

# Plasma Presepsin as a Diagnostic Marker for Early-Onset Sepsis in Newborns

**Huaqing Zhang**

Peking University Shenzhen Hospital

**Zhihao Xiao**

Peking University Shenzhen Hospital

**Lujia Yang**

Peking University Shenzhen Hospital

**Yong Xia**

Peking University Shenzhen Hospital

**Guobing Chen**

Peking University Shenzhen Hospital

**Xiaoyan Hu**

xyhu77@126.com

Peking University Shenzhen Hospital

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## Research Article

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# Abstract

## Objective

This study aimed to evaluate the diagnostic value of presepsin (P-SEP) in both umbilical cord and peripheral blood for neonatal early-onset sepsis (EOS), compare its efficacy with conventional biomarkers, and establish optimal diagnostic thresholds.

## Study design

Prospective cohort study of 355 neonates with EOS risk factors recruited from Peking University Shenzhen Hospital (June 2024-March 2026). Following stratification into EOS (n=95) and non-EOS groups (n=260), we measured P-SEP, complete blood count(CBC), C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6) in umbilical cord (n=195) and peripheral blood samples (n=338) collected within 72 hours postpartum. Diagnostic performance was assessed through ROC curve analysis and biomarker comparisons.

## Results

P-SEP concentrations were significantly elevated in EOS cases of both sample types ( $p<0.001$ ). In this prospective study, we enrolled 355 neonates (95 with EOS and 260 controls). Successful umbilical cord blood collection was achieved in 195 cases (50 EOS, 145 controls), while peripheral blood samples were obtained from 338 neonates (95 EOS, 243 controls). Our findings demonstrate significantly elevated P-SEP levels in both blood types of EOS patients compared to controls (both  $p<0.001$ ). ROC curve analysis established optimal diagnostic thresholds at 762.96 pg/mL for umbilical cord blood (sensitivity(Se) 94%, specificity(Sp) 77.2%, the area under the curve(AUC)0.937) and 752.7 pg/mL for peripheral blood (Se 78.6%, Sp 95.6%, AUC 0.935). Notably, P-SEP demonstrated superior diagnostic accuracy versus traditional biomarkers ( $p<0.001$ ). Temporal analysis revealed P-SEP's remarkable stability - maintaining diagnostic reliability for 72 hours in controls and 24 hours in EOS cases.

## Conclusion

P-SEP is a highly effective biomarker for EOS diagnosis in neonates, demonstrating superior accuracy over conventional tests. Umbilical cord blood P-SEP levels are consistently higher than peripheral samples. Moreover, peripheral blood P-SEP remains stable for 72 hours in healthy newborns and 24 hours in EOS cases, ensuring a reliable window for timely clinical intervention.

## 1 Background

Neonatal sepsis, characterized by pathogenic invasion and proliferation in the bloodstream, remains one of the leading causes of neonatal mortality worldwide. Based on time of onset, it is classified into EOS(occurring within 72 hours after birth) and late-onset sepsis (LOS). EOS primarily results from intrauterine or intrapartum infections, with an incidence of 2,202 per 100,000 live births and a mortality

rate of 11–19% [1]. The diagnostic challenge stems from neonates' immature immune systems and the non-specific clinical manifestations of EOS. Delayed diagnosis often leads to severe complications including septic shock and multiple organ dysfunction syndrome [2]. Conversely, unnecessary antibiotic use in non-EOS cases carries significant risks: increasing antibiotic resistance, disrupting microbial colonization, and elevating risks of necrotizing enterocolitis in preterm infants as well as long-term complications like asthma and obesity [3–4]. These dual risks underscore the urgent need for highly sensitive and specific inflammatory markers to facilitate timely treatment while minimizing inappropriate antibiotic exposure. While blood culture remains the diagnostic gold standard, its clinical utility is limited by prolonged turnaround times and low positivity rates [5]. Current practice relies on non-specific clinical signs and hematologic biomarkers to reduce severe EOS incidence and unnecessary antibiotic use [6]. However, conventional biomarkers including CBC, CRP, PCT, and IL-6 each have limitations in early EOS diagnosis. White blood count(WBC) is influenced by non-infectious factors, with neutrophil counts showing only 39–49% Se and 73–81% Sp [7]. CRP exhibits delayed elevation and overlapping levels between culture-positive and negative infants [8], though Se improves to 76–89% when measured at 12–24 hours after birth [7]. PCT rises within 3 hours of infection (peaking at 6 hours) [9], but its interpretation is complicated by physiological elevation during the first 72 hours of life [8, 10]. IL-6 suffers from poor specificity [1], making all these markers suboptimal for early EOS diagnosis. Recent advances highlight P-SEP as a promising alternative. Expressed on macrophages and monocytes, P-SEP rises within 2 hours of infection (peaking at 3 hours) and can be easily detected [11]. Importantly, P-SEP levels remain unaffected by perinatal inflammatory factors or neonatal age, making it an ideal candidate for early EOS diagnosis. To date, few studies have compared umbilical cord blood and peripheral blood P-SEP levels or investigated postnatal P-SEP trends. Our clinical research addresses these gaps by: (1) evaluating the diagnostic value of umbilical versus peripheral blood P-SEP; (2) comparing their performance against traditional biomarkers; (3) establishing optimal diagnostic cutoffs; and (4) analyzing P-SEP trends during the first 72 postnatal hours. This comprehensive assessment aims to establish P-SEP's clinical utility in early EOS diagnosis.

## 2 Methods

### 2.1 Study Subjects

Newborns delivered at Peking University Shenzhen Hospital between June 2024 and March 2026 who were at risk for EOS were selected as study subjects. The inclusion criteria required meeting two or more of the following four conditions:1) Prolonged rupture of membranes  $\geq 24$  hours;2) Maternal fever with temperature  $\geq 38^{\circ}\text{C}$  within 24 hours before delivery;3) Maternal WBC  $\geq 15 \times 10^9/\text{L}$  within 24 hours before or after delivery, accompanied by maternal CRP level  $\geq 10 \text{ mg/L}$ ;4) Uterine tenderness or foul-smelling amniotic fluid.Exclusion criteria included:1) Death or treatment withdrawal within 72 hours after birth;2) Presence of severe congenital malformations requiring surgical intervention;3) Lack of informed consent from guardians. Participants were divided into EOS and non-EOS groups based on whether they developed EOS.

Diagnostic Criteria for EOS(Referenced to China's latest guidelines [12]):1) Clinical Diagnosis: Newborns presenting with abnormal clinical manifestations within 72 hours after birth, including:Temperature instability, Respiratory system abnormalities, Digestive system abnormalities, Circulatory system abnormalities, Neurological abnormalities,or Pathological jaundice [13], And meeting any one of the following criteria: (1)  $\geq 2$  positive nonspecific blood tests:  $\square$  WBC  $\geq 25 \times 10^9/L$  or  $< 5 \times 10^9/L$ ;  $\square$  CRP  $\geq 3$  mg/L within 6h after birth;  $\geq 5$  mg/L at 6-24h;  $\geq 10$  mg/L after 24h;  $\square$  PCT beyond age-specific reference ranges during first 72h (considered positive) [14];  $\square$  PLT  $< 100 \times 10^9/L$ ; (2) Cerebrospinal fluid (CSF) findings consistent with purulent meningitis; (3) Detection of pathogenic bacterial DNA in blood or CSF. 2) Definitive Diagnosis: Presence of clinical manifestations and Positive blood culture, CSF culture (or culture from other sterile body cavity fluids).

Sample size calculation: The sample size was estimated based on the Se of the diagnostic test using the formula  $N = [(Z^2 \alpha / 2 \times Se \times (1 - Se))] / (L^2 \times P)$ . Parameter settings: Literature [15] reported the sensitivity of P-SEP for diagnosing sepsis as 0.8,  $\alpha$  was set at 0.05 (two-sided), the allowable error L was 0.1, and retrospective analysis of case data from our hospital over the past year showed that the probability of EOS in eligible cases was approximately 20%, with a prevalence P of 0.2. The calculated N was 310 cases. Considering a 10% dropout rate, the sample size was set at 345 cases. The actual enrolled sample size was 355 cases.

## 2.2 Research Methods

### 2.2.1 Data Collection

#### Maternal data

Maternal hospitalization records were reviewed through the electronic medical record system. Data collected included mode of delivery, group B streptococcal (GBS) colonization status of the genital tract, duration of premature rupture of membranes, amniotic fluid condition at delivery, body temperature within 24 h of delivery, pre- and postpartum white blood cell counts within 24 h, and antenatal antibiotic use.

#### Neonatal data

Data were reviewed through the electronic medical record system, including sex, gestational age, birth weight, small for gestational age (SGA) status, 1-minute and 5-minute Apgar scores, clinical symptoms and signs, and laboratory test results within 72 h after birth (CBC, CRP, PCT, IL-6, and blood culture).

### 2.2.2 Specimen Collection and Detection Methods

Umbilical cord blood collected after birth and peripheral blood samples collected within 72 h after birth were obtained for testing. Test items included CBC, CRP, PCT, IL-6, and P-SEP. Peripheral blood samples for P-SEP detection were obtained from residual samples after routine laboratory testing. Blood samples for P-SEP detection were immediately placed in a 4°C low-temperature environment and centrifuged at 3000 r/min for 5 min to separate the plasma. After stratification was complete, the upper plasma was carefully aspirated using a pipette and aliquoted into sterile 1.5 mL cryotubes, with each tube strictly labeled with the sample number and key information, and a detailed ledger was established. Finally, all samples were

rapidly transferred to a – 80°C ultra-low temperature freezer for storage, and subsequently tested for P-SEP after centralized thawing. Umbilical cord blood or venous blood samples for detection of traditional biomarkers were tested within 2 h of collection.

P-SEP, PCT, and IL-6 were detected by chemiluminescence assay, blood routine was detected by electrical impedance hematology analyzer, and CRP was detected by immunoturbidimetry.

**2.3 Ethics** This study protocol was approved by the Hospital Research Ethics Committee and registered with the Chinese Clinical Trial Registry (Ethics approval number: PKUSZH-IEC-2023-020; Chinese Clinical Trial Registry number: ChiCTR2300072525, registered on June 25, 2023. Chinese Clinical Trial Registry, <http://www.chictr.org.cn/>). Prior to study implementation, guardians of all participants were fully informed of the research purpose, procedures, and potential risks, and provided written informed consent. In addition, the entire process of sample collection and management strictly followed the relevant regulations and guidelines established by the Ethics Committee.

#### **2.4 Statistical Analysis**

Statistical analysis was performed using SPSS 27.0. Continuous variables with a normal distribution were presented as mean  $\pm$  standard deviation and analyzed using independent samples t-tests, whereas non-normally distributed data were expressed as median (interquartile range) [M (P25, P75)] and compared via the non-parametric rank-sum test. Categorical variables were summarized as frequencies and percentages (%) and assessed using  $\chi^2$  tests. For unpaired continuous variables, intra-group comparisons were performed using a generalized linear mixed model, with data visualization achieved through box plots. Additionally, ROC curves were generated, and the area under the curve (AUC) was calculated to evaluate the diagnostic efficacy of P-SEP for EOS. The optimal P-SEP cut-off value was determined based on the maximum Youden index, along with corresponding sensitivity and specificity. A two-sided P-value < 0.05 was considered statistically significant.

## **3 Results**

### **3.1 General Data**

This study enrolled a total of 355 eligible neonates, with gestational ages ranging from 29.3 to 41.7 weeks (median: 39.4 weeks). The cohort comprised 37 preterm infants (10.4%) and 318 full-term infants (89.6%). Among these, 95 neonates (26.8%) were classified into the EOS group, including 8 culture-confirmed cases (8.2%). The causative pathogens identified were *Listeria monocytogenes* (n = 2), *Escherichia coli* (n = 2), and one case each of GBS, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, and *Staphylococcus epidermidis*. The remaining 87 cases were clinically diagnosed as EOS. The non-EOS group consisted of 260 neonates (73.5%). Comparisons of baseline characteristics and perinatal factors between the two groups are presented in Table 1. Notably, maternal intrapartum fever was significantly more prevalent in the EOS group than in the non-EOS group (P < 0.05), whereas no other variables exhibited statistically significant differences (P > 0.05).

Table 1  
Analysis of perinatal and neonatal data between the two groups

| Item                                                                                                                                | Non-EOS group (n = 260) | EOS group (n = 95)  | $\chi^2/Z$ | P      |
|-------------------------------------------------------------------------------------------------------------------------------------|-------------------------|---------------------|------------|--------|
| GA (weeks)                                                                                                                          | 39.43(38.43, 40.14)     | 39.14(38.50, 39.86) | -1.474     | 0.14   |
| Birth weight (g)                                                                                                                    | 3200(2850, 3450)        | 3250(2950, 3485)    | -1.295     | 0.195  |
| Apgar score at 1 min                                                                                                                | 9 (9, 9)                | 9 (9, 9)            | -0.191     | 0.849  |
| Apgar score at 5 min                                                                                                                | 10 (10, 10)             | 10 (10, 10)         | -0.478     | 0.633  |
| PROM $\geq$ 24 h                                                                                                                    | 53 (20.4%)              | 19 (20%)            | 0.006      | 0.936  |
| Maternal GBS colonization                                                                                                           | 46 (17.7%)              | 11 (11.6%)          | 1.925      | 0.165  |
| Intrapartum maternal temperature $\geq$ 38.0°C                                                                                      | 74 (28.5%)              | 40 (42.1%)          | 5.941      | 0.015* |
| Maternal WBC $\geq$ 15 $\times$ 10 <sup>9</sup> /L                                                                                  | 54 (20.8%)              | 28 (29.5%)          | 2.968      | 0.085  |
| Turbid or foul-smelling amniotic fluid                                                                                              | 75 (28.8%)              | 28 (29.5%)          | 0.013      | 0.908  |
| Antenatal antibiotic use                                                                                                            | 140 (53.8%)             | 48 (50.5%)          | 0.308      | 0.579  |
| SGA                                                                                                                                 | 29 (11.2%)              | 6 (6.3%)            | 1.833      | 0.176  |
| Vaginal delivery                                                                                                                    | 167 (64.2%)             | 60 (63.2%)          | 0.035      | 0.852  |
| Male                                                                                                                                | 134 (51.5%)             | 45 (47.4%)          | 0.484      | 0.487  |
| Note: EOS, early-onset sepsis; categorical data are presented as n (%); continuous data are presented as M (P25, P75). $P < 0.05$ . |                         |                     |            |        |

## 3.2 Blood Specimen Collection

Peripheral blood samples were successfully obtained from 338 neonates (95 EOS; 243 non-EOS), while umbilical cord blood was collected from 195 cases (50 EOS; 145 non-EOS). The peripheral blood samples were further categorized by collection time into four intervals: T1 (0–6 h), T2 (6–12 h), T3 (12–24 h), and T4 (24–72 h). Sample distribution across these intervals showed 22 (EOS) vs 82 (non-EOS) at T1, 44 vs 60 at T2, 18 vs 51 at T3, and 1 vs 7 at T4, respectively.

## 3.3 Peripheral Blood Biomarkers

This study evaluated peripheral blood biomarker differences between 338 neonates with and without EOS, as presented in Table 2. Comparative analysis revealed significantly elevated levels of WBC, CRP, PCT, and IL-6 in the EOS group compared to non-EOS controls (all  $P < 0.05$ ). In contrast, PLT levels showed no significant intergroup difference ( $P > 0.05$ ). Notably, peripheral blood P-SEP concentrations were markedly higher in EOS cases ( $P < 0.001$ ). ROC curve analysis established diagnostic performance metrics

(Tables 3–4, Fig. 1). Traditional inflammatory markers demonstrated moderate diagnostic efficacy (Youden indices < 0.5), with significantly inferior AUC, Se, and Sp compared to P-SEP (all  $P < 0.05$ ). P-SEP emerged as the superior diagnostic biomarker, achieving an optimal cutoff of 752.73 pg/mL with outstanding performance metrics: AUC 0.935 (95% CI: 0.896–0.975), Se 78.6%, Sp 95.6%, and maximum Youden index 0.742. The corresponding positive predictive values (PPV) and negative predictive values (NPV) were 87.47% and 91.96%, respectively.

Table 2  
Comparison of peripheral blood biomarkers between the two groups

| Biomarker                                                                                                                           | Non-EOS group<br>(n = 243) | EOS group<br>(n = 95)  | Z      | P      |
|-------------------------------------------------------------------------------------------------------------------------------------|----------------------------|------------------------|--------|--------|
| P-SEP (pg/mL)                                                                                                                       | 532.21(430.34, 617.55)     | 841.98(774.0, 1089.13) | -9.63  | 0*     |
| WBC ( $\times 10^9/L$ )                                                                                                             | 18.00(13.52, 22.68)        | 21.71(17.65, 26.09)    | -3.146 | 0.002* |
| PLT ( $\times 10^9/L$ )                                                                                                             | 276.00(237.00, 312.00)     | 269.00(236.50, 308.00) | -0.715 | 0.475  |
| CRP (mg/L)                                                                                                                          | 0.32(0.20, 1.33)           | 2.41(0.94, 8.20)       | -7.375 | 0*     |
| PCT (ng/L)                                                                                                                          | 0.56(0.21, 1.14)           | 2.21(0.84, 6.07)       | -7.811 | 0*     |
| IL-6 (pg/mL)                                                                                                                        | 34.50(15.80, 87.40)        | 61.90(31.45, 451.50)   | -5.684 | 0*     |
| Note: EOS, early-onset sepsis; categorical data are presented as n (%); continuous data are presented as M (P25, P75). $P < 0.05$ . |                            |                        |        |        |

Table 3  
Diagnostic value of peripheral blood biomarkers for EOS

| Biomarker | AUC   | 95% CI       | Optimal cut-off value     | Se    | Sp    | PPV (%) | NPV (%) | Youden index |
|-----------|-------|--------------|---------------------------|-------|-------|---------|---------|--------------|
| P-SEP     | 0.935 | 0.896, 0.975 | 752.73 (pg/mL)            | 0.786 | 0.956 | 87.47   | 91.96   | 0.742        |
| WBC       | 0.662 | 0.582, 0.743 | 17.42 ( $\times 10^9/L$ ) | 0.786 | 0.474 | 36.87   | 85.00   | 0.26         |
| CRP       | 0.788 | 0.713, 0.862 | 1.87 (mg/L)               | 0.625 | 0.854 | 62.59   | 85.35   | 0.479        |
| PCT       | 0.797 | 0.725, 0.869 | 1.37 (ng/L)               | 0.679 | 0.825 | 60.26   | 86.8    | 0.504        |
| IL-6      | 0.678 | 0.597, 0.760 | 29.15 (pg/mL)             | 0.839 | 0.445 | 37.14   | 87.61   | 0.284        |

Table 4  
Comparison of diagnostic value between peripheral blood P-SEP and traditional biomarkers

| Comparison of biomarkers | AUC difference | Standard error | Z     | P         |
|--------------------------|----------------|----------------|-------|-----------|
| P-SEP vs CRP             | 0.206          | 0.0338         | 6.099 | < 0.0001* |
| P-SEP vs WBC             | 0.341          | 0.0369         | 9.231 | < 0.0001* |
| P-SEP vs IL-6            | 0.258          | 0.0331         | 7.811 | < 0.0001* |
| P-SEP vs PCT             | 0.177          | 0.0307         | 5.758 | < 0.0001* |
| Note: $P < 0.05$ .       |                |                |       |           |

### 3.4 Umbilical Cord Blood Biomarkers

This study conducted comparative analyses of umbilical cord blood biomarkers in 195 neonates (Table 5). The EOS group exhibited significantly elevated IL-6 and WBC levels compared to non-EOS controls (both  $P < 0.05$ ). Notably, cord blood P-SEP concentrations were markedly higher in EOS cases ( $P < 0.001$ ). ROC curve analysis (Tables 6–7, Fig. 2) revealed that traditional biomarkers (WBC, IL-6) showed moderate diagnostic efficacy (Youden indices  $< 0.5$ ), with significantly inferior performance metrics to P-SEP. Umbilical cord P-SEP demonstrated superior diagnostic capacity with an optimal cutoff of 762.96 pg/mL: AUC 0.937 (95% CI: 0.899–0.975), Se 94%, Sp 77.2%, and Youden index 0.712. The PPV and NPV were 58.65% and 97.40%, respectively.

Table 5  
Comparison of umbilical cord blood P-SEP and traditional biomarkers between the two groups

| Biomarker                                                                                  | Non-EOS group (n = 145) | EOS group (n = 50)       | Z      | P      |
|--------------------------------------------------------------------------------------------|-------------------------|--------------------------|--------|--------|
| P-SEP (pg/mL)                                                                              | 557.22 (461.48, 647.34) | 856.13 (766.23, 1124.64) | -6.83  | 0*     |
| WBC ( $\times 10^9/L$ )                                                                    | 16.28 (13.20, 19.75)    | 19.50 (15.83, 23.00)     | -2.563 | 0.01*  |
| PLT ( $\times 10^9/L$ )                                                                    | 230.00 (205.00, 267.00) | 226.00 (186.75, 267.75)  | -0.494 | 0.621  |
| CRP (mg/L)                                                                                 | 0.21 (0.20, 0.31)       | 0.22 (0.20, 0.37)        | -0.532 | 0.595  |
| PCT (ng/L)                                                                                 | 0.53 (0.21, 1.13)       | 0.66 (0.34, 1.15)        | -1.396 | 0.163  |
| IL-6 (pg/mL)                                                                               | 18.90 (10.25, 35.58)    | 32.75 (16.53, 114.90)    | -3.093 | 0.002* |
| Note: EOS, early-onset sepsis; continuous data are presented as M (P25, P75). $P < 0.05$ . |                         |                          |        |        |

Table 6  
Diagnostic value of umbilical cord blood biomarkers for EOS

| Biomarker | AUC   | 95% CI       | Optimal cut-off value     | Se    | Sp    | PPV (%) | NPV (%) | Youden index |
|-----------|-------|--------------|---------------------------|-------|-------|---------|---------|--------------|
| P-SEP     | 0.937 | 0.899, 0.975 | 762.96 (pg/mL)            | 0.940 | 0.772 | 58.65   | 97.40   | 0.712        |
| WBC       | 0.628 | 0.537, 0.720 | 17.35 ( $\times 10^9/L$ ) | 0.680 | 0.579 | 33.56   | 86.96   | 0.259        |
| IL-6      | 0.662 | 0.569, 0.754 | 18.90 (pg/mL)             | 0.840 | 0.441 | 31.58   | 90.24   | 0.281        |

Table 7  
Comparison of diagnostic value between umbilical cord blood P-SEP and traditional biomarkers

| Comparison of biomarkers | AUC difference | Standard error | Z     | P         |
|--------------------------|----------------|----------------|-------|-----------|
| P-SEP vs WBC             | 0.309          | 0.0458         | 6.741 | < 0.0001* |
| P-SEP vs IL-6            | 0.275          | 0.0416         | 6.614 | < 0.0001* |
| Note: $P < 0.05$ .       |                |                |       |           |

### 3.5 Comparison of P-SEP Levels Between Umbilical Cord Blood and Peripheral Blood

This study conducted a comparative analysis of P-SEP concentrations between umbilical cord blood and peripheral blood samples. In both EOS and non-EOS groups, umbilical cord blood consistently exhibited significantly higher P-SEP levels compared to peripheral blood ( $P < 0.001$ ). Detailed results are presented in Table 8.

Table 8  
Comparison of P-SEP levels between umbilical cord blood and peripheral blood within each group

| Group                                                                                      | Umbilical cord blood                 | Peripheral blood                    | F      | P      |
|--------------------------------------------------------------------------------------------|--------------------------------------|-------------------------------------|--------|--------|
| EOS group                                                                                  | 1084.505pg/mL<br>(854.270, 1228.390) | 841.980pg/mL<br>(774.000, 1089.130) | 11.649 | 0.001* |
| Non-EOS group                                                                              | 620.070pg/mL<br>(508.970, 759.550)   | 532.210pg/mL<br>(430.340, 617.550)  | 23.568 | 0.000* |
| Note: EOS, early-onset sepsis; continuous data are presented as M (P25, P75). $P < 0.05$ . |                                      |                                     |        |        |

### 3.6 Temporal Trend of Peripheral Blood P-SEP Levels at Different Time Points After Birth

To investigate postnatal P-SEP dynamics, we longitudinally analyzed peripheral blood levels at different time points after birth. Given the limited sample size (n = 1) in the EOS group’s T4 subgroup, this timepoint was excluded from analysis. Comparative analysis revealed stable P-SEP concentrations: In the EOS group, levels remained unchanged within the first 24 postnatal hours ; In the non-EOS group, levels showed no significant fluctuation through 72 postnatal hours. (**See Table 9 and Fig. 3 for detailed temporal trends**).

Table 9

Comparison of peripheral blood P-SEP at different time points within each group

| Group         | -            | T1                               | T2                               | T3                               | T4                              | F     | P     |
|---------------|--------------|----------------------------------|----------------------------------|----------------------------------|---------------------------------|-------|-------|
| EOS group     | n (cases)    | 22                               | 44                               | 18                               | 1                               | –     | –     |
| -             | M (P25, P75) | 924.94pg/mL<br>(814.55, 1092.27) | 843.89pg/mL<br>(783.29, 1017.80) | 850.03pg/mL<br>(771.49, 1009.10) | –                               | 0.431 | 0.731 |
| Non-EOS group | n (cases)    | 82                               | 60                               | 51                               | 7                               | –     | –     |
| -             | M (P25, P75) | 470.84pg/mL<br>(400.78, 566.07)  | 533.44pg/mL<br>(433.87, 598.71)  | 527.80pg/mL<br>(430.85, 585.33)  | 525.06pg/mL<br>(375.68, 574.77) | 1.813 | 0.146 |

Note: EOS, early-onset sepsis; continuous data are presented as M (P25, P75). T1–T4 represent different time points after birth. The T4 subgroup in the EOS group (n = 1) was excluded from analysis.

## 4 Discussion

EOS remains a formidable challenge in neonatal medicine despite therapeutic advancements, continuing to account for significant morbidity and mortality worldwide. The condition's characteristically rapid clinical deterioration underscores the critical need for precise diagnostic tools capable of facilitating timely interventions while minimizing unnecessary antibiotic exposure.

Blood culture, while maintaining its status as the diagnostic gold standard, presents several inherent limitations. Contemporary studies report concerningly low positivity rates, ranging from 4% in general neonatal populations to 6.7–55.4% specifically for EOS cases[12, 16], with our own data showing 8.2% positivity. This diagnostic inadequacy stems from multiple factors: the technical challenge of obtaining adequate blood volumes (typically limited to 1mL in neonates) [17], potential interference from maternal antibiotic prophylaxis, and the intrinsic limitations of microbial culturability. Furthermore, the extended processing time (typically requiring 36–48 hours) [12, 18] creates potentially dangerous therapeutic delays during this critical period. Traditional biomarkers including PCT, CRP, and IL-6 demonstrate insufficient diagnostic specificity due to their frequent elevation in non-infectious conditions. In contrast, P-SEP, the N-terminal fragment of soluble CD14 subtype (sCD14-ST), has emerged as a particularly promising alternative biomarker [19]. P-SEP exhibits favorable kinetic properties, reaching peak concentrations within

three hours post-infection while demonstrating relative resistance to perinatal confounding factors. Recent meta-analytic data corroborate its diagnostic potential, showing pooled Se and Sp of 82% (95% CI: 0.62–0.93) and 86% (95% CI: 0.73–0.93) respectively [20]. However, clinical adoption remains constrained by several unresolved issues: limited validation studies, significant heterogeneity in sampling protocols, and the absence of standardized diagnostic thresholds.

Our findings demonstrate the superior diagnostic performance of both umbilical cord blood and peripheral blood P-SEP compared to conventional biomarkers, as evidenced by their enhanced sensitivity, specificity, and AUC values. Umbilical cord blood P-SEP exhibited remarkable Se (94.0%) and Sp (77.2%), with an outstanding AUC of 0.937. Peripheral blood P-SEP showed equally impressive performance, achieving 78.6% Se and 95.6% Sp, along with an AUC of 0.935. These results not only align with prior reports [20–24] but were obtained in a significantly larger cohort (n = 355), strengthening their clinical validity.

The distinct diagnostic profiles of these sampling methods suggest optimal clinical applications: umbilical cord blood P-SEP's exceptional Se (94.0%) and NPV (97.4%) render it ideal for initial screening, while peripheral blood P-SEP's superior specificity (95.6%) combined with high PPV (87.41%) and NPV (91.96%) make it particularly suitable for confirmatory testing. This complementary performance supports implementing a two-stage diagnostic strategy ("cord blood screening + peripheral blood confirmation") to maximize EOS diagnostic accuracy.

From a clinical perspective, the cord blood P-SEP's high NPV (97.4%) provides clinicians with reliable immediate postnatal evidence to exclude infection, significantly reducing unnecessary antibiotic exposure. Its non-invasive collection further enhances its appeal as an ideal intrapartum screening tool. Conversely, peripheral blood P-SEP's strong PPV enables confident identification of true-positive cases requiring prompt therapeutic intervention.

Our comparative analysis revealed consistently higher P-SEP concentrations in umbilical cord blood versus peripheral blood, observed in both EOS and non-EOS cohorts. Currently, there are very few published studies comparing P-SEP levels between umbilical cord blood and peripheral blood. Prior research presents conflicting observations - a study of healthy neonates reporting no significant difference in P-SEP levels at birth [25], versus Dierikx et al.'s demonstration of elevated cord blood P-SEP specifically in EOS cases [26]. The observed disparity may be attributed to our study population's distinctive characteristics. Unlike previous investigations focusing on healthy neonates [25], our cohort comprised high-risk infants with established infection risk factors. We hypothesize that intrauterine stress responses triggered by conditions such as PROM and maternal fever may have significantly contributed to this P-SEP elevation pattern, potentially explaining the divergence from previously reported findings.

Our longitudinal analysis of peripheral blood P-SEP dynamics revealed clinically important temporal patterns. In non-EOS neonates, P-SEP levels remained remarkably stable throughout the first 72 postnatal hours, contrasting sharply with the well-documented physiological surge observed in PCT during this period. This temporal stability significantly enhances P-SEP's diagnostic utility for EOS by eliminating age-related variability that complicates other biomarkers.

In the EOS cohort, our observations (limited to the first 24 hours by specimen availability constraints) demonstrated similarly stable P-SEP levels, maintaining persistently elevated concentrations throughout the acute inflammatory phase. Unlike IL-6 - which rapidly declines due to its short half-life [22, 27–29] - P-SEP's sustained elevation provides a more forgiving diagnostic window, reducing the risk of false-negative results due to sampling timing variations. This characteristic offers distinct clinical advantages: (1) reduced susceptibility to physiological stress interference, (2) improved inter-timepoint comparability, and (3) enhanced diagnostic reliability across a broader sampling window - all crucial for early EOS detection. A notable limitation remains our inability to assess P-SEP kinetics between 24–72 hours in EOS cases due to specimen collection gaps. This will be a direction for further research in the future. Our study presents several key innovations: First, we conducted a comprehensive evaluation of both umbilical cord blood and peripheral blood P-SEP for EOS diagnosis, demonstrating their complementary diagnostic advantages. While umbilical cord blood P-SEP proves more valuable for initial screening, peripheral blood P-SEP offers greater confirmatory potential. By establishing optimal diagnostic thresholds for each, we show how their combined use significantly enhances diagnostic accuracy. Second, our temporal analysis revealed that peripheral blood P-SEP maintains remarkable stability during the first 24 hours postpartum, solidifying its status as an ideal early biomarker for EOS. Third, with a robust sample size of 355 cases, our study substantially surpasses previous reports in statistical power and reliability.

While this study provides valuable insights, several limitations merit consideration. First, the relatively small number of culture-confirmed sepsis cases in our cohort may introduce potential bias in assessing P-SEP's diagnostic performance. Second, the paired collection of umbilical cord blood and peripheral blood specimens was incomplete in some cases, with specimen availability varying across time points - a factor that could influence the accuracy of P-SEP trend analysis. Furthermore, our investigation focused exclusively on P-SEP's diagnostic utility for EOS, leaving its prognostic implications unexplored. These limitations highlight the need for future multicenter studies with larger sample sizes, comprehensive paired specimen collection, and longitudinal follow-up to fully elucidate P-SEP's clinical potential in EOS management.

## 5 Conclusion

This study establishes P-SEP as an optimal biomarker for early neonatal EOS diagnosis, demonstrating superior predictive performance compared to traditional biomarkers when measured in either umbilical cord or peripheral blood. Our analyses revealed an umbilical cord blood P-SEP optimal cutoff of 762.96 pg/mL (94% sensitivity, 77.2% specificity, AUC 0.937), while peripheral blood showed optimal discrimination at 752.73 pg/mL (78.6% sensitivity, 95.6% specificity, AUC 0.935). Notably, umbilical cord blood exhibited consistently higher P-SEP concentrations than peripheral blood.

A particularly valuable finding concerns P-SEP's temporal stability - levels remained remarkably consistent within 72 hours postpartum in non-EOS cases and within 24 hours in EOS cases. This stable kinetic profile significantly enhances P-SEP's clinical utility for early EOS detection, as it minimizes variability concerns associated with sampling timing.

# Abbreviations

|                                |
|--------------------------------|
| WBC                            |
| white blood count              |
| PLT                            |
| platelet                       |
| CRP                            |
| C-reactive protein             |
| IL-6                           |
| Interleukin-6                  |
| PCT                            |
| Procalcitonin                  |
| P-SEP                          |
| Presepsin                      |
| CBC                            |
| complete blood count           |
| sCD14-ST                       |
| soluble CD14 subtype           |
| EOS                            |
| Ealy Onset Sepsis              |
| LOS                            |
| late-onset sepsis              |
| PROM                           |
| Premature Rupture of Membranes |
| GBS                            |
| Group B Streptococcus          |
| CSF                            |
| Cerebrospinal fluid            |
| GA                             |
| Gestational Age                |
| SGA                            |

|                            |
|----------------------------|
| Small for Gestational Age  |
| Se                         |
| sensitivity                |
| Sp                         |
| specificity                |
| PPV                        |
| positive predictive values |
| NPV                        |
| negative predictive values |
| AUC                        |
| the area under the curve   |

## Declarations

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No funding was received for this work.

### Competing Interests

The authors have no conflicts of interest to declare.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article.

### Ethics approval and consent to participate

This research protocol has been reviewed and approved by the hospital's scientific research ethics committee (Ethical Approval Number: Peking University Shenzhen Medical University (Research) [2023] No. 020. Written informed consent was obtained from all participants prior to their participation in the study, in accordance with the Declaration of Helsinki.

### Consent for publication

Written informed consent was obtained from neonates' parents for the study's publication.

## Competing interests

The authors report no competing interests.

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## Figures

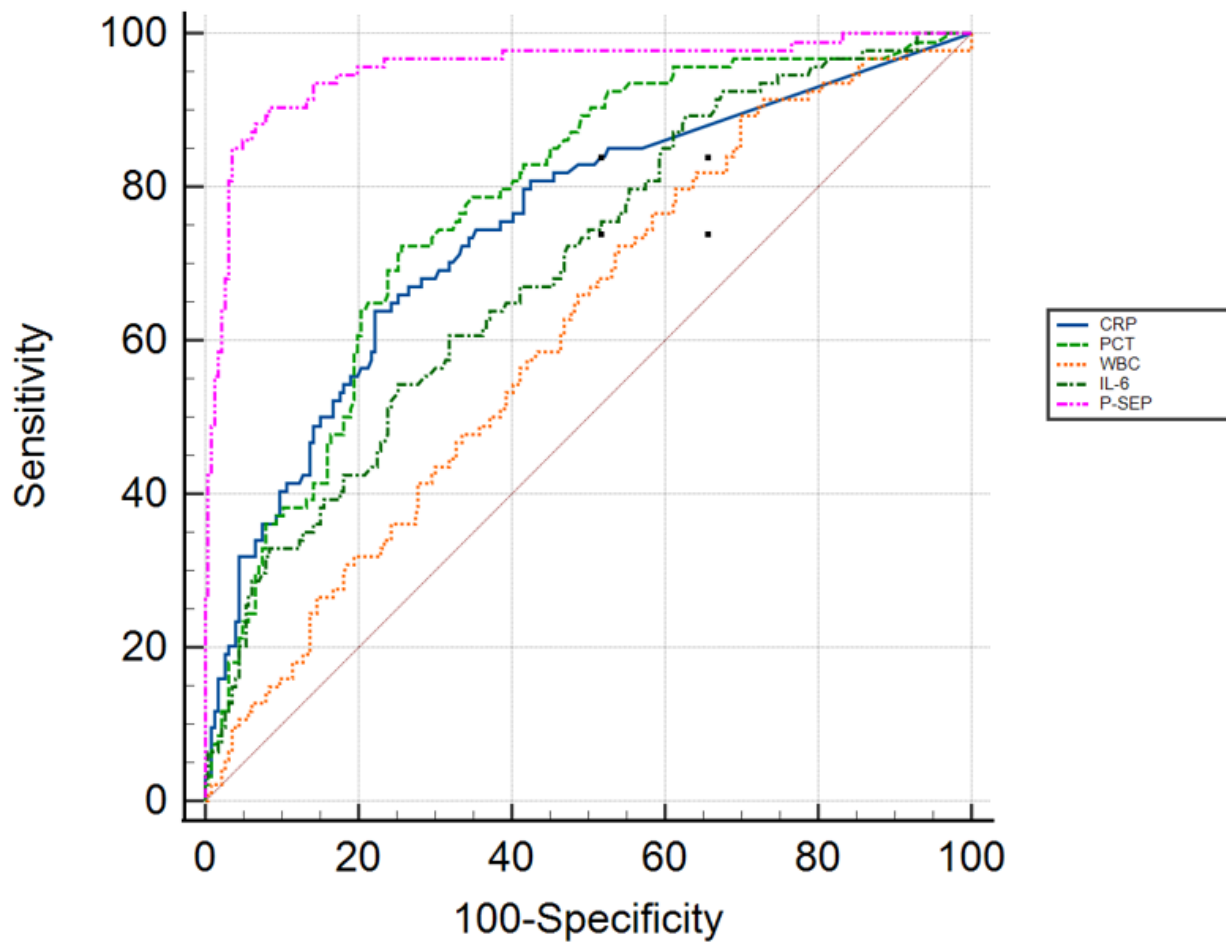


Figure 1

ROC curves of peripheral blood biomarkers for the diagnosis of EOS

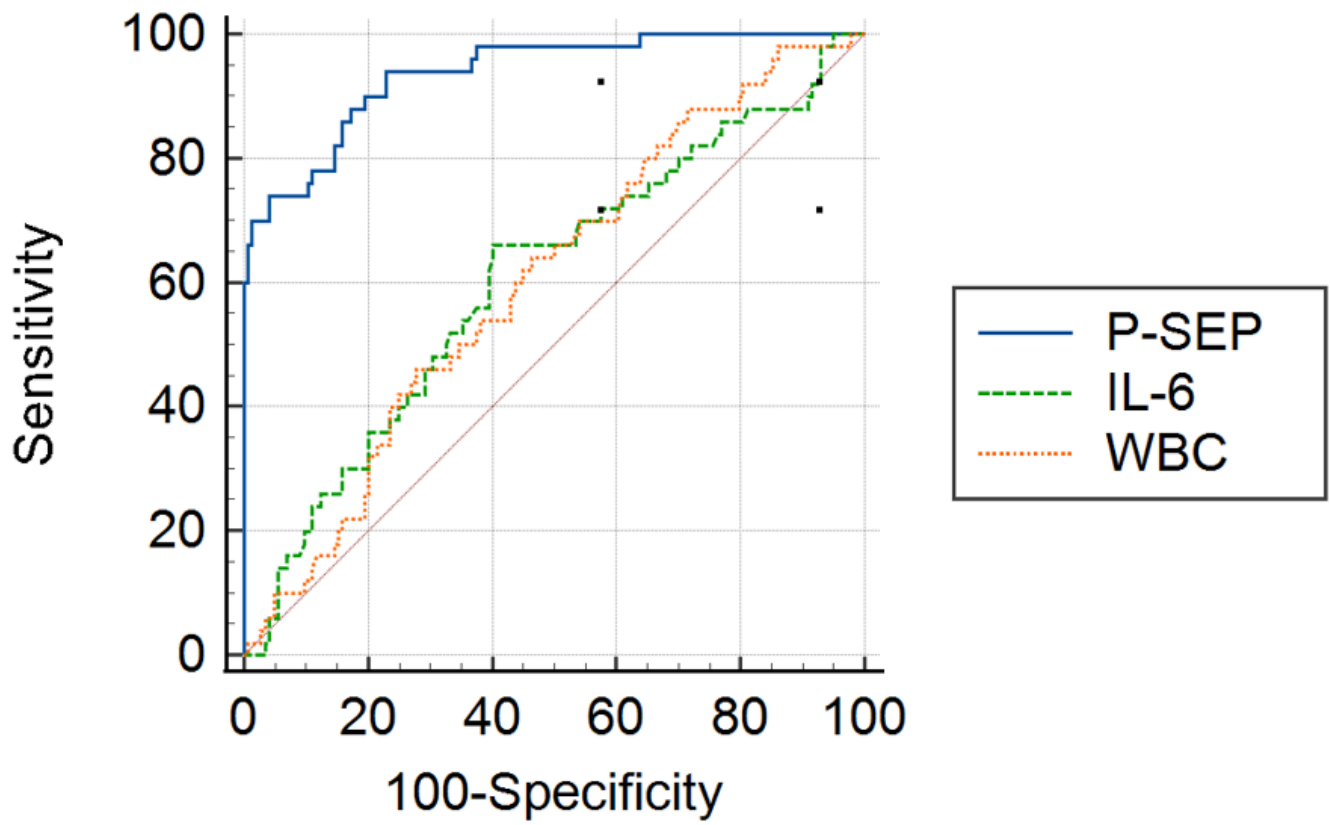


Figure 2

ROC curves of umbilical cord blood biomarkers for the diagnosis of EOS.

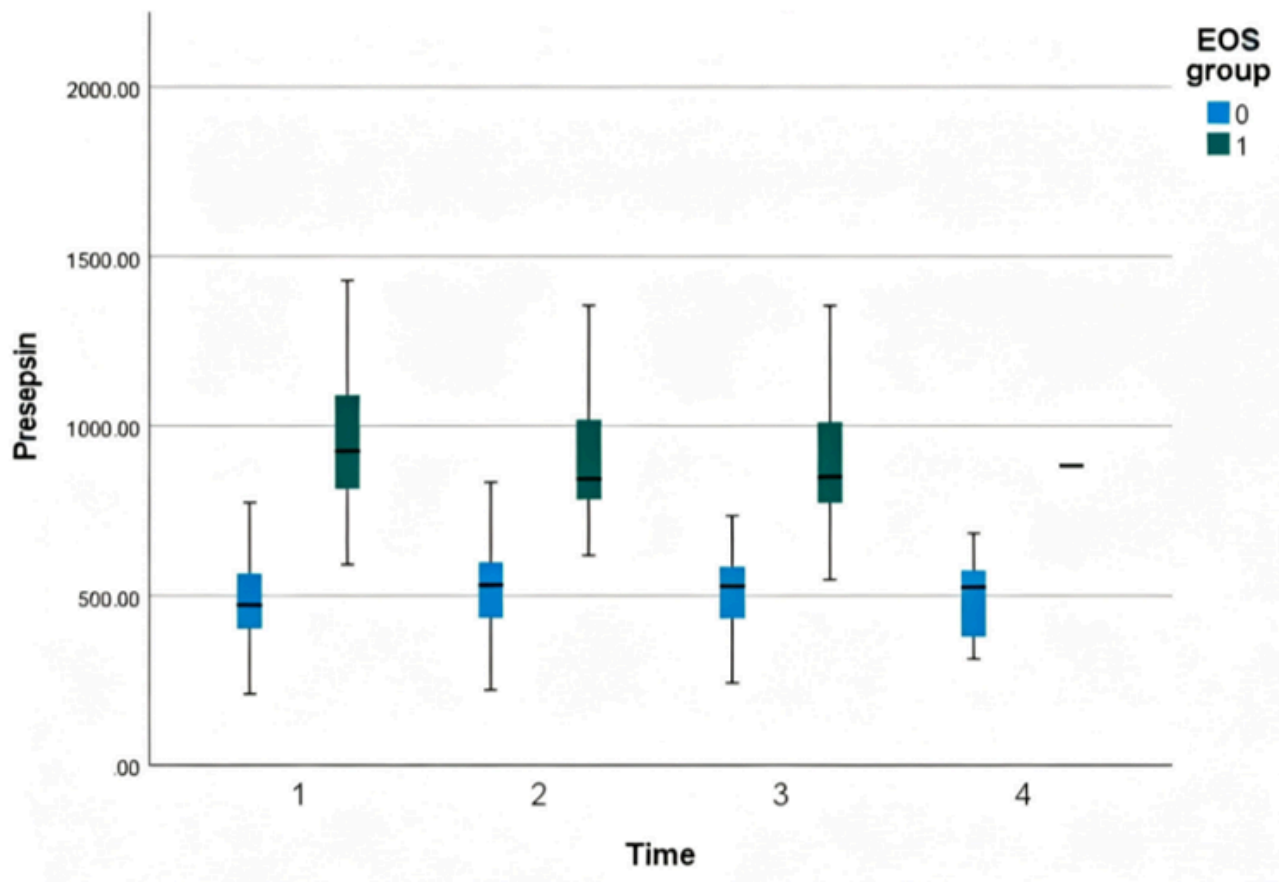


Figure 3

Peripheral blood P-SEP levels at different time points in neonates of the EOS and non-EOS groups.