

# Inflammatory Response in Preterm Newborns Born after Prolonged Premature Rupture of Membranes: Is There a Correlation with Placental Histological Findings?

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**ABSTRACT:** **Background:** Preterm birth is the leading cause of morbidity and mortality among neonates in the United States. Early recognition of sepsis in this population is a challenging task since overt clinical signs can be difficult to determine. C-reactive protein (CRP), one of the most frequently non-specific used laboratory test, can indirectly aid the diagnosis of neonatal sepsis.

**Objectives:** To evaluate the relationship between histological findings in the placenta of preterm newborns born after prolonged rupture of membranes, CRP levels, and blood cultures.

**Methods:** Medical records were reviewed of all preterm newborns born after prolonged premature rupture of membranes at a medical center in Israel between 2011 and 2014.

**Results:** Of 128 newborns with prolonged rupture of membranes, 64 had evidence of histological chorioamnionitis (HCA). Gestational age, birth weight, and Apgar scores were significantly lower, while CRP levels (on admission and 10–12 hours post-delivery) were significantly higher in preterm newborns born to mothers with histological evidence of chorioamnionitis, but values were within normal ranges. Duration of the rupture of membranes and white blood cell counts did not differ between groups.

**Conclusions:** CRP levels taken on admission and 10–12 hours after delivery were higher when HCA was present, but since there was a substantial overlap between those with and without HCA and the values for most were within normal range, the differences were not enough to serve as a tool to diagnose placental histological chorioamnionitis in preterm infants born after prolonged premature rupture of membranes and exposed to intrapartum antibiotics.

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**KEY WORDS:** C-reactive protein (CRP), fetal inflammatory response, histological chorioamnionitis (HCA), preterm newborn, prolonged premature rupture of membranes (PPROM)

## BACKGROUND

Preterm birth is the leading cause of morbidity and mortality among neonates in the United States [1]. Placental histology and fetal and neonatal autopsies showed that the most common route of early onset neonatal bacterial infection was caused by an ascending infection from maternal vaginal flora [2]. Chorioamnionitis is observed in 15–30% of women with prolonged premature rupture of membranes (PPROM) and is responsible for 3–20% of neonatal deaths [3].

C-reactive protein (CRP) is one of the most extensively studied, available, and frequently non-specific used laboratory test. It indirectly aids the diagnosis of neonatal sepsis [4]. CRP is an acute phase reactant protein that is released from the liver after stimulation predominantly by interleukin (IL)-6 and other interleukins [5]. In acute inflammation, CRP levels increase within 6 hours of infection and peak 48 hours after infection onset [6]. CRP does not cross the placenta, thus the levels that are found in the newborn's blood are endogenous [7]. In diagnosis of early-onset sepsis, previous studies reported on widely differing sensitivities and specificities of CRP ranging from 29 to 100% and from 6 to 100%, respectively [4].

Due to the potential relationship between PPRM, chorioamnionitis, and congenital infection, the aim of our study was to compare placental histological findings of premature newborns born after PPRM with blood cultures and CRP levels in the blood of those neonates.

## PATIENTS AND METHODS

We hypothesized that CRP levels taken shortly after birth and 10–12 hours later would be higher in newborns whose placenta showed signs of chorioamnionitis since an infectious and inflammatory process may be caused by PPRM.

## METHODS

The study was conducted as a retrospective medical record review of infants admitted to the neonatal intensive care unit

(NICU) at the Wolfson Medical Center, Holon, Israel, between January 2011 and October 2014. The study was approved by the ethics committee.

**ELIGIBILITY CRITERIA**

All preterm newborns (born < 37 weeks gestational age) and exposed to PPROM were included in the study. Premature prolonged rupture of membranes was defined as a rupture of membranes for more than 18 hours prior to birth. All mothers were treated with antibiotics prior to delivery according to the delivery room protocol. All the placentas were sent to the pathology department at our institution for analysis. Demographic and laboratory results were taken from the newborns' medical charts.

**PATHOLOGY**

Examination of the placenta was performed according to standard protocol. Placentas were formalin fixed and from each placenta, six tissue samples were embedded in paraffin blocks for microscopic assessment: one role of the free membranes (chorion and amnion with attached deciduas capsularis), a section of umbilical cord, and five full thickness disc samples (one at the cord insertion, one in central tissue that appeared abnormal on gross examination, two from central tissue, and one at the margin of visible abnormal areas on gross examination). All pathologic examinations were done by a single pathologist.

Placental findings consistent with chorioamnionitis were defined by the presence of inflammatory neutrophils infiltrate at two or more sites on the chorionic plate and extraplacental membrane [8].

**STATISTICAL ANALYSIS**

The two groups (with and without HCA) were compared using the *t*-test for independent samples. All tests are two-sided and considered significant at *P* < 0.05. The pathology findings were tested for correlation with CRP, WBC count, blood cultures and the membranes' rupture duration. Continuous data was described as mean ± standard deviation.

**RESULTS**

In this study 128 preterm newborns met the eligibility criteria. In 64 of the placentas, no signs of HCA were detected (Group A), while 64 placentas had findings compatible with HCA (Group B). As shown in Table 1, mean gestational age was significantly older in Group A than Group B. Consistently, both 1- and 5-minute Apgar scores were significantly lower in Group B. CRP levels on admission and at 10–12 hours after delivery were significantly higher in Group B, but the mean values were within normal range (mean CRP ≤ 1 mg/dl in both groups). Although there was a trend of elevation between the first measurement and the second sample of CRP, the mean value of the second sample was < 1 mg%. Birth weight, duration of

**Table 1.** Demographic data, C-reactive protein, and white blood cell count

	No histological chorioamnionitis (Group A)	Histological chorioamnionitis (Group B)	P-value
Gestational age (weeks)	33.7 ± 2.3	31.6 ± 3	< 0.001
Birth weight (gram)	2137.3 ± 516.6	2101 ± 1873	NS
Apgar 1 minutes	8.7 ± 1	7.6 ± 2.3	< 0.001
Apgar 5 minutes	9.88 ± 0.3	9.3 ± 1.4	< 0.005
PPROM (hour)	133.8 ± 280	198 ± 262	NS
CRP at admission (mg/dl)	0.13 ± 0.15	0.41 ± 0.7	< 0.01
CRP 10–12 hours after delivery (mg/dl)	0.2 ± 0.25	0.94 ± 1.4	< 0.001
WBC count	17690 ± 29784	15537 ± 8433	NS

CPR = C-reactive protein, PPROM = prolonged premature rupture of membranes, WBC = white blood count, NS = not significant

PPROM, and WBC counts did not differ significantly between groups. Bacteremia was detected in one preterm newborn in group B. The infected newborn was a male infant who was born 383.5 hours after rupture of membranes. Gestational age was 32 weeks and birth weight was 1740 grams. There were no special clinical signs. The first CRP levels were 0.94 mg/dl, the second 0.66 mg/dl. Blood culture was positive for *Escherichia coli*. Within the HCA group we did not find any association between the histologic staging of HCA and CRP levels.

**DISCUSSION**

Early recognition of sepsis, especially in preterm newborns, is a challenging task since overt clinical signs might be minor. Intra-amniotic inflammation is a risk factor for impending preterm delivery and adverse perinatal outcome in women with PPROM, even in the absence of documented intra-amniotic infection [9]. Having a reliable and rapidly available biomarker could give the treating physician a tool to estimate whether there was an intrauterine infection or not. The importance of such a biomarker is even greater since it takes time until the neonatologist gets the histological findings. Knowing about the existence of histologic chorioamnionitis is important because:

- The antibiotic regiment is changed according to the type of antibiotic and sometimes the length of treatment
- The known implications of chorioamnionitis on the central nervous system [10] must be taken into account while explaining the newborn's condition to the parents

Acute HCA with funisitis is associated with a significantly higher fetal and intra-amniotic inflammation response [11]; hence, we assumed that the fetal inflammatory response would begin in utero, and CRP levels shortly after birth and 10–12 hours later would be significantly abnormally increased.

In our study we found significantly increased levels of CRP both shortly after birth and 10–12 hours later in preterm

newborns born after PPROM, whose placentas showed signs of HCA compared to a cohort of preterm newborns whose placentas did not have evidence of HCA. However, the levels in both groups were within the normal range (around 1 mg/dl) [12] with large individual overlap, and thus with no ability to distinguish between the two groups. Of note, in our practice in cases in which chorioamnionitis was suspected, we changed the antibiotic regimen.

Our disappointing results in terms of assisting the clinician to diagnose HCA during the first 12 hours after admission are in accordance with studies of Leviton et al. [13], who described a subtle inflammation related protein signal (CRP, IL-8) on day one in newborns who had documented early bacteremia, and Lacaze-Masmonteil and colleagues [14] and Stein co-authors [15], who found only modest sensitivity and specificity predicting infection in 18 hour old newborns and in older neonates.

In our study the mothers were treated with antibiotics prior to delivery. The antepartum exposure to antibiotics could plausibly explain the attenuate inflammatory response, resulting in low CRP levels. Gomez et al. [16] found that a sub-group of patients with documented inflammation of the amniotic cavity demonstrated a decrease in the intensity of the inflammatory process after antibiotic administration. Other plausible explanations would be that an infection in preterm newborns induces a lower CRP levels as compared to term infants [17] and that specific reference ranges for CRP are post-natal age and gestational age dependent [12]. CRP has been proposed as a key decision parameter for guiding the duration of antibiotic therapy; however, CRP was not used as a single criterion in any infants. In fact, other criteria explicitly included in the decision of whether or not to discontinue antibiotics were clinical status, culture results, and results of other laboratory tests [4]. Thus, the current literature does not sustain CRP as the single decision parameter to discontinue antibiotics [4].

In their reviews, Trochez-Martinez and co-authors [18] and van de Laar and colleagues [19] concluded that there is no clear evidence to support the use of maternal CRP levels as an accurate diagnostic test of HCA.

Blood cultures were positive in one preterm newborn in the HCA group. Several studies have found an association between HCA and early onset sepsis. In a retrospective study in which more than 300 preterm infants were included, the presence of HCA was associated with an almost sevenfold increased risk for sepsis [20]. The results of a retrospective study, which included almost 1300 preterm infants, demonstrated an increased risk for sepsis when HCA was present, especially when fetal inflammatory response was involved [21]. Similarly, a recent prospective study including 301 preterm infants showed a more than twofold increased risk for early onset sepsis in HCA positive preterm infants [22].

Intra-amniotic inflammation is a risk factor for impending preterm delivery and adverse perinatal outcome in women

with PPROM, even in the absence of documented intra-amniotic infection [9]. There are three plausible explanations for the paucity of positive blood cultures in our study groups. First, the cause of HCA might have been viral [23]. Second, bacterial growth might have been impeded by the antibiotics administered antenatally to mothers as a preventive measure. The third explanation might be the fact that although blood culture is considered “the gold standard” for diagnosis of sepsis, there are some limitations that might cause false negative results, such as a small sample volume and the timing of taking the sample [24].

The 1- and 5-minute Apgar scores were significantly lower in Group B, perhaps attributed to significantly younger gestational age. Test and colleagues [25] found that prolonged latency is a significant risk factor for chorioamnionitis. Our results showed that the PPROM duration did not differ between the HCA group and the group with no HCA. Our study does not explain why some placentas had findings compatible with HCA while other placentas did not, despite similar duration of rupture of membranes. This issue should be investigated.

The major limitation of our study is its retrospective approach. However, HCA positive and negative infants were managed in accordance with our NICU protocols, and the caretakers were not aware of the histological findings of the placentas. Data was recorded similarly for all infants and extracted from the same database. Thus we consider the internal validity of our results to be high. However, our study has the strength of a large number of samples and a high statistical power. Thus, again, we deem the findings to be valid.

## CONCLUSIONS

Our study shows that preterm newborn infants who were exposed to PPROM and HCA, and whose mothers were treated with antibiotics prior to delivery, had a statistically higher CRP when compared to those not exposed to HCA, but with a large overlap and with values within the normal range. Thus, we conclude that CRP levels shortly after delivery and 10–12 hours later cannot assist the clinician in predicting HCA in preterm infants born after prolonged premature rupture of membranes and exposed to intrapartum antibiotics.

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## References

1. Ananth CV, Joseph KS, Oyelese Y, Demissie K, Vintzileos AM. Trends in preterm birth and perinatal mortality among singletons: United States 1989 through 200. *Obstet Gynecol* 2005; 105: 1084-91.
2. Benirschke K. Routes and types of infection in the fetus and the newborn. *AMA J Dis Child* 1960; 99: 714-21.

3. ACOG Practice Bulletin No. 80: premature rupture of membranes. Clinical management guidelines for Obstetrician-Gynecologists. *Obstet Gynecol* 2007; 109 (4): 1007-19.
4. Hofer N, Zacharias E, Müller W, Resch B. An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. *Neonatology* 2012; 102 (1): 25-36.
5. Gabay C, Kushner I. Acute phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; 340: 448-54.
6. Gutteberg TJ, Askvik K, Jorgensen T. Serum lactoferrin and C-reactive protein in mother and newborn after preterm rupture of membranes. *Acta Obstet Gynecol Scand* 1986; 65: 203-5.
7. Benitz WE, Han MY, Madan A, Ramachandra P. Serial serum C-reactive protein levels in the diagnosis of neonatal infection. *Pediatrics* 1998; 102 (4): E 41.
8. Kovo M, Schreiber L, Ben-Haroush A, et al. The placental factor in spontaneous preterm labor with and without premature rupture of membranes. *J Perinat Med* 2011; 39 (4): 423-9.
9. Shim SS, Romero R, Hong JS, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. *Am J Obstet Gynecol* 2004; 191: 1339-45.
10. Leviton A, Allred EN, Kuban KC, et al. Microbiologic and histologic characteristics of the extremely preterm infant's placenta predict white matter damage and later cerebral palsy. The ELGAN study. *Pediatr Res* 2010; 67: 95-101.
11. Park CW, Yoon BH, Park JS, Jun JK. A fetal and an intra-amniotic inflammatory response is more severe in preterm labor than in preterm PROM in the context of funisitis: unexpected observation in human gestations. *PLoS One* 2013; 8 (5): e62521.
12. Chiesa C, Natale F, Pascone R, et al. C reactive protein and procalcitonin: reference intervals for preterm and term newborns during the early neonatal period. *Clin Chim Acta* 2011; 412 (22-23): 1053-9.
13. Leviton A, O'Shea TM, Bednarek FJ, Allred EN, Fichrova RN, Dammann O. Systemic responses of preterm newborns with presumed or documented Bacteremia. *Acta Paediatr* 2012; 101 (4): 355-9.
14. Lacaze-Masmonteil T, Rosychuk RJ, Robinson JL. Value of a single C-reactive protein measurement at 18 h of age. *Arch Dis Child Fetal Neonatal Ed* 2014; 99 (1): F76-9.
15. Stein M, Schachter-Davidov A, Babai I, Tasher D, Somekh E. The accuracy of C-reactive protein, procalcitonin, and s-TREM-1 in the prediction of serious bacterial infection in neonates. *Clin Pediatr (Phila)* 2015; 54 (5): 439-44.
16. Gomez R, Romero R, Nien JK, et al. Antibiotic administration to patients with preterm premature rupture of membranes does not eradicate intra-amniotic infection. *J Matrn Fetal Neonatal Med* 2007; 20 (2): 167-73.
17. Doellner H, Arntzen KJ, Haereid PE, Aag S, Austgulen R. Interleukin-6 concentrations in neonates evaluated for sepsis. *J Pediatr* 1998; 132: 295-9.
18. Trochez-Martinez RD, Smith P, Lamont RF. Use of C-reactive protein as a predictor of chorioamnionitis in preterm prelabour rupture of membranes: a systematic review. *BJOG* 2007; 114 (7): 796-801.
19. van de Laar R, van der Ham DP, Oei SG, Willekes C, Weiner CP, Mol BW. Accuracy of C-reactive protein determination in predicting chorioamnionitis and neonatal infection in pregnant women with premature rupture of membranes: a systematic review. *Eur J Obstet Gynecol Reprod Biol* 2009; 147 (2): 124-9.
20. Dempsey E, Chen MF, Kokottis T, Vallerand D, Usher R. Outcome of neonates less than 30 weeks gestation with histologic chorioamnionitis. *AM J Perinatol* 2005; 22 (3): 153-9.
21. Lau J, Magee F, Qiu Z, Hoube J, Von Dadelszen P, Lee SK. Chorioamnionitis with a fetal inflammatory response is associated with higher neonatal mortality, morbidity and resource use than chorioamnionitis displaying a maternal inflammatory response only. *Am J Obstet Gynecol* 2005; 193 (3Pt1): 708-13.
22. Been JV, Rours IG, Kornelisse RF, et al. Histologic chorioamnionitis, fetal involvement, and antenatal steroids: effects on neonatal outcome in preterm infants. *Am J Obstet Gynecol* 2009; 201 (6): 587.e1-8.
23. Tsekoura A. adenovirus genome in the placenta: association with histological chorioamnionitis and preterm birth. *J Med Virol* 2010; 82: 1379-83.
24. Bhandari V. Effective biomarkers for diagnosis of neonatal sepsis. *J Pediatric Infect Dis Soc* 2014; 3 (3): 234-45.
25. Test G, Levy A, Wiznitzer A, et al. Factors affecting the latency period in patients with preterm premature rupture of membranes. *Arch Gynecol Obstet* 2011; 283 (4): 707-10.

**Capsule**

**A transcription factor drug for asthma**

In patients with asthma too many goblet cells in the lung differentiate and produce excess mucus in response to inflammatory signals. In mice sensitized to house dust mite allergens, Sun et al. characterized a small molecule called RCM-1 that inhibits the activity of FOXM1, a transcription factor that is critical for airway goblet cell differentiation. RCM-

1 also prevented airway hyper-reactivity and inflammation and improved lung function in these mice. This molecule may have applications for other chronic pulmonary disorders associated with mucus hyper-secretion.

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**Capsule**

**HIV reprograms progenitor cells**

Survival rates of patients with human immunodeficiency virus (HIV) have improved enormously as a result of antiretroviral therapy, but increased life expectancy is now associated with a high risk of co-morbidities. HIV-1-associated chronic obstructive pulmonary disease (COPD) often manifests as emphysema, originating around the airways and extending into lung tissue. Chung and co-authors discovered that this condition is caused by HIV binding to basal cells in the

airway and activating a tissue-destructive phenotype through a mitogen-activated protein kinase signaling cascade. HIV binding triggers up-regulation of matrix metalloproteinase 9, which is known to be elevated in COPD patients and may contribute to the degradation of extracellular matrix seen in emphysema sufferers.

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