Received: 30 October 2023 | Revised: 3 January 2024 | Accepted: 10 January 2024

DOI: 10.1111/apa.17114

ACTA PÆDIATRICA NURTURING THE CHILD WILEY

ORIGINAL ARTICLE

Maternal biomarkers in predicting neonatal sepsis after preterm premature rupture of membranes in preterm infants

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Abstract

Aim: This retrospective cohort study aimed to assess the utility of maternal C-reactive protein (CRP) and leukocyte levels in predicting neonatal sepsis after preterm premature rupture of membranes (pPROM).

Methods: We conducted a retrospective cohort study (2009–2021), encompassing preterm infants born ≤29 + 6 weeks of gestation following pPROM. The primary outcome was early-onset neonatal sepsis within the initial 72 h of life.

Results: We analysed data from 706 patients with a median gestational age at pPROM of 25.1 weeks and a median gestational age at birth of 26.4 weeks. Overall survival rate was 86.1%, with 65.7% survival without severe morbidities. These rates were significantly worse in preterm infants with sepsis. Maternal CRP and leukocyte levels correlated significantly with neonatal infection markers and sepsis. However, their predictive values, correlation coefficients, and area under the curve values were generally low. Using maternal CRP ≥2 mg/dL to predict neonatal sepsis yielded a positive predictive value of 18.5%, negative predictive value of 91.5%, AUC of 0.589, 45.5% sensitivity, and 74.5% specificity.

Conclusion: Maternal CRP and leukocyte levels were ineffective as a tool for predicting early-onset neonatal sepsis following early pPROM. Consequently, these biomarkers lack the reliability required for clinical decision-making in this context.

KEYWORDS
chorioamnionitis, mortality and morbidity, neonatal sepsis, preterm birth, preterm premature rupture of membranes

1 | INTRODUCTION

Preterm premature rupture of membranes (pPROM), characterised by the spontaneous rupture of foetal membranes prior to the initiation of labour before the 37th week of gestation, presents in roughly 3% of gestations and contributes to 30% of preterm births.[1] Approximately 30% of mothers experiencing pPROM develop chorioamnionitis, a condition that can subsequently result in

Abbreviations: AUC, area under the curve; BPD, bronchopulmonary dysplasia; c-PVL, cystic periventricular leukomalacia; CRP, C-reactive protein; EONS, early-onset neonatal sepsis; FIRS, foetal inflammation response syndrome; IL, interleukin; IVH, intraventricular haemorrhage; pPROM, preterm premature rupture of membranes; ROC, receiver operating characteristic; ROP, retinopathy of prematurity.

neonatal sepsis with a substantial contribution to both mortality and morbidity.[1]

The management of pregnancies following pPROM is one of the most important topics in perinatal medicine. Broadly, the management of pPROM is guided by two primary objectives: avoiding foetal prematurity and preventing the onset of chorioamnionitis. One of the most important issues is the correct and early diagnosis of maternal and foetal infection.

Maternal surveillance after pPROM involves assessing clinical indicators for chorioamnionitis and employing biochemical markers to detect infection. Nevertheless, the routine application of markers for inflammation or infection lacks substantial evidence.[1–4]

C-reactive protein (CRP) is an acute-phase protein secreted by the liver in response to inflammation. While it is not specific for infection, it is a marker used for the diagnosis of many inflammatory, infective and malignant conditions.[5] CRP is commonly used for the early diagnosis of chorioamnionitis despite missing supporting evidence. Systematic reviews examining the utility of CRP for the early diagnosis of histological chorioamnionitis have yielded conflicting results.[5] They indicate only a moderate capacity to predict histological chorioamnionitis, while not proving advantageous for early prediction of clinical chorioamnionitis.[6] Studies for CRP levels, using a wide range of cut-off levels between 0.7 and 8 mg/dL, have reported sensitivity and specificity rates as low as 59%–69% and 77%–83%, and for maternal leukocytes even lower corresponding rates of 51% and 65%, respectively.[3,7]

The anticipation of neonatal sepsis stands as a highly crucial concern, and unfortunately, the existing evidence falls short of addressing this topic satisfactorily. Notably, three reviews omitted neonatal sepsis as an outcome parameter,[3,5,7] while the fourth review ascertained that available data were inadequate for evaluating this aspect.[6]

Several papers on maternal CRP and neonatal outcome showed no correlation with neonatal complications,[8] cut-off values as low as 0.8 mg/dL[9] or as high as >9.5 mg/dL,[10] both unusable in clinical routine, and areas under the receiver operating characteristic (ROC) curve of only 0.61.[11] Also the combination of maternal fever, CRP and leukocytes for prediction of foetal inflammatory response syndrome (FIRS) showed an area under the curve (AUC) of only 0.66.[12]

It is noteworthy that all these studies only encompassed cases of pPROM up to 35–37 weeks of gestation. In a recent paper analysing a non-pPROM collective, maternal CRP levels were elevated in mothers of term-born neonates with sepsis, but not in mothers of preterm neonates with sepsis.[13]

Nonetheless, owing to the lack of adequate parameters to direct management following pPROM, maternal CRP and leukocytes continue to be extensively used by clinicians. This practice carries substantial implications for clinical decision-making, thus necessitating thorough evaluation.[4,11,14]

The main focus of our study was to assess the accuracy of maternal CRP and leukocyte levels in predicting neonatal sepsis after pPROM in preterm infants born ≤29 + 6 weeks of gestation, and as a secondary aspect the additional contribution of maternal white blood cell count.

Key notes

- Maternal inflammation markers are frequently used in clinical practice for management after pPROM despite lack of conclusive evidence.
- Maternal CRP and leukocyte levels were ineffective as a tool for predicting early-onset neonatal sepsis following pPROM at a median gestational age of 25.1 weeks.
- Clinical decision-making after pPROM cannot be based on maternal CRP and leukocyte levels, therefore, alternative detecting methods for intrauterine infection are urgently needed.

2 | PATIENTS AND METHODS

2.1 | Study setting

The appropriate Institutional Review Board approved the study protocol (145/2014). Due to the retrospective character of our study, no patient consent was requested. The study was conducted at our tertiary perinatal centre comprising 54 neonatal care beds with an approximate annual rate of 2500 to 2800 deliveries.[15] On average, 90 infants <1000 g and 180 infants <1500 g are treated per year with a survival rate of 82% and 90%, respectively.

The present study was a retrospective, hospital-based cohort analysis encompassing singleton and twin live-born preterm individuals with a gestational age of ≤29 + 6 weeks, born following pPROM. The study period spanned from June 1st, 2009 to December 31st, 2021. Exclusion criteria included chromosomal abnormalities, significant malformations, or inborn errors of metabolism.

2.2 | Obstetrical standard care

The diagnosis of pPROM was established through direct visualisation of amniotic fluid or the detection of insulin-like growth factor binding protein 1 or placental alpha microglobulin-1 in the vaginal fluid. Our standard management protocol entailed antenatal corticosteroid treatment using betamethasone at a dose of 12 mg, administered in two doses 24 h apart. Per our standard protocol, antibiotic therapy was administered using a 7-day regimen of 4 g ampicillin three times daily. Beginning in 2017, a single shot of 1 g azithromycin was added to the antibiotic prophylaxis.[16]

In case of bacterial vaginosis in the Gram-stained smear and/or allergy to the standard regimen, patients received 600 mg clindamycin three times daily for a 7-day period. Labour tocolysis with

atosiban was used for a minimum of 48 h. Subsequently, beginning in 2015, magnesium sulfate for foetal neuroprotection was administered within the final 24 h prior to delivery. Clinical chorioamnionitis was suspected in the presence of maternal fever of more than 38°C, maternal tachycardia >100/min, purulent vaginal discharge, uterine tenderness, or foetal tachycardia >160/min. Microbiological cultures of placental tissue and amniotic membranes were collected in case of C-section to identify bacterial and fungal growth. Growth of contaminants (e.g. *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Lactobacillus* spp.) were considered as negative results. In women delivering vaginally, no placental/amniotic cultures were obtained.

2.3 | Neonatal standard care

In the delivery room, standard management procedures included the early application of high-flow continuous positive airway pressure via Benveniste valve and the administration of surfactant via a thin catheter to spontaneously breathing infants with a gestational age of ≤27 + 6 weeks as a prophylactic measure, and ≤29 + 6 weeks as an early rescue therapy. This approach utilised the less invasive surfactant administration method known as LISA. For all infants born ≤27 + 6 weeks of gestation, blood cultures were collected, and broad-spectrum antibiotics (ampicillin and gentamicin) were initiated. Antibiotics were ceased after 48 hours if both the initial blood cultures taken at birth and laboratory biomarkers of infection remained negative. Routine blood sampling was conducted on days 1 and 3 of life.

2.4 | Descriptive data and definition of neonatal sepsis status

Obstetric and perinatal variables were extracted from patient records. Neonatal pH and serum lactate levels from the infant's blood gas analyses within the initial hour after birth were analysed. Neonatal laboratory biomarkers within the initial 72 h of life were classified as indicative of infection if two or more of the following criteria were met: CRP level ≥2 mg/dL, interleukin 8 (IL-8) concentration >100 pg/mL, interleukin 6 (IL-6) concentration >100 pg/mL, immature to total neutrophil count ratio (I/T-ratio) ≥0.2, white blood cell count either <4000 G/L or >20000 G/L. Starting in 2015, as part of our sepsis screening, we transitioned from measuring IL-8 to using IL-6. Results of blood cultures conducted within the first 72 h of the infant's life were documented. Clinical signs consistent with early-onset neonatal sepsis (EONS) were severe respiratory impairment necessitating mechanical ventilation and/or cardiovascular dysfunction requiring inotropic support, both exceeding the anticipated range for the specific gestational week. Clinical sepsis was diagnosed within the initial 72 h of life by the neonatal intensive care unit team if any of the aforementioned symptoms were observed additionally to positive biomarkers.

Neonatal sepsis status within the initial 72 h of life was classified into four distinct, non-overlapping groups: group 1: culture-positive EONS determined by positive blood culture results taken immediately after birth, accompanied by clinical signs consistent with neonatal sepsis, usually the administration of antibiotic therapy for 7 days; group 2: culture-negative EONS ('clinical sepsis') defined by the presence of positive laboratory biomarkers and clinical signs consistent with neonatal sepsis as described above, and negative blood culture results, usually administration of antibiotic therapy for 7 days; group 3: patients with positive laboratory biomarkers, yet exhibiting negative blood cultures, and absent clinical signs consistent with neonatal sepsis, usually administration of antibiotic therapy at least 5 days; group 4: patients with negative laboratory biomarkers and blood cultures, along with absent clinical signs consistent with neonatal sepsis as previously defined.

2.5 | Neonatal outcomes

Mortality prior to hospital discharge was subjected to analysis. The presence of severe cerebral morbidity was indicated if either grade III or IV intraventricular haemorrhage (IVH), or cystic periventricular leukomalacia (c-PVL) grades II-IV was diagnosed. Bronchopulmonary dysplasia (BPD) was defined as a requirement for oxygen support at 36 weeks postmenstrual age. The incidence of severe retinopathy of prematurity (ROP) classified as grade 3 or higher was assessed. Finally, severe morbidity was defined as a composite outcome involving severe cerebral morbidity, BPD, and/or severe ROP.

2.6 | Statistical analysis

For statistical analysis, we used SPSS Statistics, version 27 for Mac (IBM Corporation, New York, USA). A two-sided p-value <0.05 was considered statistically significant. Demographic data are shown as means ± standard deviation (SD) or median and interquartile range (IQR) for quantitative data and counts and percentages for qualitative data. Differences between groups were compared using t-test, Mann–Whitney U test and chi-square test. Pearson correlation coefficient (PCC) was calculated. Regarding the prediction of neonatal laboratory parameters and neonatal sepsis status, ROC curves and AUC values were calculated to determine the prognostic ability of maternal laboratory biomarkers. ROC was then used to detect sensitivity, specificity as well as positive and negative predictive values for frequently used cut-off points.

3 | RESULTS

During the study period, 721 patients were born after pPROM at a gestational age of ≤29 + 6 weeks. Data on laboratory biomarkers and clinical symptoms characterising neonatal sepsis status in the initial

72 h of life were available for 706 of these patients; any missing information was attributed to early postnatal mortality.

Tables 1 and 2 present maternal and neonatal characteristics of the four distinct categories based on neonatal sepsis status. The median gestational age at the time of pPROM was 25.1 weeks, and the median gestational age at birth was 26.4 weeks.

As shown in Table 2, among the participants, 27 infants (3.8%) had culture-positive EONS (group 1), 54 (7.6%) were diagnosed with culture-negative EONS (group 2), 111 (15.7%) had positive laboratory biomarkers without additional clinical signs consistent with neonatal sepsis (group 3), and 514 patients (72.8%) had negative laboratory biomarkers and absent clinical signs consistent with neonatal sepsis (group 4).

The majority of the identified pathogens were *E. coli* and *Klebsiella pneumonia* (21/27), the remaining pathogens were only isolated in single cases. All babies were initially treated with the empiric standard therapy, however, this was changed subsequently in the majority of cases (23/27), mainly to piperacillin/tazobactam and gentamycin. Reasons were microbial resistance ($n=21$), non-decreasing infection parameters, or severe clinical sepsis.

The overall survival rate was 86.1% with significant differences between the groups. Specifically, survival rates of groups 1 to 4 were 63.0%, 53.7%, 92.8% and 91.8%, respectively (Table 2).

The overall incidence of survival without severe morbidities was 65.7%, revealing significant variations among the groups. Specifically, survival rates without severe morbidities of groups 1 to 4 were 48.1%, 16.7%, 68.5% and 73.2%, respectively (Table 2).

Chi-square tests (Table 3) showed a significant association between maternal laboratory biomarkers and neonatal biomarkers. Yet, the corresponding Pearson correlation coefficients indicated consistently weak connections.

AUC calculations of ROC curves (Table 4) demonstrated insufficient discriminative capability for maternal laboratory biomarkers in differentiating neonatal laboratory biomarkers as well as neonatal sepsis status, as indicated by AUC values between 0.5 and 0.7, except for maternal CRP level as an acceptable predictor regarding abnormal neonatal leukocyte counts (AUC 0.718). Data separated based on maternal antibiotic prophylaxis is shown in Table S1.

The four groups of neonatal sepsis status showed significant associations with maternal laboratory biomarkers (Table 5). However, the ROC curve analysis for predicting EONS (culture-positive or negative; groups 1 and 2) compared to clinically stable infants (groups 3 and 4) revealed limited discriminatory capability for all maternal biomarkers (Figure 1A). Similarly, the ROC curve calculations for predicting positive neonatal biomarkers or culture-positive EONS (groups 1–3) in comparison to negative neonatal biomarkers (group 4) demonstrated an AUC value of less than 0.7 for all parameters (Figure 1B).

When using a threshold of maternal CRP level ≥2 mg/dL to predict neonatal sepsis (groups 1 and 2 vs. groups 3 and 4) the positive predictive value was 18.5% and the negative predictive value was 91.5%.

4 | DISCUSSION

In this retrospective study involving preterm neonates with a gestational age of ≤29 + 6 weeks, we observed a weak correlation and very poor positive predictive values between maternal CRP levels and neonatal sepsis following pPROM. Increased maternal CRP levels were only detected in half of the cases with culture-positive EONS and 43.4% of those with culture-negative EONS. On the other hand, maternal CRP levels were positive in one-fifth of neonates with negative neonatal laboratory biomarkers. Neither maternal leukocytosis nor combinations of CRP and leukocyte measurements improved the predictive power. Ultimately, maternal CRP and leukocyte levels proved ineffective as tools for predicting neonatal sepsis after early pPROM.

According to the available literature on more mature pPROM infants, our finding is not necessarily surprising. However, to our knowledge, this is the only study examining the relationship between maternal CRP, leukocytes and sepsis status exclusively in very preterm infants. Despite consensus guidelines highlighting the lack of conclusive evidence regarding the efficacy of serial biomarker monitoring for the detection of chorioamnionitis,[1] maternal inflammation markers continue to be frequently used in clinical practice, primarily due to the absence of more precise parameters for management after pPROM.[4,11,14] Given the significant clinical implications, our data underscore that maternal CRP and leukocyte levels are not reliable predictors of neonatal sepsis in preterm infants with a gestational age of less than 30 weeks.

Clinical signs of chorioamnionitis were detected in a very small percentage of mothers within our study cohort. Our perinatal center is following a proactive approach with many deliveries being performed prior to the occurrence of clinical signs of chorioamnionitis. However, in the absence of fever, other clinical criteria, such as abdominal or fundal tenderness and maternal or foetal tachycardia, show variable sensitivity and specificity for the diagnosis of chorioamnionitis.[1] Clinical chorioamnionitis detects less than half of all cases of histologic chorioamnionitis, according to previous reviews on the topic.[7]

As part of routine protocol, microbiological cultures of placental tissue and amniotic membranes are routinely taken at our institution. The overall rate of positive placental cultures was 43.7%, with significant differences in positive rates between the four groups of infants according to their neonatal sepsis status (Table 1). It is worth highlighting that even among neonates with negative biomarkers, the rate of positive placental cultures still reached 33.8%. The question of whether the womb in a healthy pregnancy constitutes a sterile environment remains a subject of debate.[17,18]

Among others, Combs et al documented the presence of 'sterile' colonisation of amniotic fluid, which, in the absence of inflammation, exhibited a relatively benign nature with perinatal outcomes similar to the negative group.[17] Amniotic fluid, umbilical cord blood and meconium may not necessarily be sterile, which was frequently shown in preterm patients with low gestational age at delivery, but also in normal pregnancies and full-term births.[19] A recent paper raises the

TABLE 1 Maternal characteristics of the four groups of infants according to their neonatal sepsis status.

	Entire cohort ($n = 721$)	Culture-positive EONS ($n = 27$, 3.8%)	Culture-negative EONS ($n = 54$, 7.6%)	Positive laboratory biomarkers ($n = 111$, 15.7%)	Negative laboratory biomarkers ($n = 514$, 72.8%)	p-value
Maternal age (years)[a]	33 [28–37] (15–48)	37 [29–40] (23–43)	34 [29–37] (25–46)	33 [29–37] (18–45)	33 [28–36] (15–48)	0.81
Maternal antibiotics (%)	512 (95.0)	16 (84.2)	33 (97.1)	86 (89.6)	366 (96.6)	0.006
Tocolytics (%)	507 (97.5)	19 (95.0)	34 (97.1)	85 (95.5)	359 (98.4)	0.360
Antenatal steroids (%)	546 (98.0)	18 (94.7)	35 (97.2)	95 (99.0)	387 (98.0)	0.664
C-section (%)	631 (87.5)	24 (88.9)	44 (81.5)	92 (82.9)	459 (89.3)	0.135
Maternal CRP mg/dL[a]	0.92 [0.35–2.29] (0.02–23.33)	1.99 [1.18–4.46] (0.05–23.33)	1.56 [0.51–5.22] (0.15–9.83)	1.64 [0.61–3.98] (0.07–13.21)	0.77 [0.30–1.60] (0.02–13.3)	**<0.001**
Maternal leukocytes G/L[a]	13.1 [10.7–16.3] (4.2–29.6)	15.1 [11.6–17.0] (4.2–26.7)	13.7 [10.6–18.3] (7.6–28.1)	14.4 [12.1–18.4] (7.5–29.6)	12.7 [10.5–15.3] (4.5–28.0)	**<0.001**
Clinical chorioamnionitis (%)	24[c] (3.3)	4 (14.8)	6 (11.1)	7 (6.3)	7 (1.4)	**<0.001**
Latency time pPROM to birth (days)[a]	3 [1–13] (0–94)	3 [0–17] (0–52)	5 [1–14] (0–56)	3 [0–9] (0–62)	3 [1–14] (0–94)	0.135
Positive placental culture (%)[b]	235 (43.7)	16 (94.1)	24 (70.6)	56 (71.8)	135 (33.8)	**<0.001**

Note: Bold value for statistical significance ($p < 0.05$).

Abbreviations: EONS, early-onset neonatal sepsis; pPROM, preterm premature rupture of membranes.

[a]Median including [IQR] and (min–max).

[b]Only patients delivered by C-section $n = 538$.

[c]Of the 24 clinical chorioamnionitis cases 13 mothers showed positive placental culture results, 6 mothers showed negative cultures and no placental cultures were obtained in 5 mothers. Only 4/24 neonates had culture-proven sepsis: in 2 cases the same germ was found in placental culture and the infants blood culture (1× *E. coli*, 1× *Klebsiella pneumonia*), in 1 case the placental culture result was negative, and in 1 case placental microbial results were missing.

TABLE 2 Patient characteristics of the four groups of infants according to their neonatal sepsis status.

	Entire cohort ($n = 721$)	Culture-positive EONS ($n = 27$, 3.8%)	Culture-negative EONS ($n = 54$, 7.6%)	Positive laboratory biomarkers ($n = 111$, 15.7%)	Negative laboratory biomarkers ($n = 514$, 72.8%)	p-value
GA at pPROM (weeks)[a]	25.1 [23.3–27.4] (12.1–29.9)	25.0 [23.1–26.3] (17.3–29.6)	23.3 [22.4–24.6] (17.7–28.6)	24.9 [23.3–26.3] (16.7–29.7)	25.7 [23.6–27.8] (12.1–29.9)	**<0.001**
GA at birth (weeks)[a]	26.4 [25.0–28.0] (23.0–29.9)	26.6 [26.0–28.1] (23.1–29.6)	25.7 [24.0–27.0] (23.0–29.6)	26.3 [25.0–27.6] (23.0–29.3)	26.6 [25.0–28.1] (23.0–29.9)	**0.005**
Gender (male, %)	419 (58.1)	14 (51.9)	26 (48.1)	59 (53.2)	313 (60.9)	0.146
Birth weight (g)[a]	900 [710–1115] (270–1780)	880 [713–980] (455–1322)	653 [580–859] (400–1500)	795 [652–1030] (495–1400)	943 [778–1152] (360–1780)	**<0.001**
Birthweight percentile[a]	53 [36–72] (0–99)	65 [40–77] (7–96)	48 [31–63] (1–99)	56 [38–78] (12–98)	54 [37–71] (0–98)	**0.037**
APGAR 5 min <7 (%)	50 (7.0)	7 (26.9)	11 (20.4)	8 (7.2)	16 (3.1)	**<0.001**
Cord blood pH[a]	7.33 [7.29–7.38] (6.71–7.60)	7.30 [7.27–7.35] (6.71–7.46)	7.33 [7.26–7.38] (6.92–7.42)	7.34 [7.30–7.37] (7.12–7.47)	7.33 [7.29–7.38] (6.87–7.60)	0.166
Neonatal pH[a]	7.19 [7.13–7.25] (6.69–7.53)	7.12 [6.99–7.16] (6.69–7.34)	7.17 [7.09–7.23] (6.82–7.38)	7.18 [7.12–7.23] (6.90–7.39)	7.20 [7.14–7.26] (6.72–7.53)	**<0.001**
Neonatal lactate (mg/dL)[a]	3.4 [2.4–4.8] (0.2–22.3)	6.5 [3.9–10.0] (1.3–22.3)	4.3 [2.8–5.8] (1.3–15.0)	3.7 [2.6–4.8] (0.2–16.1)	3.2 [2.3–4.3] (0.2–18.1)	**<0.001**
Survival (%)	621 (86.1)	17 (63.0)	29 (53.7)	103 (92.8)	472 (91.8)	**<0.001**
Survival without severe morbidity (%)	474 (65.7)	13 (48.1)	9 (16.7)	76 (68.5)	376 (73.2)	**<0.001**

Note: Bold value for statistical significance ($p < 0.05$).

Note: severe morbidity: IVH III/IV, c-PVL, BPD and/or ROP.

Abbreviations: EONS, early-onset neonatal sepsis; GA, gestational age; pPROM, preterm premature rupture of membranes.

[a]Median including [IQR] and (min–max).

TABLE 3 Pearson correlations coefficient (PCC) and significance level shown for relations between maternal laboratory biomarkers and neonatal laboratory biomarkers.

	Neonatal CRP value	Neonatal CRP ≥2 mg/dL	Neonatal IL-6/8 value	Neonatal IL-6/8 > 100 pg/mL	Neonatal leukocytes <4000 or > 20 000 G/L	Neonatal laboratory biomarkers positive for infection[a]
Maternal CRP value	**0.126** (p=0.001)	**0.120** (p=0.002)	−0.001 (p=0.985)	**0.128** (p=0.001)	**0.260** (p<0.001)	**0.270** (p<0.001)
Maternal CRP positive ≥2 mg/dL	**0.114** (p=0.003)	**0.096** (p=0.012)	−0.032 (p=0.400)	**0.134** (p<0.001)	**0.306** (p<0.001)	**0.263** (p<0.001)
Maternal CRP positive ≥5 mg/dL	**0.083** (p=0.030)	**0.096** (p=0.012)	0.002 (p=0.967)	**0.085** (p=0.027)	**0.165** (p<0.001)	**0.219** (p<0.001)
Maternal leukocytes value	0.072 (p=0.062)	**0.109** (p=0.004)	−0.016 (p=0.673)	**0.084** (p=0.029)	**0.162** (p<0.001)	**0.189** (p<0.001)
Maternal leukocytes >16 000 G/L	**0.105** (p=0.006)	**0.122** (p=0.001)	0.007 (p=0.863)	**0.097** (p=0.012)	**0.123** (p=0.001)	**0.177** (p<0.001)
Maternal CRP positive ≥2 mg/dL and leukocytes >16 000 G/L	**0.118** (p=0.002)	**0.149** (p<0.001)	−0.004 (p=0.913)	**0.110** (p=0.004)	**0.211** (p<0.001)	**0.238** (p<0.001)
Maternal CRP positive ≥5 mg/dL and leukocytes >16 000 G/L	**0.090** (p=0.020)	**0.124** (p=0.001)	0.011 (p=0.780)	−0.004 (p=0.306)	**0.127** (p=0.001)	**0.166** (p<0.001)

Note: Bold value for statistical significance ($p < 0.05$).

[a]Two or more of the following criteria positive: CRP level ≥2 mg/dL, Interleukin 8 (IL-8) concentration > 100 pg/mL, Interleukin 6 (IL-6) concentration > 100 pg/mL, immature to total neutrophil count ratio (*I*/*T*-ratio) ≥0.2, white blood cell count either <4000 G/L or >20 000 G/L.

TABLE 4 AUC calculations of ROC curves for maternal laboratory biomarkers in predicting neonatal laboratory parameters and the four groups of infants according to their neonatal sepsis status.

	Neonatal CRP ≥2 mg/dL	Neonatal IL-6/8 > 100 pg/mL	Neonatal leukocytes <4000 or > 20000 G/L	Group 1/2 vs. 3/4	Group 1/2/3 vs. 4 = Neonatal laboratory biomarkers positive for infection[a]
Maternal CRP value	0.585	0.575	0.733	0.666	0.676
Maternal CRP positive ≥2 mg/dL	0.559	0.562	0.648	0.602	0.633
Maternal CRP positive ≥5 mg/dL	0.538	0.525	0.549	0.598	0.568
Maternal leukocytes value	0.589	0.542	0.596	0.561	0.611
Maternal leukocytes >16 000 G/L	0.575	0.545	0.558	0.558	0.588
Maternal CRP positive ≥2 mg/dL and leukocytes >16000 G/L	0.565	0.537	0.572	0.573	0.585
Maternal CRP positive ≥5 mg/dL and leukocytes >16000 G/L	0.534	0.508	0.527	0.546	0.537

Note: Group 1: culture-positive early-onset neonatal sepsis (EONS), group 2: culture-negative EONS, group 3: positive neonatal laboratory biomarkers, group 4: negative neonatal laboratory biomarkers.
[a]Two or more of the following criteria positive: CRP level ≥2 mg/dL, Interleukin 8 (IL-8) concentration > 100 pg/mL, Interleukin 6 (IL-6) concentration > 100 pg/mL, immature to total neutrophil count ratio (I/T-ratio) ≥0.2, white blood cell count either <4000 G/L or >20000 G/L.

question of whether the analysis of the amniotic and placental microbiome not only illustrates the pitfalls of microbial studies at low biomasses, and thus possibly represents the result of contamination.[18] One could speculate that, in our study, the occurrence of 33.8% positive placental cultures in the group of neonates with negative biomarkers and overall favourable outcome might indicate a sterile colonisation or contamination that lacks clinical impact. However, in neonates of group 3, who only displayed positive biomarkers without clinical signs of sepsis, we observed that the majority had positive placental cultures, while perinatal outcomes were similar to the group with negative biomarkers. It remains subject of speculation why in this cohort of pregnancies complicated by pPROM, which can facilitate ascending colonisation, some foetus might undergo only subclinical reaction prior to FIRS.

FIRS, defined as an elevated concentration of foetal plasma IL-6 with histopathologic correlate of funisitis, was shown to be an independent risk factor for severe neonatal morbidity.[20,21] Notably, its prevalence rises as the gestational age at delivery decreases.[22] In the study of Combs et al, intraamniotic inflammation verified by elevated IL-6 was associated with adverse perinatal outcomes whether or not intraamniotic microbes were detected.[17] Similarly, a recent study reported an association of FIRS with neonatal morbidities, regardless of the presence (71% of cases) or absence (29% of cases) of histologic maternal-foetal inflammation in the placenta.[22] The culture-negative EONS group in our study might potentially reflect a form of FIRS.[22] It is necessary to point out that maternal CRP and leukocytes, nevertheless, showed no correlation with neonatal interleukin levels, and very weak correlations with interleukin levels >100 pg/mL (Table 3).

Our findings emphasise the elevated rates of mortality and morbidity in both the culture-positive and culture-negative EONS groups, significantly exceeding the rates of the two other groups. To optimise treatment and outcomes in these patients, there is an urgent need to develop accurate and early prenatal detecting methods for intrauterine infection or inflammation.

Urushiyama et al devised a microbiome profile test for amniotic fluid, achieving a 94% sensitivity and 79%–87% specificity in predicting chorioamnionitis.[19] Furthermore, a point-of-care test focusing on amniotic fluid IL-6 concentrations demonstrated remarkable accuracy with 97% sensitivity and 96% specificity in detecting intraamniotic inflammation.[23] Nonetheless, performing amniocentesis post pPROM can pose technical challenges due to anhydramnios. Recently, an innovative chorioamnionitis prediction scoring method, utilising next-generation sequencing to analyse the vaginal microbiome in association with amniotic cavity abnormalities, yielded an impressive AUC value of 0.849.[24]

For research purposes, further cytokines have been measured in maternal sera, vaginal secretions and amniotic fluid, with discordant results.[4,7] Proteomic analysis of amniotic fluid is innovative, but this is not yet available in clinical practice. Recently, measuring the concentration of procalcitonin,[10,25] IL-6 and tumour necrosis factor α[12] in cervicovaginal secretions showed promising results for predicting early-onset sepsis in neonates born to mothers with pPROM.

TABLE 5 Numbers (%) of patients with positive maternal laboratory biomarkers in the four groups of infants according to their neonatal sepsis status.

	Entire cohort ($n = 721$)	Culture-positive EONS (group 1) ($n = 27$, 3.8%)	Culture-negative EONS (group 2) ($n = 54$, 7.6%)	Positive laboratory biomarkers (group 3) ($n = 111$, 15.7%)	Negative laboratory biomarkers (group 4) ($n = 514$, 72.8%)	*p*-value
Maternal CRP positive ≥2 mg/dL	192 (27.6)	12 (50.0)	23 (43.4)	52 (48.1)	102 (20.6)	**<0.001 group 1/2/3 vs. 4**
Maternal CRP positive ≥5 mg/dL	56 (8.1)	5 (20.8)	14 (26.4)	14 (13.0)	22 (4.4)	**<0.001 group 1/2/3 vs. 4** **<0.001 group 2 vs. 3**
Maternal leukocytes >16000 G/L	181 (26.2)	9 (39.1)	18 (35.3)	44 (40.7)	106 (21.5)	**<0.001 group 3 vs. 4**
Maternal CRP positive ≥2 mg/dL and leukocytes >16000 G/L	78 (11.3)	6 (26.1)	12 (23.5)	25 (23.1)	33 (6.7)	**<0.001 group 1/2/3 vs. 4**
Maternal CRP positive ≥5 mg/dL and leukocytes >16000 G/L	28 (4.1)	3 (13.0)	6 (11.8)	8 (7.4)	10 (2.0)	**<0.001 group 1 vs. 3** 0.05 group 1 vs. 4

Note: Bold value for statistical significance ($p < 0.05$).

Abbreviation: EONS, early-onset neonatal sepsis.

Note: Group 1: culture-positive early-onset neonatal sepsis (EONS), group 2: culture-negative EONS, group 3: positive neonatal laboratory biomarkers, group 4: negative neonatal laboratory biomarkers.

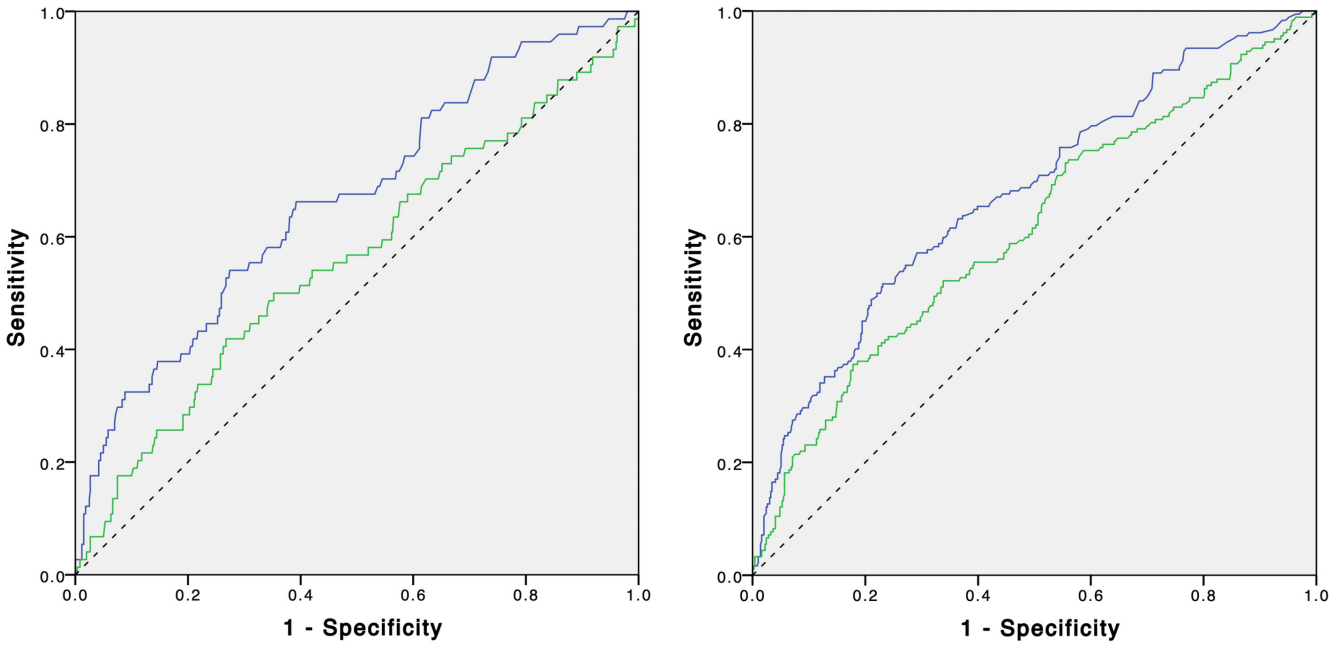

FIGURE 1 ROC curve to predict A. early-onset neonatal sepsis (group 1/2 vs. 3/4) and B. positive neonatal biomarkers (group 1–3 vs. 4): Legend: blue line (maternal CRP with an AUC of A: 0.666; B: 0.676), green line (maternal leukocytes with an AUC of A: 0.561; B: 0.611). **CRP ≥1 mg/dL** – A: sensitivity 64.9% specificity 56.0%, PPV 15.8%, NPV 92.6%; B: sensitivity 64.3% specificity 60.3%, PPV 37.7%, NPV 81.9%; **CRP ≥2 mg/dL** – A: sensitivity 45.5% specificity 74.5%, PPV 18.5%, NPV 91.5%; B: sensitivity 47.0% specificity 79.4%, PPV 46.0%, NPV 80.1%; **CRP ≥5 mg/dL** – A: sensitivity 24.7% specificity 94.0%, PPV 34.5%, NPV 90.7%; B: sensitivity 17.8% specificity 95.6%, PPV 60.0%, NPV 75.7%; **Leukocytes > 16 G/L** – A: sensitivity 36.5% specificity 75.1%, PPV 15.3%, NPV 90.6%; B: sensitivity 39.0% specificity 78.5%, PPV 40.1%, NPV 77.8%. Group 1: culture-positive early-onset neonatal sepsis (EONS), group 2: culture-negative EONS, group 3: positive neonatal laboratory biomarkers, group 4: negative neonatal laboratory biomarkers.

We acknowledge that the retrospective design of our study might have introduced biases in the data collection and subsequent analyses. Due to the lack of placental histology, the true number of chorioamnionitis could not be determined, but this parameter is typically not available for decision-making regarding delivery. The substantial sample size of extremely preterm infants along the observational period of 13 years is what we consider as a significant strength of our study.

5 | CONCLUSION

Neonatal sepsis after pPROM is associated with increased mortality and morbidity. Therefore, identifying neonates at risk for early-onset sepsis is of paramount importance. While maternal CRP and leukocyte levels exhibited a significant association with neonatal infection markers and sepsis outcome groups, their limited positive and negative predictive values, the weak Pearson's correlation coefficients and the low AUC values highlight their insufficient accuracy and reliability for clinical decision-making.

AUTHOR CONTRIBUTIONS

Katharina Goeral: Methodology; investigation; conceptualization; data curation; formal analysis; writing – original draft; visualization. **Agnes Grill:** Conceptualization; data curation; formal analysis; writing – original draft; methodology; investigation. **Harald Leitich:** Methodology; investigation; writing – review and editing; formal analysis. **Alex Farr:** Methodology; investigation; writing – review and editing; formal analysis. **Angelika Berger:** Formal analysis; supervision; writing – review and editing; investigation; methodology. **Judith Rittenschober-Boehm:** Methodology; conceptualization; formal analysis; investigation; supervision; writing – review and editing.

ACKNOWLEDGEMENTS

We appreciate the contributions of the patients and their parents to our study.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest relevant to this article to disclose.

ETHICS STATEMENT

The study protocol was approved by the Institutional Review Board of the Medical University of Vienna (EK 145/2014). Due to the retrospective character of our study no patient consent was requested.

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How to cite this article: Grill A, Goeral K, Leitich H, Farr A, Berger A, Rittenschober-Boehm J. Maternal biomarkers in predicting neonatal sepsis after preterm premature rupture of membranes in preterm infants. Acta Paediatr. 2024;00:1–11. https://doi.org/10.1111/apa.17114