ORIGINAL ARTICLE

DURATION OF PRETERM PREMATURE RUPTURE OF MEMBRANES AS PREDICTOR OF HISTOLOGIC CHORIOAMNIONITIS AND EARLY ONSET NEONATAL SEPSIS: A COHORT STUDY

Sheryl R. Del Rosario-Famadico, MD*
Ma. Esterlita V. Uy, MD*
John Anthony Tindoc, MD*

*University of the Philippines
Philippine General Hospital

Correspondence:
Dr. Sheryl R. Del Rosario-Famadico, MD
Email: delrosario_sheryl@yahoo.com

The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

Background: Preterm premature rupture of membranes (PPROM) has been associated with chorioamnionitis but studies are inconsistent on the relationship between PPROM latency and the risk of chorioamnionitis and early onset sepsis.

Objective: To define the association of PPROM latency and the risk of histologic chorioamnionitis (HCA) and early onset neonatal sepsis (EONS).

Methodology: A prospective cohort study was done at a public tertiary hospital on 569 mothers with spontaneous rupture of membranes and with fetuses <37 weeks age of gestation. The profiles of the mothers and neonates were described and the association of PPROM with HCA and EONS was defined using test of association and Receiver Operating Characteristics (ROC) curve analysis. The association of HCA with maternal and neonatal characteristics as well as adverse neonatal outcomes were also determined.

Results: A total of 569 mothers with PPROM were included. Incidence of HCA and EONS were 13% and 24% respectively. PPROM latency was significantly associated with HCA and is a fair predictor of HCA (AUC = 0.7013; 76% accuracy at 31.5-hour cut-off) but failed as a predictor of EONS (AUC = 0.4799). PPROM, platelet count, CRP, and neutrophil count were independent predictors of HCA. HCA was associated with EONS and mortality. Mortality was higher in the presence of both HCA and EONS.

Conclusion: Longer PPROM is associated with HCA and is a fair predictor of HCA at a cut-off of 31.5 hours. PPROM fails as a predictor of EONS.

KEYWORDS: *preterm premature rupture of membranes, histologic chorioamnionitis, early onset neonatal sepsis, latency period*

INTRODUCTION

The Philippines has had the 8th highest number of preterm births and 12th highest preterm rate (14.9%) worldwide in 2010[1].

Preterm premature rupture of membranes (PPROM) is defined as rupture of membranes at less than 37 weeks age of gestation. At the Philippine General Hospital, it is estimated to occur in about 30% of all preterm births that are complicated with early onset neonatal sepsis (EONS)[2]. PPROM and chorioamnionitis along with funisitis, maternal fever and low birth weight are risk factors that have been associated with EONS[3-6]. In PPROM, the rate of microbial invasion of the amniotic cavity is heightened, increasing the likelihood of EONS. Presently, EONS remains to be one of the most common causes of neonatal morbidity and mortality in the pre-term population[7].

Current researches are inconsistent on the relationship of duration of PPROM (PPROM latency) and the risk of HCA and EONS. PPROM latency of 18 hours has been associated with EONS[7] and current recommendation of the American Academy of Pediatrics (AAP) cites 18 hours as the cut-off value to merit investigation and management of potential sepsis in infants <37 weeks age of gestation. However, cut-off values of >48 hours and <4 weeks have also been associated with EONS[4,8]. A study found an association between PPROM latency period of >48 hours with HCA[9] but there were conflicting results with the study of Xie, et.al.[10]. In another observational study, a PPROM latency period >72 hours was associated with clinical chorioamnionitis[11].

This study was done to define the association between the duration of PPROM with the incidence of HCA and EONS. We hypothesized that a longer duration of PPROM is associated with an increased risk for HCA and EONS. Specifically, we aimed to (a) describe the profile of mothers with PPROM and their neonates, (b) determine the predictive ability of PPROM for HCA and EONS, (c) determine maternal and neonatal factors associated with chorioamnionitis, and (d) describe the neonatal outcomes associated with pathologic chorioamnionitis.

METHODOLOGY

A. Study Design:

This is a prospective cohort study.

B. Setting:

The study was conducted in a public tertiary hospital from October 1, 2015 to May 15, 2017.

C. Study Population:

Mothers with fetus at <37 weeks age of gestation and with spontaneous rupture of membranes in varying durations were included. Age of gestation was determined by last menstrual period or early ultrasound. There were no exclusion criteria.

D. Conduct of the study

Recruitment and informed consent

The conduct of the study started when the patient was admitted at the delivery room due to ruptured membranes. The resident or fellow informed the primary investigator of the admission. The primary investigator explained the objective and procedure of the study, and obtained verbal and written informed consent. Patients were enrolled after written consent was obtained.

Data Collection

After enrollment, the profile of the mother was obtained and recorded using a Maternal Data Record Form. Variables collected were maternal age, gravidity and parity, co-morbidities, infections, ultrasound results, and medications (specifically antibiotics and antenatal steroids). Parameters such as mode of delivery, white blood cell (WBC) counts, body temperature and PPROM latency periods were also recorded in the Maternal Data Record Form. After delivery, neonatal data were also recorded on the Newborn Data Record Form as follows: gestational age, birth weight, Apgar score, anthropometric measurements, physical examination, and vital signs. Laboratory

examinations such as complete blood count, blood culture and/or cerebrospinal fluid (CSF) culture and C-Reactive Protein (CRP) for the first 24 hours of life were obtained. All placentas were saved and sent to and examined by the pathologist on the same day (the day of delivery). The pathologist, a co-investigator in the study, examined the placenta and determined the presence or absence of chorioamnionitis. Histologic grading was also assigned once the placenta was confirmed to be positive for chorioamnionitis. The pathologist was a senior resident in his 3rd to 4th year of training during study implementation and was blinded to the maternal and neonatal data and PPROM latency.

The outcomes measured were HCA and EONS. The neonates were monitored closely over three days for clinical and laboratory features that fulfill the criteria for EONS. The clinical practice in the institution was to start on empiric antibiotics if PPROM duration is more than 24 hours. Those who met the above criteria were started on Ampicillin and Gentamycin per order of the Resident-in-charge under the supervision of a Fellow. The antibiotics were continued or stopped based on the blood culture results. The Residents and Fellows who were not part of the study team were oriented on the conduct of the study.

The Flow Diagram of the study is presented in Figure 1.

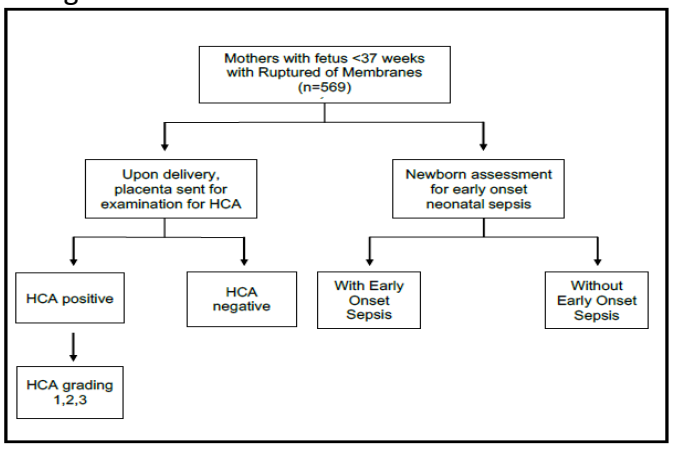

Figure 1. Study flow diagram

E. Sample Size:

To compute for the sample size, we calculated for the minimum sample required for both HCA and for EONS and the higher sample size was followed in this study. The values used for this sample size computation was based on a study by Daunoravičienė et al. in 2014[12]. The calculations are presented below.

Calculation 1 based on PPROM and chorioamnionitis:

For HCA, a minimum sample size of 111 subjects are required for this study. This value gives 80% power to detect an effect size of 45.11 at 0.05 α-level of significance.

Legend:

n = minimum sample

q1= proportion of mothers who developed chorioamnionitis = assumed as 0.5 to obtain a sample size adequate for any proportion of chorioamnionitis, since the said proportion is unknown for this population.

q2= proportion of mothers who did not develop chorioamnionitis = 0.5 (1 – q1)

Zα = 1.96

Zβ = 0.842

E= effect size = 45.11 based on Daunoravičienė et al. 2014[12].

S=standard deviation of the membrane rupture duration (hours) of women with chorioamnionitis = 84.72 based on Daunoravičienė et al. 2014[12].

Sample size formula:

$$d = \frac{E}{S} = \frac{45.11}{84.72} = 0.532$$

$$N = \frac{\left(\frac{1}{q_1} + \frac{1}{q_2}\right) \times \left(z_\alpha + z_\beta\right)^2}{d^2}$$

$$N = \frac{\left(\frac{1}{0.5} + \frac{1}{0.5}\right) \times \left(1.96 + 0.842\right)^2}{0.532^2}$$

$$N = 111$$

Calculation 2 Based on PPROM and Sepsis:

A minimum sample size of 539 subjects are required for this study. This value gives 80% power to detect an effect size of 15.7 at 0.05 α-level of significance. The values used for this sample size computation were based on a study by Daunoravičienė et al. 2014[12].

Legend:

n = minimum sample

q1= proportion of neonates who had sepsis = assumed as 0.5 to obtain a sample size adequate for any proportion of sepsis, since the said proportion is unknown for this population.

q2= proportion of neonates who did not develop sepsis = 0.5 (1 – q1)

Zα = 1.96

Zβ = 0.842

E= effect size = 15.7 based on Daunoravičienė et al. 2014[12].

S= standard deviation of the membrane rupture duration (hours) of women who gave birth to neonates with infection = 65.04 based on Daunoravičienė et al. 2014[12].

Sample size formula:

$$d = \frac{E}{S} = \frac{15.7}{65.04} = 0.241$$

$$N = \frac{\left(\frac{1}{q_1} + \frac{1}{q_2}\right) \times \left(z_\alpha + z_\beta\right)^2}{d^2}$$

$$N = \frac{\left(\frac{1}{0.5} + \frac{1}{0.5}\right) \times (1.96 + 0.842)^2}{0.241^2}$$

$$N = 539$$

In this study, the sample size followed was the higher value obtained which is a minimum of 539 mothers/preterm infants.

F. Statistical Analysis:

Descriptive statistics was used to summarize the clinical characteristics of the mothers and infants. Frequency and percentages were used for nominal variables while median and range for ordinal variables. For interval or ratio variables, mean and standard deviation (SD) were computed or, if not normally distributed, the median and range were computed instead.

Independent t-test was used to compare the means of the 2 groups. Chi- square was used to compare the frequencies between groups. If ≥25% of cells have expected value <5, then Fisher Exact test was used instead.

A receiver operating curve was used to compute area under the curve of the different PPROM latencies and presence of HCA as well as different PPROM latencies and presence of EONS. AUC more than 0.90 is considered an excellent predictor of subsequent histologic chorioamnionitis. AUC of 0.80 - <.90 is considered a good predictor but an AUC of 0.70-<0.80 is considered a fair predictor. AUC of <0.70 is a poor predictor of outcome.

All valid data were included in the analysis. Missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05 α-level of significance. STATA 15.0 was used for data analysis.

G. Definition of Terms:

Preterm premature rupture of membranes (PPROM) is defined as spontaneous pre-labor rupture of membranes before 37 weeks gestation diagnosed by the obstetrician using sterile speculum examination to confirm amniotic fluid pooling in the vagina[5].

Latency period or PPROM latency is defined as the time from rupture of membranes to the time of delivery.

Early onset neonatal sepsis (EONS) is defined by the World Health Organization as blood or cerebrospinal fluid culture-proven infection occurring in the newborn at ≤3 days of life.

Histologic chorioamnionitis (HCA) is defined as inflammation of the placental chorionic disk and extraplacental membranes. Grading of histologic chorioamnionitis is as follows: Stage 1 (Mild); Acute subchorionitis/acute chorionitis, neutrophils in

subchorionic fibrin or interface between deciduas and chorion. Stage 2 (Moderate); Acute chorioamnionitis, neutrophils in connective tissue plane between chorion and amnion. Stage 3 (Severe); necrotizing chorioamnionitis, necrosis, amnion sloughing, thickening of amnion basement membrane and neutrophilic karyorrhexis, multifocal abscess may be present.

H. Ethical considerations:

The study was conducted according to the principles of the International Conference on Harmonization - Good Clinical Practice. It was approved for implementation by the Institution and Hospital Research Ethics Board Review Panel. Informed consent was obtained prior to enrollment of each subject. The study records were locked in the Office of the Section of Newborn Medicine. Data confidentiality was maintained throughout the study and access was allowed only to parties permitted by the principles of ICH-GCP and by laws/regulations including the Data Privacy Act of 2012. Archived study files will be destroyed by paper shredder ten years after completion of the study.

RESULTS

Profile of mothers with PPROM and their neonates

We analyzed 569 mothers with PPROM. Five hundred and eighty-two eligible, consecutive mothers who achieved the inclusion criteria were asked to participate. Thirteen declined leaving 569 mothers (98%) who all gave informed consent for the study. The clinical characteristics of women are presented in Table 1.1. They had a mean (± standard deviation [SD]) maternal age of 19.7 ± 6.8 years, BMI of 23.3 ± 3.8 kg/m2, gravidity of 2 to 3, and parity of 1 to 2. Those with comorbidities of diabetes mellitus (DM), thyroid disease, preeclampsia or eclampsia, anemia, and/or TB represented 30% of the study participants (details not shown in table). Medications taken during the prenatal period included antibiotics and steroids in 46% and 24%, respectively. Twenty-four (4%) were alcohol drinkers, while 19 (3%) were smokers. Deliveries were spontaneous in 65% and via Caesarean section in the rest. The mean (±SD) of women's Amniotic Fluid Index (AFI) was 1.9 ± 0.3 cm (not shown in table). Mean PPROM latency among patients was around 16 hours (range 0.1 to 398 hours) and 44% had PPROM latency of >18 hours. Chorioamnionitis developed in 13% (n = 75) of mothers. Of these, 36% and 59% had HCA grades 1 and 2 respectively.

Table 1.1 Clinical characteristics of 569 mothers who delivered with a history of PPROM

	Frequency (%); Mean ± SD
Age (years)	29.67 ± 6.84
BMI	23.33 ± 3.82
Gravidity	
1	163 (28.65)
2 – 3	259 (45.52)
4 and up	147 (25.83)
Parity	
0	165 (20.9)
1 – 2	243 (42.71)
3 and up	161 (28.3)
With co-morbidities	169 (29.7)
Lifestyle	
Alcohol drinker	24 (4.22)
Smoker	19 (3.33)
Prenatal medications*	
Antibiotics	264 (46.40)
Steroids	134 (23.55)
Mode of delivery	
SVD	373 (65.38)
CS	195 (34.62)
PPROM latency count (hours)	15.88 (0.08 to 397.7)
< 18 hours	318 (55.9)
≥ 18 hours	251 (44.1)
Chorioamnionitis	
Positive	75 (13.18)
Grade 1	27 (36.0)
Grade 2	44 (58.7)
Grade 3	4 (5.3)
Negative	494 (86.82)

** - Multiple Responses*

The mean neonatal age at delivery was 33.2 ± 2.6 weeks, and the median birthweight was 1780 grams (range 500 to 4800). The mean (±SD) of Ballard scores was 32.6 ± 3.3. Those with APGAR scores ≥7 at the 1st and 5th minutes comprised 73% and 99% of infants respectively (Table 1.2).

Table 1.2 Clinical characteristics of 569 neonates who were delivered due to PPROM

	Frequency (%); Mean ± SD; Median (Range)
Birth weight (grams)	1780 (500 to 4800)
Age of gestation (weeks)	33.20 ± 2.63
Total Ballard score	32.55 ± 3.30
Abdominal circumference (cm)	26.67 ± 2.91
Chest circumference (cm)	27.79 ± 2.99
Head circumference (cm)	30.40 ± 2.31
Neonate sex	
Male	203 (35.68)
Female	366 (64.32)
APGAR score	
At 1st minute	
≥ 7	413 (72.6)
< 7	156 (27.4)
At 5th minute	
≥ 7	565 (99.3)
< 7	4 (0.7)

The median length of hospital stay of infants was 34 days, with a median of 5 antibiotic days (Table 1.3). Early onset sepsis occurred in 24% of the neonates, respiratory distress syndrome (RDS) in 30%, retinopathy of prematurity (ROP) in 18%, and bronchopulmonary dysplasia (BPD) in 5%. There were 33 (6%) neonates who died. Blood cultures were positive in 23% of the neonates, and CSF cultures were positive in five (<1%) neonates. Ninety-four percent of infants had CRP levels below 6 mg/L and 79% had WBC between 10 – 20 x 10^9/L. Three fourths had a neutrophil percentage of the total WBC of >0.7 (not shown in table). In 18%, the platelet counts were below 100 x10^9/L.

Table 1.3 Clinical outcomes of 569 neonates who were delivered due to PPROM

	Frequency (%); Median (Range)
Length of hospital stay (days)	34 (12 to 44)
Number of days given antibiotic	5 (3 to 21)
Adverse outcomes*	
Early onset sepsis	138 (24.25)
RDS	171 (30.05)
ROP	101 (17.75)
BPD	31 (5.45)
Mortality	33 (5.8)
Blood CS	
With growth	128 (22.5)
No growth	441 (77.5)
CSF CS	
Positive	5 (0.88)
Negative	564 (99.12)
CRP	
> 6	35 (6.15)
< 6	534 (93.85)
WBC	
0 – 10	122 (21.44)
10 – 20	447 (78.56)
20 and above	0
Platelet count (x10^9/L)	
> 250	439 (77.15)
250 – 100	25 (4.39)
100 and below	105 (18.45)

Predictive ability of PPROM latency for HCA

To determine the optimal cut-off for latency of PPROM to predict HCA, we constructed a receiver operating characteristic (ROC) curve (Figure 2). The ROC curve has an area under the curve (AUC) of 0.7013 which means that the said test has fair accuracy in terms of predicting HCA. Nevertheless, not a single cut-off point had a sensitivity and specificity that were both ≥ 80%. Looking at the different cut-off values, the optimal cut-off of 31.5 hours has the highest accuracy when the sensitivity and specificity are combined (sensitivity of 53%, specificity of 80%, accuracy of 76%) as well as the highest Youden's Index (0.3329) among all other cut-off values. The standard cut-off of 18 hours has a lower accuracy (61%) and Youden's Index (0.2905).

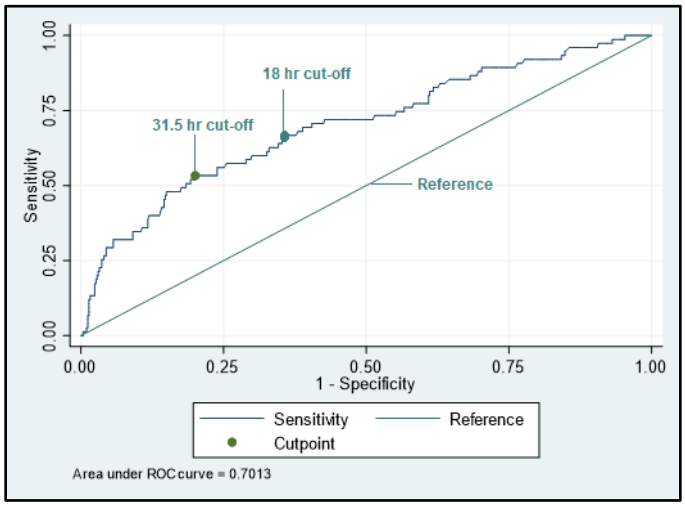

Figure 2. Receiver operating characteristic curve of PPROM latency as predictor of histologic chorioamnionitis

To determine the optimal cut-off for latency of PPROM to predict EONS, we constructed a receiver operating characteristic (ROC) curve (Figure 3). The ROC has an AUC of 0.4799 which means that the said test fails in terms of predicting EONS. An AUC which is close to 0.5, as it is in this case, indicates that the diagnostic test is similar to "chance alone" in predicting the condition (in this case EONS). It is not recommended to use PPROM

as a diagnostic test for EONS and as such, identifying an optimal cut-off value will not be useful.

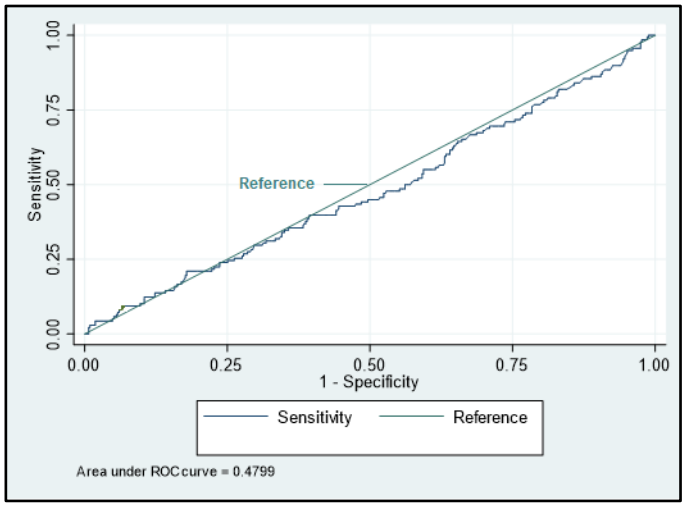

Figure3. Receiver operating characteristic curve of PPROM latency as a predictor of early onset neonatal sepsis

The association of PPROM at different cut-off values with HCA and EONS are presented in Tables 2 and 3. The cut-off used were the standard cut-off of 18.0 hours and the cut-off identified in the ROC in Figure 1, which is 31.5 hours. Table 2 shows that at both cut-off values, there is a significant association of HCA for both 18 and 31.5 hours (both p-values <0.001). However, the odds ratio for the 31.5-hour cut-off was higher than the 18.0-hour cut-off (4.56 vs. 2.86), indicating that the higher cut-off is a better predictor of HCA, as also shown by the ROC analysis in the previous section.

Table 2. Association of PPROM duration with histologic chorioamnionitis

Cut-offs for PPROM	Positive for HCA	%	OR	95% CI	p-value*
Less than 18	23	7.20	2.86	1.80-4.54	<0.001
18 or more	52	20.70			
Less than 31.5	35	8.10	4.56	2.75-7.55	<0.001
31. 5 or more	40	28.80			

**Statistical test: Chi square*

On the other hand, Table 3 shows that at both cut-off values (18 hours and 31.5 hours), there is no evidence of a significant association between PPROM latency and EONS (p-values = 0.925 and 0.934 for 18 - and 31.5-hour cut-offs, respectively and the odds ratios are not different [both 0.98]). This indicates that neither cut-off is predictive of EONS as also revealed by the ROC analysis in the previous section.

Table 3. Association of PPROM duration with early onset neonatal sepsis

Cut-offs for PPROM	Positive for EONS	%	OR	95% CI	p-value*
Less than 18	86	27.00	0.98	0.75-1.29	0.925
18 or more	67	26.70			
Less than 31.5	116	27.00	0.98	0.63-1.51	0.934
31. 5 or more	37	26.60			

**Statistical test: Chi square*

Maternal and Neonatal Factors Associated with HCA

Neonatal factors crudely associated with developing HCA were having PPROM latency period ≥ 18 hours (OR 3.352, 95% CI 1.99 - 5.65), PPROM latency period ≥ 31.5 hours (OR 4.56, 95% CI 2.75 - 7.55), WBC counts of 10-20 x10^9/L (OR 0.455, 95% CI 0.27 - 0.77), and platelet levels greater than 250 x10^9/L (OR 0.277, 95% CI 0.16 - 0.47). No significant associations with maternal characteristics were found. (Table 4.1)

Table 4. 1 Binary logistic regression for predictors of chorioamnionitis

	HCA Positive (n=75)	HCA Negative (n=494)	Crude Odds ratio (95% CI)	*p*-value
	Frequency (%)			
Maternal characteristics				
Age	30.59 ± 6.83	29.53 ± 6.83	1.024 (0.99 - 1.06)	0.213
Parity				
0	25 (33.3)	140 (28.3)	(reference)	-
1 – 2	31 (41.3)	212 (42.9)	0.819 (0.48 - 1.45)	0.491
3 and up	19 (25.3)	142 (28.7)	0.749 (0.39 - 1.42)	0.377
Mode of delivery				
SVD	48 (64.0)	324 (65.6)	(reference)	-
CS	27 (36.0)	170 (34.4)	1.072 (0.65 - 1.78)	0.788
Lifestyle				
Alcoholic drinker	5 (6.67)	19 (3.85)	1.786 (0.65 - 4.94)	0.264
Smoker	4 (5.33)	15 (3.04)	1.799 (0.58 - 5.57)	0.309
Pre-natal meds				
Antibiotics	33 (44)	231 (46.76)	0.895 (0.55 - 1.46)	0.655
Steroids	15 (20)	119 (24.09)	0.788 (0.43 - 1.44)	0.438
Co-morbs				
With at least one co-morbidity	24 (32.0)	145 (29.4)	1.133 (0.67 - 1.91)	0.64
Diabetes mellitus	8 (10.67)	37 (7.49)	1.475 (0.66 - 3.3)	0.345
Hypertension	7 (9.33)	34 (6.88)	1.393 (0.59 - 3.27)	0.446
Thyroid	4 (5.33)	23 (4.66)	1.154 (0.39 - 3.43)	0.797
Preeclampsia	0 (0)	18 (3.64)	1	-
Eclampsia	1 (1.33)	11 (2.23)	0.593 (0.08 - 4.66)	0.62
Anemia	0 (0)	13 (2.63)	1	-
Tuberculosis	0 (0)	12 (2.43)	1	-
Others	8 (10.67)	64 (12.96)	0.802 (0.37 - 1.75)	0.579
Neonatal characteristics				
Age of gestation				
< 34 weeks	35 (46.67)	230 (46.56)	1.004 (0.62 – 1.63)	.986
≥ 34 weeks	40 (53.33)	264 (53.44)	0.996 (0.61 - 1.62)	0.986
Birth weight (grams)				
< 2000 grams	44 (58.67)	323 (65.38)	(reference)	-
≥ 2000 grams	31 (41.33)	171 (34.62)	1.331 (0.81 - 2.18)	0.258
PPROM duration				
< 18 hours	23 (30.67)	295 (59.72)	(reference)	-
> 18 hours	52 (69.33)	199 (40.28)	3.352 (1.99 - 5.65)	<0.001
PPROM 31.5				
< 31.5 hours	35 (46.67)	395 (79.96)	(reference)	-
> 31.5 hours	40 (53.33)	99 (20.04)	4.56 (2.75 - 7.55)	<0.001
CRP				
< 6	73 (97.33)	461 (93.32)	(reference)	-
> 6	2 (2.67)	33 (6.68)	0.383 (0.09 - 1.63)	0.194
WBC				
0 – 10	26 (34.67)	96 (19.43)	(reference)	-
10 – 20	49 (65.33)	398 (80.57)	0.455 (0.27 - 0.77)	0.003
Platelet count				
≤ 100	29 (38.67)	78 (15.38)	(reference)	-
100 – 250	4 (5.33)	21 (4.25)	0.499 (0.16 - 1.56)	0.237
> 250	42 (56)	397 (80.36)	0.277 (0.16 - 0.47)	<0.001
Neutrophil				
< 0.7	19 (25.33)	125 (25.3)	(reference)	-
0.7	43 (57.33)	317 (64.17)	0.892 (0.5 - 1.59)	0.7
> 0.7	13 (17.33)	52 (10.53)	1.645 (0.76 - 3.57)	0.209
IT ratio				
< 20	72 (96)	478 (96.76)	(reference)	-
> 20	3 (4)	16 (3.24)	1.245 (0.36 - 4.36)	0.733

Controlling for the effect of other variables, significant predictors of HCA are having platelet count >250 x10^9/L (adjusted OR [aOR] 0.215, 95% CI 0.12–0.38), PPROM duration ≥ 31.5 hours (aOR 5.058, 95% CI 2.95 - 8.68), CRP level above 6 (aOR 0.186, 95% CI 0.04 - 0.85), and having a neutrophil percentage of the total WBC greater than 0.7 (aOR 2.95, 95% CI 1.4 – 6.2). While controlling for other variables PPROM duration ≥31.5 is around 5 times more likely among those with HCA and neutrophil >0.7 is around 3 times more likely among those with HCA. On the other hand, those with platelet count >250 and CRP>6 are less likely (20% and 19% likelihood respectively) among those with HCA. A platelet count <250 and CRP<6 are associated with higher HCA. (Table 4.2)

Table 4.2 Significant predictors of chorioamnionitis

	Adjusted Odds ratio [aOR] (95% CI)	*p*-value
Platelet count > 250	0.215 (0.12 - 0.38)	**<0.001**
PPROM duration ≥ 31.5 hours	5.058 (2.95 - 8.68)	**<0.001**
CRP >6	0.186 (0.04 - 0.85)	**0.031**
Neutrophil >0.7	2.946 (1.4 - 6.2)	**0.004**

R2=15.79%, p-value <0.001

Neonatal Outcomes Associated with Pathologic Chorioamnionitis

Histologic chorioamnionitis was associated with 2.55 times the odds of having early onset neonatal sepsis and 4.97 times the odds of mortality. We had insufficient evidence to demonstrate a significant association between HCA with RDS, BPD, and ROP (Table 5). Histologic chorioamnionitis carried 2.6 odds for early-onset neonatal sepsis, and 5.0 odds for a fatal outcome.

Table 5 Association between histologic chorioamnionitis and selected neonatal outcomes

	HCA Positive (n=75)	HCA Negative (n=494)	Crude Odds Ratio (95% CI)	p-value
	Frequency (%)			
Early onset neonatal sepsis (EONS)	31 (41.33)	107 (21.66)	2.548 (1.53 - 4.23)	**<0.001**
Respiratory Distress Syndrome (RDS)	20 (26.67)	151 (30.57)	0.826 (0.48 - 1.43)	0.493
Broncho Pulmonary Dysplasia (BPD)	3 (4)	28 (5.67)	0.693 (0.21 - 2.34)	0.555
Retinopathy of Prematurity (ROP)	16 (21.33)	85 (17.21)	1.305 (0.72 - 2.38)	0.385
Mortality	13 (17.33)	20 (4.05)	4.969 (2.36 - 10.49)	**<0.001**

Having either or both HCA and EONS increased the probability of mortality compared to having none of these (P<.0001). Deaths occurred in 23% of neonates with both HCA and EONS, higher

than in cases with positive HCA but no EONS (14%), with positive EONS but no HCA (9%), and neither EONS nor HCA (3%) (Table 6). There was no association between HCA and RDS, BPD, or ROP.

Table 6. Neonates with early onset sepsis and with positive chorioamnionitis

	With EONS; With HCA (n = 31)	No EONS; With HCA (n = 44)	With EONS; No HCA (n = 107)	No EONS; No HCA (n = 387)	*P* value
	Median (Range); Frequency (%)				
Length of hospital stay (days)	34 (15 – 41)	33 (15 – 42)	34 (12 – 43)	34 (12 – 44)	0.839*
Duration of antibiotics (days)	10 (7 – 14)	4 (3 – 14)	7 (3 – 21)	5 (3 – 14)	0.143*
Adverse outcomes					
With RDS	10 (32.26)	10 (22.73)	33 (30.84)	118 (30.49)	0.738‡
With BPD	2 (6.45)	1 (2.27)	6 (5.61)	22 (5.68)	0.852§
With ROP	5 (16.13)	11 (25.0)	20 (18.69)	65 (16.8)	0.564‡
Mortality	7 (22.58)	6 (13.64)	10 (9.35)	10 (2.58)	**<0.0001**‡

EOS – Early Onset Sepsis; HCA – Histologic Chorioamnionitis
*Statistical tests used: * - Kruskal Wallis test; § - Fisher's exact test; ‡ - Chi square test*

DISCUSSION

We analyzed women with different durations of PPROM by sending the placenta for histologic examination after delivery and assessing maternal and neonatal outcomes including HCA and EONS. The incidence of HCA and EONS were 13% and 24%, respectively. PPROM latency was significantly associated with HCA and is a fair predictor of HCA (AUC =0.7013; 76% accuracy at 31.5-hour cut-off) but failed as a predictor of EONS (AUC of 0.4799). PPROM, platelet count, CRP, and neutrophil count were independent predictors of HCA. HCA was associated with EONS and mortality with mortality being higher in the presence of both HCA and EONS.

The incidence of HCA was 13% among these patients with PPROM. Curiously, this is lower than those found in other studies among PPROM patients where incidences as high as 68% and 70% have been reported[9,10]. Conversely, the incidence of EONS in this study (24%) was higher than the other studies mentioned (both 6.5%)[9,10].

We determined if the duration of membrane rupture could be a predictor of HCA and EONS. ROC curve analysis was done to determine whether there is another cut-off point that can give a better sensitivity and specificity, compared with the cut-off of 18 hours that is used in standard practice. An area under the curve of 0.7013 showed that the test (PPROM latency) could fairly predict HCA. Comparing the different levels of PPROM latency, the cut-off at 31.5 hours versus the standard 18 hours showed a higher accuracy. However, for EONS, PPROM duration had an area under the curve of 0.4799, indicating that PPROM duration cannot be used to predict EONS.

The association of longer PPROM latency with HCA has also been noted in other studies. Xie, et.al., found that a PPROM >48 hours was associated with HCA[9]. Daunoraviciene et.al., studied in retrospect 135 pairs of neonates and their mothers who had PPROM at 32 to less than 34 weeks of gestation. Women with inflammation and neonates with congenital infection had longer latency periods and higher CRP values compared to those women with no inflammation and neonates with no infection, concluding that a longer latency period and higher maternal CRP can be used as prognostic indicators of intrauterine infection and congenital infection[12].

In the crude analysis, the 31.5-hour cut-off had a stronger association with HCA than the standard cut-off of 18 hours. Furthermore, even after controlling for the effect of other variables, it is notable that PPROM at ≥31.5-hour cut-off was most strongly associated with HCA among the significant predictors identified. Consistent with the results of the ROC curve and the test of association of the 2 cut-off values with HCA, this study shows that PPROM at the cut-off of 31.5 hours is an independent predictor of HCA. Based on the results of this study, the PPROM cut-off of 18 hours may lead to over-treatment with antibiotics in preterm infants whose mothers did not have chorioamnionitis and a cut-off 31.5 hours may decrease overtreatment. However, since PPROM latency per se is only a fair predictor of HCA, it would be prudent for the physician to look into other associated factors or predictors when making their

assessment, rather than relying on PPROM latency alone.

When managing patients with PPROM, it is important, as much as possible, to avoid early termination of pregnancy while preventing possible complications of prolonged PPROM. Comparing the different levels of PPROM duration, the cut-off of 31.5 hours versus the recommended 18 hours, the longer latency showed the optimal specificity and accuracy of predicting histologic chorioamnionitis. This suggests that the 31.5-hour cut-off may be considered for serial testing in identifying mothers with PPROM who will need to be observed and treated, especially taking into consideration those with lower age of gestation to prevent earlier termination of pregnancy. Despite previous research findings that EONS is significantly associated with PPROM latency, in this research, there is no significant evidence of PPROM latency predicting EONS, hence not supporting its use in diagnosing EONS.

This study revealed that PPROM, platelet count, CRP, and neutrophil count were independent predictors of HCA. HCA was associated with EONS and mortality with mortality being higher in the presence of both HCA and EONS. Various factors have been cited in the literature on PPROM as being associated with chorioamnionitis and early onset sepsis. Alam MM et.al., retrospectively investigated 428 neonates born to mothers with premature rupture of membranes for more than 18 hours. The results showed that the risk factors associated with the development of culture-proven EONS include maternal fever, PPROM>48 hours, neonatal prematurity, and low birth weight, along with neonatal thrombocytopenia and raised CRP[13]. In a study of 838 preterm infants born at less than 30 weeks gestational age by Strunk, *et.al.* increased risk of EONS was associated with HCA[14]. Nayot et.al., studied 1535 singleton pregnancies with PPROM and associated a PPROM latency of >72 hours with clinical chorioamnionitis[11]. But somewhat counterintuitively, it also associated PPROM latency of ≤72 hours combined with <32 weeks gestational age with a two-fold higher incidence of severe neonatal morbidity[11].

In the association between HCA and mortality, mortality was significantly higher in the presence of both HCA and EONS. This is not surprising considering that infection in HCA could predispose to EONS, which in turn is associated with higher risk of mortality. Management-wise this stresses the importance of early detection of HCA with a higher index of suspicion at PPROM latency of ≥ 31.5 hours especially in the presence of other associated factors, with the physician anticipating the need for more aggressive management to prevent poor neonatal outcomes. The combination of HCA and EONS is a special concern in this case as it is associated with a significantly higher mortality than the presence of only one of either condition. However, the results of this study do not suggest any drastic change in what is considered as the optimal treatment of infants with EONS, which is broad spectrum antimicrobials (ampicillin and an aminoglycoside) and adjustment of coverage once a pathogen has been identified[7]. Aside from neonates with histologic chorioamnionitis and early onset neonatal sepsis having a high mortality rate (22.6%), there is also high mortality among neonates with HCA but no signs of EONS (13.6%) compared with neonates with neither HCA nor EONS (2.58%). This may indicate that even initially asymptomatic preterm infants who have HCA, need antibiotic therapy. The AAP recommendation is to start antibiotic therapy among preterm infants with maternal chorioamnionitis[7]. This result supports what is believed to be the current optimal management of clinical chorioamnionitis, which includes antibiotic therapy and delivery[15].

CONCLUSION

The mothers with PPROM had a mean age of 19.7 years, with 1 to 2 children, and a 13% incidence of HCA. Their neonates averaged 1,780 grams

birthweight, 33 weeks AOG, 34 days in the hospital, and had a 24% incidence of EONS.

PPROM latency at 31.5-hour cut-off value was a fair predictor of HCA (76% accuracy; AUC of 0.7013) and was a better predictor of HCA than the 18-hour cut-off (61% accuracy). However, PPROM latency failed as a predictor of EONS (AUC of 0.4799).

After controlling for other variables PPROM ≥ 31.5 hours, platelet count <250, CRP <6, and neutrophil count >0.7 were shown to be independent predictors of HCA. HCA was also associated with adverse neonatal outcomes, particularly EONS and mortality, with mortality being higher in the presence of both EONS and HCA.

RECOMMENDATION

In the context of PPROM, it is recommended to include placental examination in clinical practice. Without it, there may be underdiagnosis or late diagnosis of chorioamnionitis and possible complications and neonatal outcomes such as early onset sepsis.

Future research is also recommended to validate the cut-off of 31.5 hours and for further evaluation of the effects of histologic chorioamnionitis on neonatal outcomes.

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