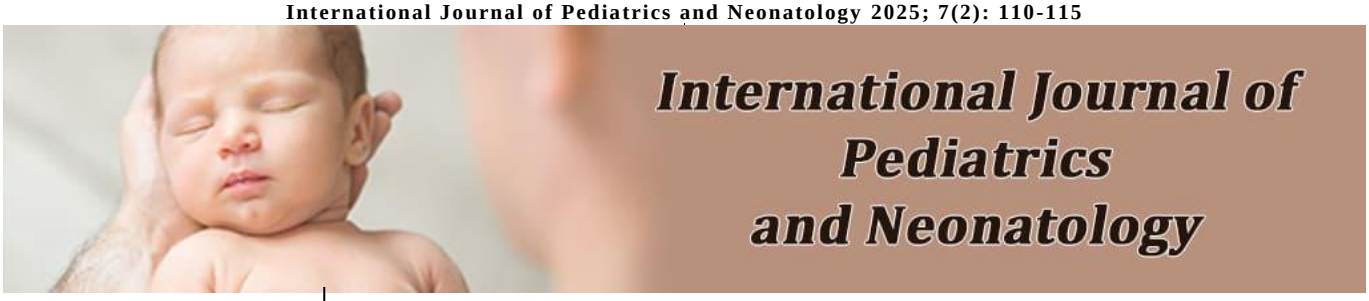

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Procalcitonin as a prognostic marker in preterm infants with late-onset sepsis

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Abstract
Background: Late-Onset Sepsis (LOS) remains a major cause of morbidity and mortality in preterm and extremely low birth weight neonates. Accurate early identification of neonates at higher risk of severe outcomes is essential for timely intervention. While C-reactive protein (CRP) and procalcitonin (PCT) are widely used biomarkers in neonatal sepsis diagnostic, their comparative prognostic value in LOS remains insufficiently defined.
Objective: To evaluate the prognostic value of PCT compared to CRP in preterm neonates with LOS.
Methods: We conducted a retrospective cohort study including preterm and extremely low birth weight neonates (≤ 32+6 weeks gestational age and/or ≤ 1500 g birth weight) diagnosed with LOS in a level III neonatal intensive care unit between 01/01/2012 and 31/12/2024. CRP and PCT values at the time of sepsis diagnosis were analyzed in relation to severity indicators, including platelet count, need for invasive ventilation, inotropic support, neonatal Sequential Organ Failure Assessment (nSOFA) score, and 30-day mortality. ROC curves, regression models, and multivariable analyses were performed.
Results: Among 100 neonates included, PCT demonstrated superior prognostic accuracy for mortality (AUC 0.89) compared to CRP (AUC 0.84), although not statistically significant. PCT was independently associated with nSOFA score (p<0.001), while CRP predicted the need for invasive ventilation and inotropic support. Both biomarkers showed limited predictive value for thrombocytopenia. Multivariable models confirmed PCT as a stronger predictor of disease severity, particularly for nSOFA score.
Conclusion: Procalcitonin demonstrated higher prognostic utility than CRP in assessing disease severity and predicting mortality in preterm neonates with LOS. These findings support the integration of PCT into neonatal sepsis management strategies and emphasize the need for further prospective validation.

Keywords: C-Reactive Protein, sepsis, procalcitonin, infant, premature, prognosis

Introduction

Neonatal sepsis is a complex clinical entity resulting from a dysregulated systemic inflammatory response to infection, occurring within the first 28 days of life [1]. It is typically classified as early- or late-onset based on the timing of symptom onset. Late-onset neonatal sepsis (LOS) is generally defined as sepsis manifesting after the first 72 hours of life and is often associated with nosocomial pathogens and risk factors such as prolonged hospitalization, invasive procedures, and immunological immaturity of the neonate [2, 3]. According to the World Health Organization, there are approximately 1.3 to 3.9 million cases of neonatal sepsis annually, resulting in 400,000 to 700,000 deaths worldwide [4].

Preterm neonates and/or those with very low birth weight exhibit increased susceptibility to LOS. Studies have shown that LOS in this group is associated with deleterious neurodevelopmental outcomes, including an increased risk of cerebral palsy, cognitive impairment, and motor delay [5, 6].

Thus, the diagnosis of LOS and the assessment of its severity and prognosis should be performed as early as possible, identifying patients at highest risk of complications, so that appropriate treatment and monitoring can be instituted. However, the often nonspecific clinical presentation makes this task particularly challenging [1, 3, 7].

C-reactive protein (CRP) and procalcitonin (PCT) have been studied as markers to aid in the diagnosis and monitoring of patients with LOS.

CRP, despite its wide availability, exhibits slower kinetics-typically rising 12-24 hours after infection onset and shows limited specificity, as it may be elevated in various non-infectious inflammatory conditions; there are studies in which CRP proved non-predictive for LOS diagnosis [8, 9]. In contrast, PCT rises earlier 6-8 hours after bacterial stimulus and its levels correlate with the intensity of the inflammatory response [10, 11].

Additionally, the neonatal Sequential Organ Failure Assessment (nSOFA) score, which quantifies organ dysfunction based on respiratory, cardiovascular, and coagulation parameters, can be used to assess LOS severity [12, 13]. Studies have shown that elevated nSOFA scores are associated with increased mortality and risk of complications [14, 15].

However, studies investigating the role of biomarkers in LOS prognosis remain limited [16]. Therefore, this study aimed to evaluate the prognostic value of PCT compared to CRP in preterm and extremely low birth weight neonates with LOS.

Materials and Methods

This was a retrospective analytical cohort study conducted in the Neonatal Intensive Care Unit of Braga Local Health Unit, approved by the institution's Ethics Committee.

Participants

We included neonates meeting the following criteria:

Inclusion criteria

- Preterm infants with gestational age ≤ 32 weeks + 6 days and/or birth weight ≤ 1,500 g
- Born in or transferred to the Braga Local Health Unit within the first 24 hours of life between 01/01/2012 and 3/12/2024
- Diagnosis of LOS.

Exclusion criteria

- Analytical evaluation without a PCT value
- Major malformations.

Definitions

Sepsis was defined according to NICHD Neonatal Research Network criteria [17]. LOS was defined as a positive blood culture obtained after 72 hours of life plus intention to treat with antibiotics for ≥ 5 days. Sepsis with negative blood culture was also considered when CRP exceeded 10 mg/L within 2 days of culture collection, with intent to treat with antibiotics for ≥ 5 days and clinical signs consistent with sepsis.

Severity of LOS was assessed by applying the nSOFA score 12 hours after diagnosis. A high nSOFA score was defined as > 4 points.

Invasive ventilation was defined as requirement for intubation within 3 days of suspicion of sepsis.

Inotropic support was defined as initiation of inotropes/vasopressors within 3 days of suspicion of sepsis.

Thrombocytopenia was defined as platelet count < 150,000/µL.

Statistical Analysis

Normality of the quantitative variables CRP and PCT was assessed via skewness, kurtosis, and Kolmogorov-Smirnov and Shapiro-Wilk tests. As these variables were not normally distributed, nonparametric tests were used. The Mann-Whitney U test compared these markers between two independent groups. The Kruskal-Wallis test was applied for comparisons across three or more groups, followed by pairwise comparisons with Bonferroni correction to identify statistically significant differences. Spearman's rank correlation coefficient evaluated associations between these markers and other quantitative variables.

Univariate and multivariate regression analyses were then performed: Linear regression for quantitative outcomes and binary logistic regression for categorical outcomes. Gestational age, birth weight, postnatal age at LOS diagnosis, and etiological agent shown to relate to the variables under study, so were included as covariates in regression models when appropriate. For linear regression, assumptions of independence of observations, absence of multicollinearity, absence of influential outliers, and normality of residuals were verified. For binary logistic regression, key assumptions checked were absence of multicollinearity and absence of influential outliers affecting the model.

ROC curves and corresponding area under the curve [AUC] were generated to evaluate the discriminative capacity of CRP and PCT for the categorical outcomes under study, considering AUC values of 0.6-0.7 as poor, 0.7-0.8 as moderate/acceptable, 0.8-0.9 as good, and > 0.9 as excellent [18]. The Youden index was used to identify the optimal cut-off the ROC point maximizing sensitivity and specificity; a Youden index ≥ 0.50 was deemed acceptable for diagnostic accuracy [19, 20]. Differences between ROC curves were assessed using the Hanley & McNeil method [21, 22].

Results

After applying the inclusion criteria, 155 neonates were initially selected. Of these, 55 were excluded based on exclusion criteria: 46 due to lack of PCT measurement in the analytical evaluation and 9 due to major malformations. Therefore, the final sample comprised 100 neonates, Table 1. Among them, 62% were male, with a mean gestational age of 27 weeks and 5 days 23 weeks and 6 days-34 weeks and 4 days. The mean birth weight was 964.4 g (430g-1,500g), and 54% of the sample were classified as extremely low birth weight. The median postmenstrual age at the time of LOS diagnosis was 18 days, and the most common etiologic agent was *Staphylococcus epidermidis* (37%), followed by *Enterococcus faecalis* (8%). The most common Gram-negative agent was *Escherichia coli.* Blood cultures were negative in 31% of cases.

Relationship between CRP, PCT, and Potential Confounding Variables

Median CRP and PCT values were very similar between female and male patients, with no statistically significant differences observed $p>0.05$. In contrast, CRP and PCT differed significantly according to the category of etiologic agent (P=0.011 and P=0.002, respectively). Pairwise comparisons with Bonferroni correction revealed significant differences between Gram-positive and Gram-negative pathogens for both CRP (P=0.009) and PCT (P=0.001), with higher values in Gram-negative cases. Statistically significant positive correlations were found between CRP-but not PCT-and gestational age (P=0.001), birth weight (P=0.030), and postnatal age at diagnosis (P=0.021), indicating that higher gestational age, birth weight, and

postnatal age at diagnosis were associated with elevated CRP levels.

Platelet Count

The ROC curves for CRP and PCT in predicting platelet counts < 150,000/µL yielded areas under the curve (AUC) of 0.62 and 0.63, respectively, indicating poor discriminative ability, with no statistically significant difference between the two markers (P=0.920). The Youden index was below the recommended threshold of 0.50 for both.

The multivariable model adjusting for confounding variables was not statistically significant, F(7, 90)=1.82, P=0.093, explaining 6% of the variance in platelet count (adjusted R^2=0.06). Neither CRP nor PCT were statistically significant independent predictors of platelet count, p>0.05.

Need for Invasive Ventilation

The ROC curve results for CRP and PCT showed similar AUC values of 0.70 and 0.71, respectively, indicating moderate discriminative ability, with no statistically significant difference between the two markers (P=0.906). The Youden index was below the recommended threshold of 0.50 for both.

The multivariable logistic regression model adjusting for confounding variables was statistically significant, χ^2 [7] =20.22, P=0.005, explaining 26% of the variance in the need for invasive ventilation (Nagelkerke R^2=0.26; percentage of correctly classified cases=74.5%). Only CRP remained a statistically significant independent predictor (P=0.024), with higher CRP values associated with an increased likelihood of requiring invasive ventilation (OR=1.02).

Need for Inotropic Support

The ROC curve results for CRP and PCT showed higher AUC for PCT (AUC=0.77 vs. 0.63 for CRP), although this difference was not statistically significant (P=0.104). Thus, CRP demonstrated poor discriminative ability, whereas PCT was classified as having moderate discriminative ability for predicting the need for inotropic support. Notably, the Youden index for PCT exceeded the recommended threshold of 0.50, with a cutoff of 1.31 ng/mL corresponding to a sensitivity of 89% and specificity of 62%.

The multivariable logistic regression model adjusting for confounding variables was statistically significant, χ^2 [7]=27.39, p < 0.001, explaining 36% of the variance in the need for inotropic support (Nagelkerke R^2=0.36; percentage of correctly classified cases=83.7%). Only CRP remained a statistically significant independent predictor (P=0.003), with higher CRP values associated with increased likelihood of requiring inotropic support (OR=1.03).

nSOFA Score

ROC curves were generated to evaluate the predictive ability for an nSOFA score > 4. The results for CRP and PCT showed similar AUC values of 0.60 and 0.69, respectively, indicating poor discriminative ability for both markers in relation to nSOFA > 4, with no statistically significant difference between them (P=0.504).

A multivariable model adjusting for confounding variables was applied to nSOFA as a continuous outcome. This model was statistically significant, F(7, 90)=4.05, P=0.001, and explained 18% of the variance in nSOFA score (adjusted R^2=0.18). Both CRP and PCT were independent, statistically significant predictors of nSOFA score (P=0.040 and p<0.001, respectively), with PCT being the stronger predictor (β=0.41); higher biomarker levels were associated with higher nSOFA scores (Table 2).

30-day Mortality

The ROC curve results for CRP and PCT showed similar AUC values of 0.84 and 0.89, respectively, indicating good discriminative ability, with no statistically significant difference between the two markers (P=0.617). The Youden index exceeded 0.50 in both analyses. For CRP, a cutoff of 50.6 mg/L was identified, corresponding to a sensitivity of 80% and specificity of 83%. For PCT, a cutoff of 10.53 ng/mL was established, with sensitivity of 80% and specificity of 86% (Table 3). Multivariable analysis adjusting for confounding variables could not be performed due to the small sample size for this outcome (N=5).

Discussion

This study highlights PCT as the biomarker with the greatest prognostic potential in preterm neonates with LOS. Although CRP retained some association with clinical deterioration, PCT demonstrated stronger and more consistent correlations with organ dysfunction severity (nSOFA) and mortality.

Our main findings showed that elevated PCT levels were independently associated with higher nSOFA scores (β=0.41, p<0.001) and exhibited superior discriminative capacity for mortality (AUC 0.89), compared to CRP. In contrast, CRP while linked to the need for invasive ventilation and inotropic support had more modest predictive performance for life-threatening outcomes. These results underscore PCT's earlier kinetics and greater infection specificity, rendering it a more reliable prognostic marker in this high-risk population.

Some other studies in preterm infants suspected of LOS corroborate these observations. For instance, in a study of neonates born before 32 weeks with suspected LOS, IL-6 and PCT but not CRP were significantly associated with 7-day mortality, whereas CRP did correlate with severity indicators such as need for inotropic support but with lower discriminative accuracy than PCT [23].Additionally, Ruetsch *et al*. conducted a pilot cohort study I n very preterm infants (< 32 weeks) with LOS and found that, although PCT at the moment of diagnosis did not differ significantly between survivors and non-survivors, PCT levels during the first 24 hours after diagnosis were significantly higher in those who died [24].

Nonetheless, CRP remains readily available and inexpensive; its consistent association with respiratory or circulatory support needs suggests it still has a supportive role within a multimodal assessment. In practice, PCT measurement can complement CRP and clinical scoring [e.g., nSOFA], particularly when early risk stratification is critical. The PCT cutoff of 10.53 ng/mL for mortality in our cohort is in a similar range with other findings in the literature and may be useful in clinical practice but given the small number of deaths (N=5), this requires prospective validation [24].

Our multivariable analyses adjusted for gestational age, birth weight, postnatal age at diagnosis, and pathogen confirm PCT's robustness as a prognostic indicator beyond these neonatal-specific confounders. This supports

incorporating PCT into sepsis protocols in neonatal units, especially for preterm/low-birth-weight infants.

Limitations and Future Directions

The retrospective, single-center design and limited mortality events constrain definitive conclusions on PCT cutoffs. Additionally, timing variability of biomarker sampling relative to symptom onset may influence levels. Future prospective, multicenter studies should standardize sampling times, validate PCT thresholds, and assess combinations of biomarkers (e.g., IL-6, presepsin) with PCT. Additionally, cost-effectiveness analyses are needed to justify routine PCT use in resource-limited settings.

Clinical Implications

Given PCT's superior prognostic performance, neonatal units should consider implementing PCT-guided risk stratification alongside CRP and clinical scores. Early identification of infants at highest risk may prompt timely escalation of monitoring and therapy. However, CRP remains valuable as a widely accessible marker reflecting inflammatory burden, to be interpreted in context rather than used in isolation.

Conclusion

In this cohort of preterm neonates with late-onset sepsis, PCT demonstrated superior prognostic performance compared to CRP, in relation to organ dysfunction and mortality. While CRP retained predictive value for clinical deterioration, including the need for respiratory and circulatory support, PCT showed stronger and more consistent associations with nSOFA scores and 30-day mortality, highlighting its potential as a key marker for early risk stratification. These findings support the integration of PCT into sepsis management protocols in neonatal intensive care settings. Further prospective, multicenter studies are warranted to validate optimal cut-off values and assess the clinical utility of combining PCT with other biomarkers and severity scores.

Tables

Table 1: Sample characterization

Sex, n (%)	
Male	62 (62)
Female	38 (38)
Gestational age (days), M (SD)	194,1 (14,9)
Birth weight (g), M (SD)	964,4 (248,4)
0-500g, n (%)	1 (1)
500-1000g, n (%)	53 (53)
1000-1500g, n (%)	45 (45)
1500-2000g, n (%)	1 (1)
Age at diagnosis (days), Mdn (IQR)	18 (22)
Weight at diagnosis (g), Mdn (IQR)	1103,0 (559)
Etiologic agent n (%)	
Staphylococcus epidermidis	37 (37)
Staphylococcus aureus	3 (3)
Staphylococcus capitis	3 (3)
Staphylococcus haemolyticus	2 (2)
Staphylococcus warneri	3 (3)
Staphylococcus hominis	1 (1)
Staphylococcus caprae	1 (1)
Enterococcus faecalis	8 (8)
Streptococcus agalactiae	1 (1)
Escherichia coli	3 (3)
Klebsiella pneumoniae	2 (2)
Serratia marcescens	2 (2)
Enterobacter cloacae	1 (1)
Candida parapsilosis	1 (1)
Candida albicans	1 (1)
Negative cultures	31 (31)
CRP at diagnosis (mg/L), Mdn (IQR)	13,0 (38,9)
PCT at diagnosis (ng/ml), Mdn (IQR)	1,5 (4,0)
Platelet count (x10^3/uL), M (SD)	246,8 (120,1)
Need for invasive ventilation, n (%)	
Yes	35 (35)
No	65 (65)
Need for non-invasive ventilation, n (%)	
Yes	58 (58)
No	42 (42)
Need for inotropic support, n (%)	
Yes	26 (26)
No	74 (74)
nSOFA score, Mdn (IQR)	0,5 (2)
> 4, n (%)	11 (11)
≤ 4, n (%)	89 (89)
Mortality within 30 days of diagnosis, n (%)	
Yes	5 (5)
No	95 (95)

M = Mean; DP = Standard deviation; Mdn = median; IQR = interquartile range; n = absolute frequency

Table 2: Univariate and multivariable linear regression for the nSOFA score.

	Univariate			Multivariable		
	B (EP)	**β**	**p**	**B (EP)**	**β**	**P**
CRP	0,02 (0,01)	0,34	0,001	0,01 (0,01)	0,22	0,040
PCT	0,03 (0,01)	0,39	< 0,001	0,03 (0,01)	0,41	< 0,001

Table 3: Discriminative capacity of CRP and PCT regarding mortality

Parameter	CRP	PCT
AUC (EP)	0,84 (0,08)	0,89 (0,06)
CI 95%	0,68; 1,00	0,77; 1,00
Youden´s index	0,63	0,66
Cut-off	50,60	10,53
Sensitivity	0,80	0,80
Specificity	0,83	0,86

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