, it also gets elevated in non-infectious conditions like respiratory distress syndrome, meconium aspiration syndrome, fetal asphyxia, intraventricular hemorrhage and post-surgical period. Its sensitivity and specificity are 69% and 77%, respectively.[7,8]

Procalcitonin (PCT) was first described in 1993 as a novel sepsis marker, rising significantly from negligible normal levels in patients having systemic inflammatory response to bacterial, fungal and parasitic infections. It rises within 2-3 hours of onset of infection and peaks by 6-12 hours, much earlier than the rise of CRP.[9] Unlike CRP, its levels remain unchanged in viral infections and other non-infectious inflammatory diseases. PCT appears to be more reliable for the early diagnosis of neonatal sepsis and also correlating with severity of infection, particularly bacterial infection.[10] A 2019 meta-analysis showed higher mean sensitivity of PCT as compared to CRP in the EONS, LONS and EONS+LONS groups.[8] Moreover, the mean specificity was similar. But this meta-analysis revealed paucity of literature with reference to preterm, VLBW neonates.[8] Only four studies have been performed so far in this specific population group. The purpose of the present study was to compare the sensitivity and specificity of PCT with that of CRP in predicting sepsis in preterm VLBW neonates, to compare PCT and CRP levels in gram positive and negative sepsis and to compare PCT and CRP levels in early and late onset sepsis.

METHODS

A comparative study was conducted in the Neonatal Intensive Care Unit (NICU), Department of Pediatrics, over a period of one year. Study was approved by institutional ethics committee. All neonates with suspected neonatal sepsis and being gestational age of <37 weeks and birth weight <1500 grams were included in study after taking written informed consent from the parents.

Neonates with gross congenital malformations, or neonates who were already on antibiotics prior to admission were excluded. Detailed antenatal and obstetric history was taken for risk factors associated with early onset sepsis in case a neonate presented within 72 hours of life i.e. spontaneous preterm labor, foul-smelling liquor or meconium-stained liquor, febrile illness in the mother during or within two weeks of delivery, rupture of membranes >24 hours, single unclean or >3 sterile vaginal examinations during labor, prolonged perinatal asphyxia. In neonates presenting after 72 hours of life, the risk factors for community acquired late onset sepsis were enquired. Those already admitted in NICU and suspected of nosocomial sepsis were analyzed for various factors in the NICU viz. mechanical ventilation, central venous access, use of stock solution etc. Neonates with blood culture positive were considered as culture positive sepsis. Others were considered as clinical sepsis, which includes various systemic infections such as septicemia, pneumonia, meningitis, osteomyelitis, arthritis and urinary tract infections.

On the development of first sign/symptom of sepsis and before starting antibiotics, under all aseptic precautions venous blood sample was collected. 1 ml of blood was collected for blood culture in brain heart infusion (BHI) broth medium. It is the gold standard for diagnosing sepsis. 1 ml blood was collected in plain vacutainer for PCT and CRP estimation. It was centrifuged within 2 hours to separate serum. Serum was analyzed immediately. If not, it was stored at -20ºC till analysis was done. Another 2ml blood was collected in EDTA vial and sample was analyzed for TLC, DLC, ANC, platelet count using 3-part cell counter working on the principle of optical impedance. Peripheral blood film examination for Immature/Total neutrophil ratio was done. Micro ESR was performed in heparinized capillary tubes. The neonates were managed as per NICU protocols. Cut-off levels for CRP were taken as 10 mg/L, whereas for PCT it was 2 ng/ml. Blood culture was considered as gold standard investigation.

We divided enrolled neonates into 3 groups, Group I-clinical sepsis + sepsis screen negative + blood culture negative; Group II-Clinical sepsis + sepsis screen positive + blood culture negative; Group III -Clinical sepsis+ blood culture positive. Outcomes were recorded.

CRP and PCT was estimated using fluorescence Immunoassay for quantitative determination with Boditech Ichroma™ kits in Biochemistry lab. The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized antibody on test strip. The more antigen in sample results in formation of more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument to show CRP and PCT concentration in sample.

Sample size

As per previous 18 months unit data, around 128 preterm VLBW neonates were admitted, so considering 30-40%

neonates will get sepsis, we took a convenient sample size of 50.

Statistical analysis

Data were described in terms of range, mean (standard deviation) (SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups was done using ANOVA and Mann-Whitney U test for non-parametric data. For categorical data, Chi square ($\chi 2$) test was performed and fisher exact test was used when the expected frequency is less than 5. Receiver operator characteristics (ROC) curve was obtained, and criterion values were estimated. Sensitivity, specificity, accuracy, positive predictive value, and negative predictive value was calculated. Area under curve (AUC) was also measured. Correlation test was used to see the association between continuous variables. A probability value (p value) less than 0.05 was considered statistically significant.

RESULTS

A total of 50 newborns were enrolled after evaluating inclusion and exclusion criteria. The study flow is attached as Figure 1. Demographic data is shown in Table 1. In the study population, out of 50 neonates, 23 (46.0%) neonates were successfully discharged. The mean birth weight and mean gestational age of the study population was 1240.86 (205.74) grams and 30.94 (2.13) weeks respectively.

The antenatal and postnatal risk factors in the neonates of the study population are shown in Table 2. Out of the 50 total cases, 35 (70.00%) had EONS while 15 (30.00%) had LONS. 18 (36.0%) neonates had a positive sepsis screen. 19 (38.0%) neonates had culture positive sepsis. Among Gram-negative organisms, Burkholderia Cepacia was the most common organism isolated. Figure 2 shows organisms isolated from the blood culture.

Table 3 compares various parameters among 3 groups within study population based on positive/negative sepsis screen and positive/negative blood culture. The median value of CRP in group I, II and III was 5.00(2.20-7.00), 25.95 (10.11-71.45) and 12.00(3.30-21.00) mg/l respectively(p=0.013). The median value of PCT in group I, II and III was 7.30(3.30-11.50), 13.40(6.92-77.50) and 3.91(1.30-14.00) ng/ml respectively (p=0.002). The difference was statistically significant for both CRP and PCT with Group II (Clinical sepsis + sepsis screen positive + blood culture negative) having maximum values. Significant differences were seen only in I/T ratio (p=0.001), CRP (p=0.013) and PCT (p=0.002) among the three groups.

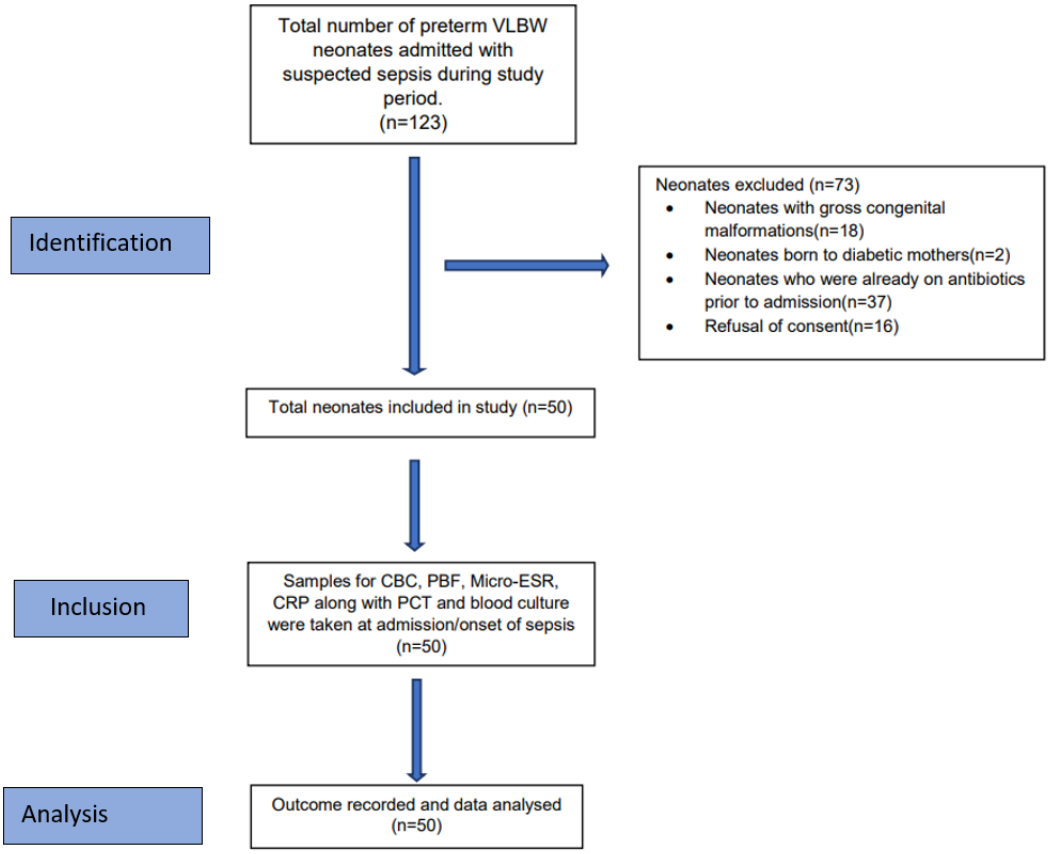


Figure 1: Study flow.

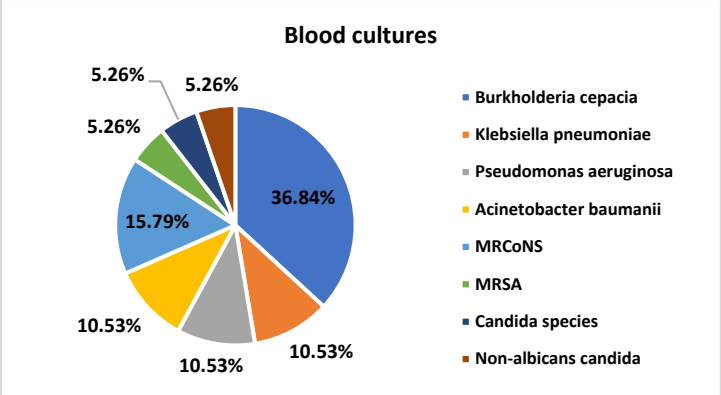


Figure 2: Pie diagram showing organisms isolated from blood culture of sepsis cases.
MRCoNS-Methicillin Resistant Coagulase Negative Staphylococcus; *MRSA-Methicillin Resistant Staphylococcus Aureus*.

Table 1: Various data of the study population (n=50).

Variables		Number	Percentage
Gender	Male	29	58.00
	Female	21	42.00
Birth weight (grams)	1499-1000	43	86.00
	<1000	7	14.00
Gestational age (weeks)	34-<37	12	24.00
	32-33+6	9	18.00
	<32	29	58.00
Mode of delivery	Vaginal delivery	34	68.00
	LSCS	16	32.00
Place of delivery	Inborn	42	84.00
	Outborn	8	16.00
APGAR	≤3	5	11.90
	4-6	8	19.05
	≥7	29	69.05

*LSCS- lower segment caesarean section.

Table 2: Presence of antenatal and postnatal risk factors in the neonates of the study population (n=50).

Variables			Number of neonates	Percentage
Antenatal risk factors	LPV > 24 hours		22	44.00
	Pre-eclampsia/Eclampsia		13	26.00
	BPV/Abruptio/Placenta previa		4	8.00
	PIH		4	8.00
	Gestational diabetes		2	4.00
	> 3 sterile vaginal examinations during labor		2	4.00
	Foul smelling liquor		1	2.00
	Infection/fever>38 F two weeks before		1	2.00
	Single unclean vaginal examination		0	0.00
Postnatal risk factors`	Need for inotropes		34	68.00
	Central Catheters		31	62.00
	Requirement of blood products		37	74.00
	Maximum respiratory support requirement	Oxygen by prongs	3	6.00
		CPAP	6	12.00
		NIV	5	10.00
		Mechanical ventilation	36	72.00

*Abbreviations: LPV-Leaking per vaginum, PIH- Pregnancy Induced Hypertension, BPV- Bleeding per vaginum, CPAP-Continuous Positive Airway Pressure, NIV-Non-Invasive ventilation

Table 3: Comparison of various parameters among 3 different groups within study population based on positive/negative sepsis screen and positive/negative blood culture.

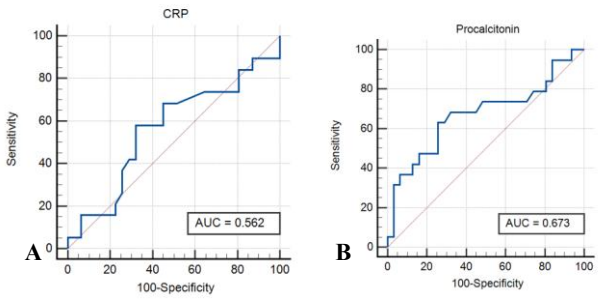
Parameters	Groups			P value
	Clinical sepsis + sepsis screen negative + blood culture negative (Group-I) (n=21)	Clinical sepsis + sepsis screen positive + blood culture negative (Group-II) (n=10)	Clinical sepsis+ blood culture positive (Group-III) (n=19)	
Birth weight a (grams)	1257.19 ± 215.64	1257.90 ± 235.48	1213.84 ± 185.99	0.775*
Gestational age a (weeks)	31.29 ± 2.37	31.10 ± 2.33	30.47 ± 1.74	0.478*
TLC a (cells/µl)	9718.81 ± 4694.10	15427.40 ± 8301.81	12666.53 ± 6108.83	0.050*
ANC a (cells/ µl)	6101.71 ± 3538.32	9812.40 ± 5010.74	8176.32 ± 4131.50	0.056*
I/T Ratio >0.2 (n, %)	1,4.80%	6,60.00%	3,15.80%	0.001**
Micro-ESR[a] (mm in 1st hour)	9.10 ± 2.86	12.20 ± 3.79	10.37 ± 3.44	0.056*
CRP [b] (mg/l)	5.00(2.20-7.00)	25.95(10.11-71.45)	12.00(3.30-21.00)	0.013*
PCT [b] (ng/ml)	7.30(3.30-11.50)	13.40(6.92-77.50)	3.91(1.30-14.00)	0.002*

*Test applied: Anova test; p value-<0.05- Significant, <0.001 – highly significant; **Test applied: Chi-square test; p value-<0.05- Significant, < 0.001- highly significant; a: Mean ± SD; b: Median (IQR); *Abbreviations: ng/ml- nanograms per milliliter, mg/L- milligram per liter, cells/µL- cells/microliter, TLC -Total leucocyte count, ANC-Absolute Neutrophil Count, I/T ratio- immature/Total neutrophil ratio, PCT-Procalcitonin, CRP- C-Reactive protein.

Table 4: Statistical parameters of CRP at cut-off value of 10 mg/l, PCT at 2ng/ml and sepsis screen with blood culture as gold standard.

	Sensitivity	Specificity	Positive likelihood ratio	Negative likelihood ratio	Positive predictive value	Negative predictive value
CRP 10 mg/l (95% CI)	57.89% (33.50- 79.75)	64.52% (45.37- 80.77)	1.63 (0.89 - 3.00)	0.65 (0.36 to 1.18)	50.00% (35.20- 64.80)	71.43% (58.13-81.83)
PCT 2 ng/ml (95%CI)	68.40% (43.45 87.42)	03.20% (0.08 -16.70)	0.71 (0.52 - 0.97)	9.87 (1.27-75.18)	30.20% (24.08-37.19)	14.30% (2.12 - 56.14)
Sepsis screen	42.11% (20.25- 66.50)	67.74% (48.63-83.32)	1.31 (0.63 - 2.72)	0.85 (0.54 to 1.35)	44.44% (27.75- 62.49)	65.62% (54.80- 75.04)

*CRP- C-Reactive protein, PCT-Procalcitonin.



Figures 3 (A and B): Receiver operating characteristic (ROC) curves for CRP and PCT prepared against blood culture as gold standard.

Area under curve for PCT was 0.673 (0.526-0.799; 95% CI; p value=0.0401) which is higher than that of CRP (0.562 {0.414-0.702; 95% CI; p value=0.4763}) and also significant (Figure 3 A and B). It was seen that the specificity, PPV, and NPV of CRP was more than that of PCT. Sensitivity of PCT was higher than that of CRP and sepsis screen (Table 4).

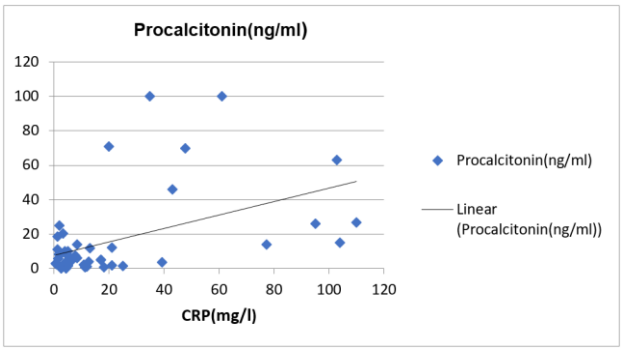


Figure 4: Scatter plot of correlation of PCT (ng/ml) and CRP (mg/l) levels of patients.

Pearson correlation was found to be 0.333(p=0.018), representing positive correlation between PCT and CRP

which is statistically significant (Figure 4). Median value of PCT was found to be higher in gram negative sepsis [4.00(1.51-23.25) ng/ml] as compared to gram positive and fungal sepsis [2.25(1.38-3.98) and 2.57(0.41-2.57) ng/ml respectively], but this difference was not statistically significant, (p=0.419). Median value of CRP was also found to be higher in gram negative sepsis [12.70(4.60-49.10) mg/l] as compared to gram positive sepsis and fungal sepsis [5.60(1.70-20.30) and 9.92(2.73-9.92) mg/l respectively] but this difference also did not reach statistical significance (p=0.472). Median of PCT was higher in LONS group as compared to EONS group [15.00(7.3-6.67) vs 4.60(2.01-8.50) ng/ml], but the difference was not statistically significant (p=0.178). Median value of CRP was also higher in LONS group as compared to EONS group [39.20(5.20-95.00) vs 5.00(2.73-13.00) mg/l respectively] which is statistically highly significant (p=0.001).

DISCUSSION

This was a comparative study done on 50 VLBW neonates. The mean (SD) of birth weight of our study population was 1240.86 (205.74) grams. In a study on 36 neonates by Turner et al mean (SD) of birth weight was observed to be 1682 (500) grams, which is higher than our study as they included all preterm population ≤36 weeks of gestational age.[11] Distefano et al in their study on preterm neonates with sepsis found a mean birth weight of 1778 (541) grams in cases and 1832 (352) grams in controls, which is also higher than our study for the same reasons.[12] The mean (SD) of gestational age of our study population was 30.94 (2.13) weeks. Similarly study population of Bustos et al and Turner et al had a mean gestational age of 30 weeks (range 23-35 weeks) and 32 (2.9) weeks respectively.[11,13]

Spontaneous vaginal deliveries were higher than LSCS in our study. In contrast, in the study by Naher et al caesarean sections were higher in number (56%) than vaginal deliveries (44%).[14] They observed that this was due to the fact that more high-risk pregnancies were been admitted to their hospital requiring caesarean section. The proportion of in-borns was more as such cases are usually transported in-utero to our referral institute. Similar to our study, Naher et al also found premature rupture of membranes as the most important risk factor in their study (24.00%).[14] However, in a study by Kurul et al pregnancy induced hypertension/pre-eclampsia was found in 22.30% cases followed by premature rupture of membranes (17.70%) (67). All 50 neonates required respiratory support whereas in a study by Kurul et al which included only LONS neonates with <32 weeks gestation, 17% neonates required respiratory support and 14% required inotropic support.[15] This difference suggests that our population was sicker as compared to theirs.

On first suspicion of clinical sepsis, a sepsis screen was performed in our study. We found it to be positive in only 18 (36.00%) neonates. Similar results were also seen in the studies by Thayi et al and Turner et al.[11,16] The former noted sepsis screen to be positive in 22/60 (36.67%) neonates and the later reported a positive sepsis screen in 6/85 (7.00%) sepsis episodes.

In our study, 19 (38%) neonates with sepsis were culture positive. Similar results were found in a study by Vazzalwar et al where 18 (35.29%) out of 51 neonates included were blood culture positive.[4] Conversely a study by Naher et al, exhibited blood culture positivity only in 3 cases out of 50.[14] They proposed that it could be due to late arrival, culture sample taken after giving antibiotic dose or wrong method of sample collection.

In our study, among Gram-negative organisms, Burkholderia cepacia (36.84%) was the most common organism isolated. In the study by Vazzalwar et al, *Klebsiella pneumoniae* was maximally found among gram negative organisms.[4] The microbiological profile of NICU isolates varies from region to region and hospital to hospital. Hence, this difference is plausible.

In our study, we took samples for PCT and CRP at the onset of clinical sepsis. The median (IQR) value of PCT was 6.70(2.59-14.25) ng/ml whereas the same for CRP was 6.97(3.97-21.00) mg/l. Turner et al measured PCT serially in preterm neonates at the first suspicion of sepsis and 25-48 hours later.[11] The values obtained were 2.7(0.8-5.4) ng/ml and 6.90 (4.70-16.30) ng/ml respectively. The latter values are very similar to our results. Median CRP was found to be higher in their study as compared to ours being 19 (10-80) mg/l. This was performed once only at onset of sepsis.[11] The results obtained by Kurul et al in 480 episodes of sepsis occurring in 208 neonates at onset of sepsis were much lower as compared to our study [0.67(0.02-100) ng/ml for PCT and 3.45(0.3-309.0) mg/l for CRP].[15] This difference is because of the reason that in our study, the proportion of EONS was much higher. Further our population was very sick as is obvious from the fact that 36 (72.00%) neonates required mechanical ventilation as the maximum respiratory support. On comparison of values of PCT and CRP among the three groups formed in our study we found that the median value of both CRP and PCT with values highest in Group II (Table 3). Thayi et al also formed three groups in their study on both term and preterm neonates similar to ours.[16] They found highest mean value of CRP and PCT in group-III, followed by group-II and group -I (p value = <0.005 for both).[16] This difference could be because of our smaller sample size or because of methodology of the tests performed.

The ROC curves for CRP and PCT were obtained against blood culture as gold standard. AUC for PCT was found to be higher than that of CRP and also significant(p=0.041). These results are similar to those obtained by Kurul et al in their study [AUC 0.52(0.40-0.65) for CRP and 0.74(0.64-0.84) for PCT].[15] Bustos and

Araneda also found higher AUC for PCT being 0.83(0.70-0.92) vs 0.51(0.37-0.65) for CRP.[13]

In our study sensitivity and specificity of PCT was 68.40% and 03.20 % respectively whereas for CRP it was 57.89% and 64.52% respectively. Thayi et al and Ramesh et al found sensitivity and specificity of 88.88% and 75.00% respectively for PCT vs 66.66% and 76.19% for CRP.[16] The wide variations in sensitivity and specificity could be due to the difference in gestational age, birth weight, day of onset of sepsis i.e., whether EONS or LONS and timing of sampling in different studies. There is a well-documented physiological increase in both CRP and PCT after birth with maximum concentrations reaching at 24 hours of life and normalizing by 48 hours.[17-19] The PCT peak at 24 hours is reported as 10-20 ng/ml for terms and 50-60 ng/ml for preterms. This physiological increase may be one of the reasons for the difference of results, as majority (35/50 neonates) of our cases were EONS and sampling was done within 24-48 hours of life. Moreover, the levels of both CRP and PCT are affected by birth weight and gestational age.[17] As the birth weight and gestational age increases, PCT levels fall while CRP levels increase.[17] Our study population was preterm VLBW neonates only and levels of PCT and CRP may have been different from other studies with different sets of population.

Few studies have determined the 95th percentile for procalcitonin concentration for both term and preterm infants at different hours of life staring from birth to 96 hours, which can be used as cut-offs. It is pertinent to note that in the preterm population, these cut-offs are 1ng/ml at birth, increase to 12-60 ng/ml at 24 hours and then come down to 1-2 ng/ml at 96 hours.[17-19] The values at 12, 24 and 36 hours of life are three times more than term infants and at one time-point varied by a factor of 4-5 times between studies. With so much variation in normal levels according to day of life and gestational age, variations in diagnostic accuracy of these markers are bound to occur.

Pearson correlation was found to be 0.333(p=0.018), representing positive correlation between PCT and CRP which is statistically significant. Naher et al also found a positive correlation between these two variables with Pearson correlation of 0.448 (P<0.01).[14] As both markers are elevated in neonatal sepsis, this correlation represents their utility in such populations.

Median values of both PCT and CRP were higher in gram negative sepsis as gram-negative bacterial sepsis produces more sustained inflammatory response probably because of the lipopolysaccharide stimulation of gram-negative bacteria in multiple organ dysfunction syndrome and can lead to higher levels of markers, especially PCT.

In our study median of PCT and CRP were higher in LONS group as compared to EONS group. None of the studies reviewed so far had compared these parameters in these two groups. The difference in PCT not reaching statistical significance could be because we included only preterm, VLBW neonates and PCT levels are very variable in this population group as already discussed above. Higher mortality rate of our study could be explained by the fact that our present study included more EONS cases and majority of them were very sick.

Strengths of the study

This study directly compares diagnostic efficacy of CRP and PCT in preterm VLBW population.

Limitations of the study

Small sample size, no control group, most sample taken within 24 hours, post-natal age and gestation specific nomograms of procalcitonin were not taken in account.

CONCLUSION

Procalcitonin could not be labeled as a better marker compared to C-reactive protein for the diagnosis of neonatal sepsis in preterm, very low birth weight neonates. Further research has been suggested to be conducted on this topic using a larger sample size, including a control group, and considering post-natal age and gestation-specific nomograms for procalcitonin, especially in the preterm, very low birth weight population.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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