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Histological chorioamnionitis could be predicted with high accuracy in preterm labor with intact membranes before delivery

Xianhu Fu, Xiaobo He, Daifei Sun, Qihui Fan, Bailei Zhang, Qian Wang, Yinfen Wang, Huili Chen

Department of Obstetrics and Gynecology, Ningbo Women and Children's Hospital, Ningbo (China)

Summary

Objective: Histological chorioamnionitis was associated with adverse outcomes. The objective of this study was to develop a prediction model for histological chorioamnionitis in preterm labor with intact membranes. *Materials and Methods:* Data were obtained from 307 women with singleton preterm labor (gestational age 28-33^{+6} weeks) of the intact membranes between October 2011 and July 2014 in the Ningbo Women and Children's Hospital, Ningbo, China. Histological chorioamnionitis (HC) prediction model was developed with maternal independent risk factors before delivery. *Results:* Multivariable Logistic regression analysis showed that serum C-reactive protein (CRP) (OR=1.175, p = 0.0015, 95% confidence interval (CI) 1.064~1.297), and procalcitonin (PCT) (OR=9.736, p = 0.0117, 95% CI 1.658~57.166) were independent risk factors of HC. When PCT ≥ 0.05 ng/ml and CRP > 7.3 mg/L or PCT < 0.05 ng/ml and CRP > 21.4 mg/L, HC could be detected. HC was predicted with 91.1% accuracy, yielding an area under receiver operating characteristic (ROC) curve of 0.938 (95% CI 0.857~0.996), a positive predictive value of 84.6% (95% CI 65.1~95.6%), and a negative predictive value of 96.7% (95% CI 82.8~99.9%). *Conclusion:* Combined with CRP and PCT, HC could be predicted with high accuracy in preterm labor with intact membranes before delivery. Further studies should evaluate the value of this model to guide early treatment.

Key words: Histological chorioamnionitis; Serum C-reactive protein; Procalcitonin.

Introduction

Histological chorioamnionitis (HC) is a subclinical intrauterine infection and inflammation. HC increases the risk of adverse outcomes including severe postpartum hemorrhage, peripartum hysterectomy, stillbirths, necrotizing enterocolitis, retinopathy, and cerebral palsy [1-8]. A recent study has suggested that HC has degrees of severity that correlate with adverse outcomes [9, 10]. So early detection of HC has important clinical significance, which can improve pregnancy outcome through timely intervention.

The gold standard for diagnosing HC is placental histological examination. However the results of placental pathology cannot be obtained before delivery. Current guidelines have the standards of clinical chorioamnionitis before delivery [11]. However the overall detection frequency of clinical chorioamnionitis was low and most intrauterine infection and inflammation was histological chorioamnionitis [12-14], but now it lacks the standards of HC.

Currently most researches focus on the amniotic fluid cytokine profile, such as interleukin (IL)-6 to detect chorioamnionitis in preterm prelabor rupture of membranes [15, 16]. However we cannot non-invasively obtain amniotic fluid for the intact membranes before delivery. Recently some researchers explored maternal demographic inflammatory and hematological markers and clinical findings to detect HC. However their use is experimental, and sensitivity and specificity have generally shown to be low or moderate [17-21].

In the current study the authors aim to develop a clinical prediction model for histological chorioamnionitis of the intact membranes with 28-33^{+6} weeks.

Materials and Methods

This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Ningbo Women and Children's Hospital. Written informed consent was also obtained from all participants.

The clinical prediction model was developed in a prospective cohort. Pregnant women, who delivered between October 2011 and June 2014 in the Ningbo Women and Children's Hospital, Ningbo, China, at a gestational age of 28-33^{+6} weeks, were eligible for the study. Multiple births, preterm premature rupture of membranes, use of antibiotics, and other infections were excluded from analysis. All clinical variables were obtained within 12 hours before birth. Placentas and membranes were fixed in formalin directly after delivery. Sampling was done according to a standard protocol and included at least two membrane rolls, two cross-sections of the cord, and three representative blocks of the placental disk. Tissues were embedded in paraffin until examination. A single pathologist examined all placentas in a blinded fashion for presence of chorioamnionitis. Histological chorioamnionitis was diagnosed by the presence of inflammatory cells (predominantly neutrophils) in the chorionic plate and/or chorioamnionotic membranes according to widely accepted, reproducible semi-quanti-

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Table 1. — *Risk factors of HC.*

Variable	Group	n	Mean	SD	Median	Min	Max	T	*p*
Gestational age	Non-HC	225	31.75	1.59	32.10	28.00	33.60	2.32	0.0204
	HC	82	31.24	1.70	31.50	28.00	33.60		
Maternal age	Non-HC	225	26.81	5.90	26.00	16.00	46.00	0.21	0.8332
	HC	82	26.65	5.16	26.00	16.00	42.00		
T	Non-HC	225	37.00	0.24	37.00	36.20	38.20	1.46	0.1431
	HC	82	36.65	4.11	37.00	0.00	38.90		
HR	Non-HC	225	82.90	7.94	80.00	10.40	110.00	-0.52	0.6014
	HC	82	83.55	13.21	80.00	0.00	140.00		
WBC	Non-HC	225	11.62	3.50	11.50	4.60	22.70	4.01	<0.0001
	HC	82	13.98	4.61	13.50	6.90	26.30		
N (%)	Non-HC	225	78.93	10.43	80.00	6.90	94.00	4.19	<0.0001
	HC	82	83.59	8.01	86.00	63.00	96.00		
Hb	Non-HC	225	11.40	1.41	11.50	6.40	15.10	2.22	0.0265
	HC	82	11.00	1.44	11.10	5.10	14.30		
CRP	Non-HC	209	8.78	8.00	6.00	1.00	62.00	-8.61	<0.0001
	HC	80	24.81	23.68	17.65	1.00	124.00		
Serum total protein	Non-HC	225	62.35	6.30	63.20	46.90	75.30	1.99	0.0462
	HC	82	64.16	5.87	64.60	41.60	86.30		

Abbreviations: T=temperature, HR=maternal heart rate, WBC=maternal leukocytosis, N=neutrophils, Hb=hemoglobin, CRP=C-reactive protein (serum).

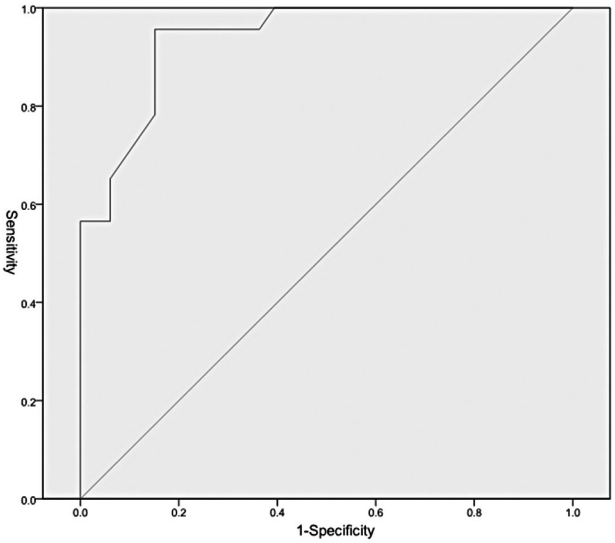

Figure 1. — ROC cure of prediction model for histological chorioamnionitis.

tative scoring system [22]. Variables were considered as potential predictors associated with HC: maternal age, gestational age, parity, abortion, mullitus, hypertension in pregnancy (four categories: 1) pre-eclampsia-eclampsia, 2) chronic hypertension (of any cause), 3) chronic hypertension with superimposed preeclampsia, and 4) gestational hypertension) [23], HELLP syndrome (clinical presentation of intravascular haemolysis, elevated liver enzymes, and a low platelet count), antenatal steroid administration (dexamethasone), ritodrine, vaginal bleeding, uterine tenderness, maternal temperature, maternal heart rate, maternal leukocytosis, neutrophils, hemoglobin, serum C-reactive protein (CRP), procalcitonin (PCT), maternal serum total protein, malodorous vaginal discharge, vaginal discharge culture, fetal heart rate, umbilical artery S/D value, and amniotic fluid volume.

Univariable analyses were performed to identify relevant clinical variables that differed between the groups ("no HC" *vs*. 'HC''), using χ^2-test, Student *t*-test, where appropriate (alpha, 0.05). These were entered into a backward logistic regression model to predict HC. The final model was selected using the likelihood ratio method with an alpha level of 0.05. It was used to develop a clinical prediction model for HC. Receiver operating characteristic (ROC) curves were computed to determine the optimum cutoff value for prediction. Analyses were performed using SPSS 16.0 software.

Results

Six hundred seventy-two placenta were examined in this study. Of these, 102 had HC, and the incidence rate of HC was 15.2%. According to the study criteria, 307 subjects were selected and among them, 82 had HC.

Single factor analysis showed that gestational age, maternal leukocytosis, maternal neutrophils, hemoglobin, serum CRP, maternal serum total protein, parity, hypertension in pregnancy, vaginal bleeding, ritodrine, procalcitonin, and fetal heart rate were associated with HC ($p < 0.05$) (Tables 1 and 2).

Multivariable logistic regression analysis showed that CRP (OR=1.175, $p = 0.0015$, 95% confidence interval (CI) 1.064~1.297), and PCT (OR=9.736, $p = 0.0117$, 95% CI 1.658~57.166) were independent risk factors of HC (Figure 1).

The prediction model was computed, yielding the following formulas: logit (taipbl) = -3.21+0.1609×CRP+2.2758×PCT. The most discriminative cut-off value was 0.243111. Through

Table 2. — *Risk factors of HC.*

Variable	Category	HC		Non-HC		χ^2	*p*
		Case (n)	Constituent ratio (%)	Case (n)	Constituent ratio (%)		
Parity	0	42	51.22	121	53.78	11.15	0.0249
	1	34	41.46	83	36.89		
	2	2	2.44	19	8.44		
	3	4	4.88	1	0.44		
	4	0	0.00	1	0.44		
Abortion	0	46	56.10	114	50.67	5.13	0.5276
	1	16	19.51	60	26.67		
	2	13	15.85	27	12.00		
	3	4	4.88	12	5.33		
	4	1	1.22	10	4.44		
	5	1	1.22	1	0.44		
	6	1	1.22	1	0.44		
GDM	0	53	88.33	142	86.06	0.20	0.6574
	1	7	11.67	23	13.94		
Hypertension in pregnancy	0	76	92.68	169	75.11	11.79	0.0027
	1	0	0.00	6	2.67		
	2	6	7.32	50	22.22		
HELLP syndrome	0	81	98.78	222	98.67	0.00	1.0000
	1	1	1.22	3	1.33		
Dexamethasone	0	58	70.73	161	71.56	0.02	0.8877
	1	24	29.27	64	28.44		
Vaginal bleeding	0	44	53.66	153	68.00	5.38	0.0204
	1	38	46.34	72	32.00		
Ritodrine	0	73	89.02	219	97.33	7.23	0.0072
	1	9	10.98	6	2.67		
Procalcitonin	0	11	47.83	31	91.18	13.30	0.0003
	1	12	52.17	3	8.82		
Malodorous vaginal discharge	0	82	100.00	223	99.55		1.0000
	1	0	0.00	1	0.45		
Vaginal discharge culture	0	26	76.47	39	81.25	0.28	0.5989
	1	8	23.53	9	18.75		
Fetal heart rate	0	74	90.24	221	98.22	8.17	0.0043
	1	8	9.76	4	1.78		
Umbilical artery S/D	0	78	95.12	210	93.33	0.33	0.5650
	1	4	4.88	15	6.67		
Amniotic fluid volume	0	77	93.90	207	92.00	0.60	0.7418
	1	1	1.22	6	2.67		
	2	4	4.88	12	5.33		

Note: GDM 0=no, 1=yes; hypertension in pregnancy 0=no, 1=gestational hypertension, 2=pre-eclampsia-eclampsia; HELLP syndrome 0=no, 1=yes; dexamethasone 0=not used, 1=used; vaginal bleeding 0=no, 1=yes; ritodrine 0=not used, 1=used; procalcitonin 0 ≤ 0.05 ng/ml, 1=PCT ≥ 0.05ng/ml; malodorous vaginal discharge 0=no, 1=yes; vaginal discharge culture 0=negative, 1=positive; fetal heart rate 0=110-160 bpm, 1 ≥ 160 bpm; umbilical artery S/D 0=normal, 1=abnormal; amniotic fluid volume 0=normal, 1=polyhydramnios, 2=oligohydramnios.

computing,when PCT ≥ 0.05 ng/ml and CRP > 7.3 mg/L or PCT < 0.05 ng/ml and CRP > 21.4 mg/L, it was diagnosed as HC. ROC curves showed high ability for prediction model, with an area under the curve of 0.938 (95% CI 0.857~0.996) for HC sensitivity 0.9565 [95% CI 0.7805-0.9989], specificity 0.8788 [95% CI 0.718~0.966], a positive predictive value of 84.6% (95% CI 65.1-95.6%), and a negative predictive value of 96.7% (95% CI 82.8~99.9%), respectively) (Tables 3 and 4, Figure 1).

Discussion

In this study, the authors developed a prediction model for histological chorioamnionitis. Combined with CRP and PCT, HC could be predicted before delivery with high accuracy.

CRP and PCT are biomarkers that may be used as adjunctive tests in the diagnosis of inflammation, sepsis, and infection. However whether they can be used as markers predicting HC has conflict. Erdemir *et al.* found that maternal CRP values were not indicative of HC of mothers who delivered earlier than 35 gestational weeks [24]. However Popowski *et al.* concluded that maternal CRP was as-

Table 3. — *Independent risk factors of HC.*

Variable	DF	Coefficient	SE	Wald Chi-square	*p*	Standardized coefficient	OR	95%CI	
Constant	1	-3.2106	0.7911	16.4687	<.0001				
CRP	1	0.1609	0.0506	10.1198	0.0015	1.853	1.175	1.064	1.297
PCT	1	2.2758	0.9032	6.3492	0.0117	0.5607	9.736	1.658	57.166

Note: Logit(taipbl)= -3.21+ 0.1609× CRP+ 2.2758× PCT.

Table 4. — *Prediction model test characteristics.*

	Value	95% CI	
Area under ROC curve	0.938	0.857	0.996
Sensitivity	0.9565	0.7805	0.9989
Specificity	0.8788	0.718	0.966
Positive predictive value	0.8462	0.6513	0.9564
Negative predictive value	0.9667	0.8278	0.9992
Positive likelihood ratio	7.891914	3.136224	19.85901
Negative likelihood ratio	0.049499	0.007249	0.337997
Cut-off value	0.243111		
Coincidence rate	0.9107	0.8038	0.9704

sociated with clinical and histological chorioamnionitis in cases of premature rupture of membranes at or after 34 weeks of gestation [14]. In the present study, the authors found that maternal CRP was an independent risk factors of HC (OR=1.175, $p = 0.0015$).

PCT is a peptide prohormone of calcitonin physiologically produced by C-cells in the thyroid gland. In response to endotoxin and inflammatory cytokines, PCT is also synthesised by non-thyroidal tissues, including monocytes, renal and pancreatic cells, adipose tissue, and hepatocytes [25]. PCT levels can accurately reflect the real time extent of inflammation and may be useful in the early diagnosis of infection and monitoring of disease. However, Howman *et al.* found that there was no statistically significant relationship between HC and maternal PCT. However they found that the median cord PCT level was significantly higher in subjects with HC [26]. In the present study, the authors found that maternal PCT was an independent risk of HC (OR=9.736, $p = 0.0117$).

The marker of prediction for clinical chorioamnionitis included uterine tenderness, malodorous vaginal discharge, maternal leukocytosis (WBC 15,000 cells/ml), raised serum CRP >15 mg/L, maternal tachycardia (>100 bpm), and fetal tachycardia (>160 bpm) [11]. However the present study found these markers were not the risk of HC except for CRP. It was further confirmed that HC was a subclinical intrauterine infection and inflammation. If we use the standards of clinical chorioamnionitis to predict the HC, it perhaps has a certain degree inaccuracy. However Tokumasu *et al.* found that clinical chorioamnionitis is a useful predictor of histological chorioamnionitis in extremely premature infants < 28 weeks' gestational age [27]. The positive predictive value of clinical chorioamnionitis for histological chorioamnionitis was 86.6% (681/786; 95% CI: 84.4~88.6%), sensitivity was 60.3% (681/1129; 95% CI: 58.8~61.7%), and specificity was 92.2% (1236/1341; 95% CI: 90.9~93.3%). The discrepancy may result from different gestational ages in this study and the intact membranes or not. Therefore further studies are needed with larger samples.

Markers in this study included maternal age, gestational age, parity, abortion, GDM, hypertension in pregnancy, HELLP syndrome (clinical presentation of intravascular hemolysis, elevated liver enzymes, and a low platelet count), antenatal steroid administration, ritodrine, vaginal bleeding, vaginal discharge culture, umbilical artery S/D value, amniotic fluid volume, but these were not independent risk factors of HC.

Recently, Been *et al.* used a clinical prediction rule composed of clinical variables available at birth to predict HC with good test characteristics in preterm newborns [11]. Others ultilised amniotic fluid IL-6 to identify the presence in PROM or shortly after birth [15]. However these ways were not available to the patients with the intact membranes before delivery. Currently some researchers began to make use of maternal inflammation inducers to predict the presence of HC. Gulati *et al.* found that maternal serum IL-6 can be used as a biomarker to predict preclinical asymptomatic infection in PPROM. A cut-off value of IL-6 of 8 pg/ml was found to correctly diagnose 19 out of 23 patients with infectious morbidity and showed the best sensitivity (82.6%) and specificity (86.3%) [28]. Popowski *et al.* established a prediction model with CRP in women with premature rupture of membranes at or after 34 weeks. Their study suggested that CRP was associated with histological chorioamnionitis, with areas under the ROC curve of 0.62 (95% CI [0.47, 0.74]) [14]. Oludag *et al.* exploited maternal PCT levels to predict subclinical intra-amniotic infection in PPROM [17]. They found that ProCT levels were significantly higher in patients with PPROM. At a cut-off of 0.054 ng/ml, the sensitivity and specificity of ProCT to predict histological chorioamnionitis were 92.3% and 68.4%, respectively. In the present study, the authors can firstly provide a model of predicting HCA with the intact membranes before delivery combined with CRP and PCT. The PPV was 84.6% and NPV 96.7% with an area under the curve of 0.938. Negative predictive value was particularly well preserved and the present test characteristics were superior to others. The prediction model carries future potential in facilitation of early intervention strategies.

In conclusion, the authors developed a prediction model for histological chorioamnionitis with the intact membranes before delivery. The prediction model presented here could guide individualisation of therapy.

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Corresponding Author:
DAIFEI SUN, M.D.
Department of Obstetrics and Gynecology
Ningbo Women and Children's Hospital
No. 339 Liuting Street, Haishu District
Ningbo 315000 (China)
e-mail: daifeisun@163.com