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# PRESEPSIN IN NEONATAL SEPSIS: A SINGLE-CENTER EVALUATION IN COMPARISON TO CONVENTIONAL MARKERS

**Mojca Kavčič[1], Petja Fister[2], Aneta Soltirovska Šalamon[1, 3], Joško Osredkar[4, 5], Darja Paro Panjan[1, 3]**

[1] Department of Neonatology, Division of Pediatrics, University Medical Center Ljubljana, Ljubljana, Slovenia
[2] Department for Intensive Care, Division of Pediatrics, University Medical Center Ljubljana, Ljubljana, Slovenia
[3] University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia
[4] Institute of Clinical Chemistry and Biochemistry, University Medical Center Ljubljana, Ljubljana, Slovenia
[5] University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia

**Corresponding author**: Mojca Kavčič, MD, Department of Neonatology, Division of Pediatrics, University Medical Center Ljubljana, Zaloška cesta 2, 1000 Ljubljana, Slovenia,Phone: +386 [41223006], Email: mojca.kavcic@kclj.si

## ABSTRACT

**Objective**: Neonatal sepsis is a leading cause of morbidity and mortality in newborns. Timely and accurate diagnosis remains a challenge due to the nonspecific clinical signs and the limited sensitivity and specificity of conventional biomarkers such as C-reactive protein (CRP), procalcitonin (PCT), and white blood cell (WBC) count. Presepsin, a soluble CD14 subtype, has been proposed as a promising early marker of bacterial infection.

This study aimed to assess the diagnostic value of serum presepsin in comparison with traditional inflammatory markers in neonates with suspected sepsis.

**Methods**: In a prospective observational study conducted at a single tertiary care center, 18 neonates with clinical signs of sepsis were evaluated. The patients were divided into culture-proven (n=7) and culture-unproven (n=11) sepsis groups. Presepsin levels were measured using a chemiluminescent assay and compared to CRP, PCT, and WBC counts.

**Results**: The median presepsin levels were higher in culture-proven cases (740 ng/L vs. 393 ng/L), but without statistical significance. Similar trends were observed for CRP and PCT, while WBC counts showed no diagnostic value.

**Conclusion**: Although presepsin demonstrated potential as an early biomarker, its diagnostic performance in this small cohort was inconclusive. Combined use of presepsin with conventional markers and clinical evaluation may improve early sepsis diagnosis in neonates.

**Keywords**: neonatal sepsis, presepsin, inflammatory markers, procalcitonin, C-reactive protein

## INTRODUCTION

Sepsis remains a leading cause of mortality in newborns worldwide, with an estimated incidence of 2202 per 100 000 live births, with mortality between 11% and 19% [1–3]. Neonatal bacterial sepsis is defined as a clinical syndrome characterized by systemic signs of infection ac-

companied by bacteremia during the first month of life [4]. Identified risk factors include: low birth weight, preterm birth (<37 weeks' gestation), premature rupture of membranes (PROM), intrapartum complications such as perinatal asphyxia, low socioeconomic status, poor sanitation, malnutrition, and overcrowding [3,5]. Sepsis is classified as early-onset (occurring within the first 72 hours of life) or late-onset (occurring between 3–28 days post-birth) [4,6].

Neonatal sepsis is a serious condition with diagnostic challenges, primarily due to the subtle and nonspecific nature of the clinical signs. Early initiation of empirical antibiotic treatment in cases of unconfirmed sepsis carries the risk of fostering antibiotic resistance, and it is associated with longer hospital stays and an increase in the health care costs [7,8]. Antibiotic exposure in early life influences the developing microbiome and may be associated with an increased risk of asthma, allergy, overweight, diabetes, and inflammatory bowel disease later in life [9]. The diagnostic approach typically combines standard laboratory markers, such as: C-reactive protein (CRP), procalcitonin (PCT), and leukocyte differentials, with blood culture, as the microbiological gold standard [2,7]. According to studies, CRP has sensitivity of 0.6 to 0.84, and specificity 0.84 to 1.00, and PCT has sensitivity of 0.77, and specificity of 0.62. PCT also has a higher positive likelihood ratio and lower negative likelihood ratio than CRP [10,11]. However, blood cultures often have limited utility. The small sample volume and prior antibiotic administration can yield false negatives, positive cultures may reflect contamination, and the results of the microbial culture are not available for at least 24 to 72 hours [6]. Fleiss et al. found that obtaining two blood cultures did not improve diagnostic utility [12].

These limitations underscore the need for more reliable and specific biomarkers for neonatal sepsis.

Presepsin (sCD14-ST), a soluble fragment of glycoprotein CD14, has emerged as a promising early biomarker for sepsis. CD14 is a receptor involved in recognizing bacterial components (e.g. lipopolysaccharides) and initiating the innate immune response. It exists in two forms: membrane-bound (mCD14) on monocytes/macrophages, and soluble (sCD14) in plasma. Soluble CD14 is cleaved into a 13 kDa fragment, known as presepsin, during the activation of the plasma protease cascade [13-15].

Presepsin is highly specific to bacterial infections, and is unaffected by factors such as delivery type, surgical interventions, or viral infections. Its levels typically increase within 2–12 hours of infection and decline rapidly with effective treatment, often within 24 hours. Severe infections are associated with higher presepsin levels, while persistent infections show little or no decline. Despite its promise, there is no consensus on threshold presepsin values, with significant variability in cut-off values, sensitivity, and specificity across studies [15].

The primary aim of our study was to evaluate the diagnostic value of serum concentrations of presepsin in comparison to the traditional inflammatory markers, including serum concentrations of CRP, PCT, and WBC count. Specifically, the study sought to determine whether presepsin could enable earlier detection of bacterial infections in neonates, assess infection severity, distinguish between sepsis that has been culture-proven and sepsis that has not, and provide added value in guiding clinical decision-making, facilitating more targeted antibiotic use and reducing unnecessary treatment for those without infections.

## MATERIALS AND METHODS

This study was conducted as a comparative observational analysis at the Neonatal Department, Division of Paediatrics, University Clinical Centre Ljubljana from January to March 2022.

The study population included neonates suspected of having sepsis on the basis of clinical presentation or risk factors [4,16]. Blood samples were taken as part of the standard diagnostic procedure as soon as sepsis was suspected. To ensure minimal influence on infant health, 100 µL of whole blood was collected into EDTA tubes in order to limit the sample volume. To maintain the integrity of the biomarker, all samples were processed immediately.

The PATHFAST chemiluminescence-based immunological analyzer (Mitsubishi Chemical Medience Corporation) was used to measure the levels of presepsin. For neonatal testing, its low blood volume requirement of 100 µL proved especially helpful. Analytical precision was evaluated through repeatability and reproducibility studies. Repeatability testing was performed using whole blood and plasma samples at four concentration

levels, each measured in 20 replicates. The coefficients of variation (C.V.) ranged from 2.5% to 7.9% across all levels. Reproducibility was assessed using plasma samples tested in duplicate over 40 runs (2 runs/day for 20 days) on a single instrument with one reagent lot, following the CLSI EP5-A2 protocol. Total C.V. values ranged from 4.1% to 5.0%. Analytical sensitivity was defined by a limit of blank (LoB) of 2.53 pg/mL, a limit of detection (LoD) of 8.86 pg/mL, and a limit of quantitation (LoQ) of 38.4 pg/mL at 10% C.V.

The measurements of serum concentrations of CRP, PCT, and WBC were performed simultaneously to allow direct comparisons with serum concentrations of presepsin.

For each patient, clinical data were documented, including gestational age, birth weight, and perinatal history. The sepsis classification was based on the clinical and microbiological findings, with blood cultures serving as the reference standard for confirmed bacterial infections.

The study protocol was reviewed and approved by the Slovenian National Medical Ethics Committee, approval number 0120-401/2021/3, and written informed consent was secured from the parents or legal guardians of all participating neonates.

## RESULTS

A total of eighteen neonates diagnosed with sepsis were included in the study. The participants were stratified into two groups for analysis: culture-proven sepsis (N=7) and culture-unproven sepsis (N=11). Among the study cohort, 50% were male. The median gestational age of the neonates was 39 weeks (range: 23–40 weeks), and the median chronological age at the time of diagnosis was 11 days (range: 1 day to 16 weeks) (Table 1). The isolated bacteria were: *Klebsiella aerogenes, Staphylococcus haemolyticus, Staphylococcus epidermidis, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus,* and *Lactobacillus gasseri*.

Although there was a difference in the median serum concentrations of presepsin levels between culture-proven (740 ng/L) and culture-unproven patients (393 ng/L), it was not statistically significant (p = 0.537) (Table 2). The culture-proven group had significantly higher serum concentrations of CRP (47 mg/L) than the culture-unproven group (9 mg/L), with a trend towards statistical significance (p = 0.080). The serum concentrations of PCT in the culture-proven group were almost significantly higher (1.5 µg/L vs. 0.17 µg/L, p = 0.052). The groups' WBC levels were comparable (11.5 × 10^9/L vs. 9.5 × 10^9/L, p = 0.931).

## DISCUSSION

Our study assessed the diagnostic utility of serum concentrations of presepsin in newborns with sepsis in comparison to serum concentrations of conventional inflammatory markers (CRP, PCT, and WBC). The culture-proven group had significantly higher serum concentrations of CRP than the culture-unproven group, with a trend towards statistical significance (p = 0.080). This discrepancy implies that CRP may still have

**Table 1.** *Clinical characteristics of patients.*

| Clinical characteristics | N=18 |
|---|---|
| Male | 9 (50%) |
| Culture-proven | 7 (39%) |
| Gestational age (mean value, weeks) | 39 (23-40) |
| Chronological age (mean value) | M 11 days (1 day-16 weeks) |

**Table 2.** *Results of serum concentration of C-reactive protein (CRP), procalcitonin (PCT), and white blood cell (WBC) count in analyzed groups.*

| | Culture-proven sepsis | Culture-unproven | p value |
|---|---|---|---|
| CRP | 47 mg/L | 9 mg/L | p = 0.080 |
| PCT | 1.5 µg/L | 0.17 µg/L | p = 0.052 |
| Presepsin | 740 ng/L | 393 ng/L | p = 0.537 |
| WBC | 11.5 × 10^9/L | 9.5 × 10^9/L | p = 0.931 |

valuable discriminatory power in newborns with confirmed bacterial infections, despite being a slower-reacting marker. However, its usefulness as a stand-alone marker is diminished by its delayed response and concentration overlap. Given that serum concentrations of PCT increase quickly after infection, this study implies that it may be useful for early identification of bacterial sepsis, but it is important to consider the physiologically higher concentrations of PCT in the first 72 h after birth. With a larger sample size, the likely trend indicated by the near-significant p-value could become significant. In accord with their recognized functions as markers of bacterial infection and inflammation, CRP and PCT also showed trends towards increased serum concentrations in sepsis that was culture-proven. However, in neonatal populations, their independent diagnostic usefulness is limited by their delayed response (CRP) or variability due to non-infectious variables (PCT) [17,18]. According to this study, the WBC count appears to have no discriminating value for newborn sepsis. This supports earlier research showing that in newborns with sepsis, the WBC count alone is frequently inaccurate due to fluctuation.

Neonates with culture-proven sepsis had higher median serum concentrations of presepsin, CRP, and PCT than those with culture-unproven sepsis, according to the results. However, these differences were not statistically significant. The lack of statistical significance may be due to the limited sample size or serum concentrations that overlap between groups. In spite of this, the culture-proven group's higher median is consistent with previous research, indicating that presepsin may be a biomarker for bacterial sepsis. It is especially useful in neonatal populations due to its quick increase in concentration and low sample volume requirement. In culture-proven sepsis, both concentrations of PCT and CRP exhibited tendencies towards increased values, with PCT approaching significance. Even though the trend was not statistically significant, presepsin may be a useful adjunct to PCT and CRP, especially in the early stages of diagnosis. According to these results, presepsin and other markers may be useful in the diagnosis of newborn sepsis, but, given the limitations of this small cohort, their sensitivity and specificity are still unclear.

Due to its early increase in response to sepsis and low blood volume requirement, which make it especially appropriate for neonates, presepsin has been suggested as a viable biomarker for bacterial infections. Prior research has demonstrated that presepsin is a reliable marker for differentiating bacterial sepsis from non-infectious diseases in both neonates and adults, which is in line with the higher median presepsin levels seen in culture-proven sepsis [19,20]. The lack of statistical significance in this study, however, may suggest that larger sample numbers are required to validate its diagnostic value.

The neonates in this study ranged greatly in both chronological age at diagnosis (1 day to 16 weeks) and gestational age (23–40 weeks). Significant variations in inflammatory responses, infection susceptibility, and immune system maturity resulted from this heterogeneity. For example, cytokine and biomarker production is known to be lower in preterm infants than in term neonates (6). These variations might have affected presepsin levels, making it more difficult to compare cases that have been culture-proven with those that have not. Future research should concentrate on more homogeneous populations, or stratify patients by gestational and chronological age.

Presepsin levels have been shown to increase quickly within hours of the commencement of an infection, and to fall with successful treatment [21]. The observed variations in presepsin levels were probably caused by variations in the time of the sample collection in relation to the onset of signs of sepsis and the start of treatment. Its kinetics and potential as a dynamic marker of infection severity and responsiveness to therapy could be better understood through longitudinal studies that monitor presepsin levels over the course of illness.

The timing of presepsin measurement relative to the onset of clinical symptoms and treatment initiation is another factor that could influence the findings. Presepsin levels are known to rise rapidly within hours of infection onset and decrease with effective treatment. In this study, variations in the timing of sample collection may have contributed to the observed variability in presepsin levels. Longitudinal monitoring of presepsin over the course of illness may provide a clearer picture of its diagnostic and prognostic utility.

Despite these drawbacks, the results offer important new information about the possible function of presepsin in the treatment of newborn sepsis. Presepsin may help guide diagnostic and therapy choices by supplementing current biomarkers such as CRP and PCT, as ev-

idenced by its capacity to identify bacterial infections early, and its correlation with treatment response. However, the lack of high precision in differentiating between culture-proven and culture-unproven sepsis underscores the need for more investigation.

## FUTURE RESEARCH

Increasing the sample size and including a range of clinical contexts would boost the findings' generalizability and statistical power. Presepsin levels may be correlated with certain bacterial diseases by the use of sophisticated diagnostic methods, such as multiplex pathogen detection assays or next-generation sequencing, which would provide better knowledge of its diagnostic specificity [22]. One obstacle to its broad use is the variation in presepsin thresholds between studies and clinical settings. It is crucial to standardize cut-off values according to the clinical situation, infection onset, and gestational and chronological age. Presepsin should be assessed using a multi-marker approach that includes clinical risk factors, PCT, CRP, and other markers in order to create algorithms for the early and precise diagnosis of sepsis. Presepsin's prognostic potential and usefulness in therapy monitoring will become clearer when its dynamics are examined over time in connection with treatment response and clinical outcomes.

## CONCLUSIONS

Our study's conclusions highlight the difficulties involved in correctly detecting newborn sepsis and the drawbacks of depending on just one biomarker. Despite its potential as an early marker of bacterial infection, presepsin's diagnostic efficacy in this group was unclear. These findings emphasize how important it is to combine several biomarkers and clinical factors in order to improve diagnostic accuracy.

Larger, more varied study populations should be the focus of future research, and long-term monitoring of presepsin in conjunction with other markers, such as PCT and CRP, should be taken into consideration. Additionally, the prompt and precise diagnosis of sepsis in newborns could be greatly enhanced by creating standardized diagnostic algorithms that use presepsin and cutting-edge pathogen detection techniques. We can minimize needless antibiotic exposure and improve outcomes for this susceptible group by improving our diagnostic capabilities.

*Conflict of Interest Statement*

The authors declare no conflict of interest

*What Is Already Known on This Topic*

Neonatal sepsis is a leading cause of morbidity and mortality in newborns, yet its diagnosis remains difficult due to its nonspecific clinical signs and the limited accuracy of conventional laboratory markers. C-reactive protein and procalcitonin are widely used but are hindered by delayed responses, variability, or confounding factors. Blood culture is the diagnostic gold standard, but it suffers from low sensitivity, long turnaround time, and susceptibility to contamination. Presepsin (sCD14-ST), a soluble CD14 subtype released during bacterial infection, has emerged as a promising early biomarker of sepsis in adults and children. Evidence from meta-analyses suggests the good diagnostic performance of presepsin in neonatal sepsis, but the reported thresholds vary widely, and only a few prospective, single-center studies have been conducted in neonatal populations. Further data are needed to clarify its added value compared with the existing inflammatory markers.

*What This Study Adds*

This single-center study prospectively evaluated presepsin in neonates with suspected sepsis, and compared it with CRP, procalcitonin, and white blood cell count. Presepsin levels were higher in culture-proven cases, consistent with previous literature, although not statistically significantly in this small cohort. The findings confirm the feasibility of presepsin testing in neonates, highlighting its potential as an adjunct to conventional markers, and underscoring the need for larger, standardized studies to establish clinically useful cut-offs and diagnostic algorithms.

*Authors' contributions*

Conception and design: MK, JO, DPP; Acquisition, analysis and interpretation of data: MK, JO; Drafting the article: MK, JO; Revising

it critically for important intellectual content: MK, JO, DPP; Approved final version of the manuscript: MK, JO, ASŠ, PF, DPP.

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**Резиме**

## ПРЕСЕПСИН КАЈ НЕОНАТАЛНАТА СЕПСА: ЕВАЛУАЦИЈА ВО ЕДЕН ЦЕНТАР ВО СПОРЕДБА СО КОНВЕНЦИОНАЛНИТЕ МАРКЕРИ

**Мојца Кавчич[1], Петја Фистер[2], Анета Солтировска Шаламон[1, 3], Јошко Осредкар[4, 5], Дарја Паро Панјан[1, 3]**

[1] Клиника за неонатологија, Оддел за педијатрија, Универзитетски медицински центар Љубљана, Љубљана, Словенија

[2] Клиника за интензивна нега, Оддел за педијатрија, Универзитетски медицински центар Љубљана, Љубљана, Словенија

[3] Медицински факултет, Универзитет во Љубљана, Љубљана, Словенија

[4] Институт за клиничка хемија и биохемија, Универзитетски медицински центар Љубљана, Љубљана, Словенија

[5] Универзитет во Љубљана, Фармацевтски факултет, Љубљана, Словенија

**Цел**: Неонаталната сепса е водечка причина за морбидитет и морталитет кај новороденчињата. Навремената и точна дијагноза останува предизвик поради неспецифичните клинички знаци и ограничената чувствителност и специфичност на конвенционалните биомаркери, како што се С-реактивниот протеин (CRP), прокалцитонин (PCT) и бројот на белите крвни клетки (WBC). Пресепсинот, растворлив поттип CD14, е предложен како ветувачки ран маркер на бактериска инфекција.

Оваа студија имаше цел да ја процени дијагностичката вредност на серумскиот пресепсин во споредба со традиционалните воспалителни маркери кај новороденчињата со сомневање за сепса.

**Методи**: Во проспективната опсервациска студија спроведена во еден центар за терцијарна здравствена заштита, беа евалуирани 18 новороденчиња со клинички знаци на сепса. Пациентите беа поделени во групи со докажана култура (n = 7) и недокажана култура (n = 11) сепса. Нивоата на пресепсин беа мерени со помош на хемилуминисцентен тест и споредени со бројот на CRP, PCT и WBC.

**Резултати**: Средните нивоа на пресепсин беа повисоки кај случаите докажани со култура (740 ng/L наспроти 393 ng/L), но без статистичка значајност. Слични трендови беа забележани за CRP и PCT, додека бројот на леукоцити не покажа дијагностичка вредност.

**Заклучок**: Иако пресепсинот покажа потенцијал како ран биомаркер, неговата дијагностичка ефикасност во оваа мала кохорта беше неубедлива. Комбинираната употреба на пресепсин со конвенционални маркери и клиничка евалуација може да ја подобри раната дијагноза на сепса кај новороденчиња.

**Клучни зборови**: неонатална сепса, пресепсин, воспалителни маркери, прокалцитонин, С-реактивен протеин