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Current opinion

The combination of maternal blood and amniotic fluid biomarkers improves the predictive accuracy of histologic chorioamnionitis

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ABSTRACT

Introduction: This study was performed to determine whether the combination of maternal blood and amniotic fluid biomarkers can improve the predictive accuracy of histologic chorioamnionitis (HC).

Methods: This retrospective study included 80 singleton pregnant women who were suspected to have intrauterine infection and underwent measurement of two maternal blood biomarkers [maternal white blood cell count (mWBC) and maternal C-reactive protein level (mCRP)] and three amniotic fluid biomarkers [amniotic white blood cell count (aCell), amniotic glucose level (aGlucose), and amniotic lactate dehydrogenase level (aLDH)]. We divided the patients into two groups based on the presence or absence of HC and assessed the predictors of HC using logistic regression models: Model 1, combination of mWBC and mCRP; Model 2, combination of Model 1 and aGlucose; and Model 3, combination of Model 2, aCell, and aLDH.

Results: The multivariable analysis showed that aCell was the only significant predictor of HC [odds ratio, 1.24; 95% confidence interval (CI), 1.06–1.68] independent of mWBC, mCRP, aGlucose, and aLDH. The c-statistics were higher in Model 3 (0.803; 95% CI, 0.701–0.905) than Model 1 (0.634; 95% CI, 0.511–0.758) and Model 2 (0.785; 95% CI, 0.684–0.887).

Discussion: We found that the combination of maternal blood and amniotic fluid biomarkers can improve the predictive accuracy of HC. Therefore, our data provide relevant information to support counseling with regard to improving the predictive accuracy of HC in patients with suspected intrauterine infection.

1. Introduction

Chorioamnionitis is a type of intrauterine infection that is mainly caused by ascending bacterial vaginosis and cervicitis [1,2]. Postpartum placental pathology examination findings in patients with intrauterine infection are classified as histologic chorioamnionitis (HC) [3,4]. Chorioamnionitis has become recognized as a multiorgan disease of the fetus [5] and is reportedly associated with systemic adverse neonatal outcomes [6–11]. Chorioamnionitis is sometimes difficult to diagnose during pregnancy because the inflammatory reaction is often clinically silent.

Lencki et al. [12] described the evaluation of clinical chorioamnionitis based on maternal body temperature, maternal clinical symptoms, and maternal blood test results. However, some cases have already progressed to advanced HC before meeting the diagnostic criteria of clinical chorioamnionitis [13]. Early diagnosis of HC is considered difficult when it is based only on the maternal inflammatory response.

Abbreviations: HC, histologic chorioamnionitis; WBC, white blood cell count; mWBC, maternal white blood cell count; mCRP, maternal C-reactive protein level; aCell, amniotic white blood cell count; aGlucose, amniotic glucose level; aLDH, amniotic lactate dehydrogenase level; OR, odds ratio; CI, confidence interval; IL, interleukin

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Table 1
Clinical characteristics of patients.

Characteristics	HC (n = 49)	non-HC (n = 31)	P
Age (year), mean ± SD	30.9 ± 5.8	32.2 ± 4.0	0.30
Median (range)	31 (22–42)	31 (25–43)	
Gravidity, Median (range)	2 (1–8)	2 (1–8)	
Parity, Median (range)	1 (0–4)	0 (0–4)	
Height (cm), mean ± SD	157.6 ± 6.6	158.1 ± 5.6	0.53
Body weight at delivery (kg), mean ± SD	58.7 ± 9.8	56.9 ± 8.8	0.39
Body weight before pregnancy (kg), mean ± SD	52.6 ± 8.4	50.2 ± 9.3	0.12
BMI before pregnancy (kg/m2), mean ± SD	21.1 ± 3.1	20.0 ± 3.1	0.10
Case of ruptured membranes, n (%)	26 (53.1)	9 (29.0)	0.03
Maternal temperature at amniocentesis (degree Celsius), Median (range)	37.0 (35.8–37.9)	36.9 (36.2–37.9)	
Gestational age at amniocentesis (week), mean ± SD	28.9 ± 3.7	29.5 ± 3.9	0.38
Gestational age at delivery (week), mean ± SD	29.3 ± 3.7	34.3 ± 4.3	< 0.01
Delivery mode			
Cesarean delivery, n (%)	20 (40.8)	10 (32.3)	0.58
Trans-vaginal delivery, n (%)	29 (59.2)	21 (67.7)	
Birth weight at delivery (kg), mean ± SD	1,317 ± 559	2,212 ± 796	< 0.01
Apgar score			
at 1 min < 5, n (%)	14 (28.6)	3 (9.7)	0.06
at 1 min < 7, n (%)	21 (42.9)	7 (22.6)	0.10
at 5 min < 5, n (%)	2 (4.1)	0 (0.0)	0.27
at 5 min < 7, n (%)	10 (20.4)	1 (3.2)	0.04
Placental weight (g), mean ± SD	376 ± 100	481 ± 123	< 0.01
Median (range)	358 (150–625)	470 (230–760)	

SD, standard deviation. BMI, body mass index

Early diagnosis of HC is important for protection against neonatal morbidity and mortality. Construction of a diagnostic prediction model of HC is essential in clinical practice. Therefore, this study was performed to determine whether the combination of maternal blood and amniotic fluid biomarkers can improve the predictive accuracy of HC.

2. Methods

2.1. Study design and population

This single-center retrospective study was conducted at Fukuoka University Hospital, which is a tertiary perinatal center in Fukuoka, Japan. The institutional review board of Fukuoka University Hospital approved the study (15-2-08, approved 13 December 2017), and written informed consent was obtained from all participants.

In total, 882 pregnant women were transported to our center by ambulance because of preterm labor from April 2005 to April 2018. Of these 882 women, 80 singleton pregnant women were suspected to have intrauterine infection but did not meet the diagnostic criteria for clinical chorioamnionitis [12]; i.e., they had a maternal body temperature of < 38 °C and did not satisfy all of the following four conditions: maternal tachycardia (maternal heart rate of ≥100 beats/min), uterine tenderness, foul-smelling vaginal discharge or amniotic fluid, or a white blood cell count (WBC) of ≥15,000 cells/mm^3. All eligible pregnant women underwent maternal blood testing, amniocentesis, and placental pathological examination after delivery.

Maternal blood samples were measured for the maternal WBC (mWBC) and maternal C-reactive protein level (mCRP). The samples were analyzed using an automated hematology analyzer (XN-1000 or XN-3000; Sysmex, Kobe, Japan). Amniotic fluid was obtained by transabdominal ultrasound-guided amniocentesis using a 25G disposable puncture needle (Hanaco Medical Co., Ltd., Saitama, Japan). Amniotic fluid samples were measured for the amniotic WBC (aCell), amniotic glucose level (aGlucose), and amniotic lactate dehydrogenase level (aLDH) at a biochemical laboratory. aCell was determined using an upright microscope (ECLIPSE *Ci*-L; Nikon, Tokyo, Japan) and is expressed as the number of cells per cubic millimeter. aGlucose and aLDH were analyzed automatically using a LABOSPECT 008 (Hitachi High-Technologies Corporation, Tokyo, Japan).

HC was defined as the presence of acute inflammatory lesions of the decidua, chorion, or amnion according to Blanc's criteria [14]: stage I (sub-chorionitis): patchy or diffuse accumulation of neutrophils within the sub-chorionic plate or decidua; stage II (chorionitis): more than a few scattered neutrophilic infiltrates in the chorionic plate or membranous chorionic connective tissue; and stage III (chorioamnionitis): neutrophilic infiltrates reaching the sub-amniotic connective tissue and amniotic epithelium. We divided the pregnant women into two groups based on Blanc's classification of placental inflammation severity: the HC group (stage II–III) (n = 49) and the non-HC group (stage 0–I) (n = 31).

We obtained the following clinical data from the electronic medical records at our center: maternal age, gravity, parity, maternal height, maternal body weight before pregnancy, body weight at delivery, body mass index before pregnancy, occurrence of ruptured membranes, gestational age and maternal temperature at amniocentesis, gestational age at delivery, delivery mode, and birth weight at delivery.

2.2. Statistical analysis

Continuous variables are summarized as mean ± standard deviation or median and range, and categorical variables are summarized as number and percentage. We assessed the predictors of HC using logistic regression models: Model 1, combination of mWBC and mCRP; Model 2, combination of Model 1 and aGlucose; and Model 3, combination of Model 2, aCell, and aLDH. Odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated using logistic regression models. The ability of the statistical models to discriminate between individuals with and without HC was evaluated using c-statistics. All statistical analyses were conducted using the JMP software program, version 9 (SAS Institute, Cary, NC, USA) and SPSS version 16.0J for Windows Base System SC (SPSS Japan, Tokyo, Japan). A P-value of < 0.05 was considered statistically significant.

3. Results

The clinical characteristics of the patients are shown in Table 1. Significant differences in case of ruptured membranes (P = 0.03), gestational age at delivery (P < 0.01), birth weight at delivery

Table 2
Crude and multivariable OR for histologic chorioamnionitis.

	HC (n = 49)	non-HC (n = 31)	Crude OR (95% CI)	Multivariable OR (95% CI)		
				Model 1	Model 2	Model 3
Maternal blood biomarker						
mWBC ($\times 10^3$/μL), mean ± SD	13.64 ± 4.86	11.34 ± 3.16	1.14 (1.02–1.29)	1.14 (1.01–1.29)	1.12 (0.99–1.30)	1.09 (0.90–1.37)
mCRP, mean ± SD	3.15 ± 2.95	2.41 ± 3.85	1.07 (0.93–1.26)	1.02 (0.88–1.20)	1.08 (0.92–1.31)	0.98 (0.78–1.22)
Amniotic fluid biomarker						
aGlucose ($\times$ 10 mg/dl), mean ± SD	1.92 ± 1.97	3.61 ± 2.18	0.67 (0.85–1.49)		0.66 (0.49–0.85)	1.08 (0.74–1.58)
aCell ($\times$ 10/mm^3), mean ± SD	78.46 ± 120.63	1.41 ± 1.83	1.31 (1.10–1.75)			1.24 (1.06–1.68)
aLDH ($\times 10^2$ IU/L), mean ± SD	17.06 ± 21.76	4.18 ± 7.74	1.15 (1.05–1.32)			1.04 (0.95–1.17)

mWBC, maternal white blood cell count; mCRP, maternal C-reactive protein level; aCell, amniotic white blood cell count; aGlucose, amniotic glucose level; aLDH, amniotic lactate dehydrogenase level; HC, histologic chorioamnionitis; SD, standard deviation; OR, odds ratio; CI, confidence interval.
Model 1: combination of mWBC and mCRP.
Model 2: combination of Model 1 and aGlucose.
Model 3: combination of Model 2, aCell, and aLDH.

($P < 0.01$), and placental weight ($P < 0.01$) were observed between HC and non-HC groups. Moreover, the incidence of Apgar score < 7 at 5 min was significantly higher in the HC group than in the non-HC group ($P = 0.04$).

The effects of potential predictors of HC are shown in Table 2. The pregnant women with HC had higher levels of mWBC, mCRP, aCell, and aLDH and lower levels of aGlucose than those without HC. According to the crude analyses, mWBC (OR, 1.14; 95% CI, 1.02–1.29), aCell (OR, 1.31; 95% CI, 1.10–1.75), and aLDH (OR, 1.15; 95% CI, 1.05–1.32) were significantly associated with an increased risk of HC. According to the multivariable analysis with information from maternal blood biomarkers (Model 1), mWBC was a significant predictor of HC (OR, 1.14; 95% CI, 1.01–1.29). With further adjustment for aGlucose (Model 2), the effects of mWBC became statistically non-significant, but aGlucose was associated with a lower risk of HC (OR, 0.66; 95% CI, 0.49–0.85). With further adjustment, aCell was the only significant predictor of HC (OR, 1.24; 95% CI, 1.06–1.68) independent of mWBC, mCRP, aGlucose, and aLDH.

To assess the value of diagnosis using a combination of the three models, these models were subjected to multivariate logistic regression analysis by the JMP software program. Each probability of the models were as follows: Model 1 probability = $1/(1 + \exp(-1.186 + 0.127 \times \text{mWBC} + 0.022 \times \text{mCRP}))$, Model 2 probability = $1/(1 + \exp(-0.097 + 0.117 \times \text{mWBC} + 0.080 \times \text{mCRP} - 0.414 \times \text{aGlucose}))$, and Model 3 probability = $1/(1 + \exp(-2.521 + 0.087 \times \text{mWBC} - 0.017 \times \text{mCRP} + 0.021 \times \text{aCell} + 0.081 \times \text{aGlucose} + 0.044 \times \text{aLDH}))$.

Receiver operating characteristic curves to demonstrate the ability of the statistical models to discriminate pregnant women with HC from those without are shown Fig. 1 and Table 3. The c-statistics were higher in Model 3 (0.803; 95% CI, 0.701–0.905) than in Model 1 (0.634; 95% CI, 0.511–0.758) and Model 2 (0.785; 95% CI, 0.684–0.887). The accuracy of HC based on Model 3 had the best sensitivity (87.5%), specificity (74.0%), positive predictive value (80.8%), and negative predictive value (91.4%).

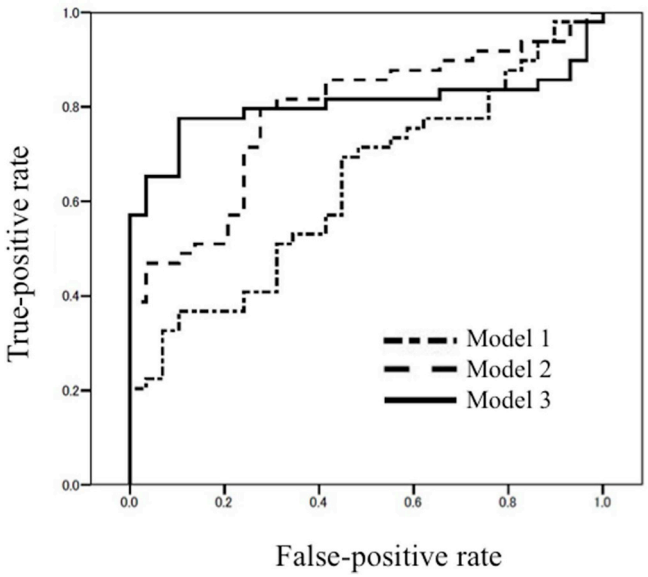

Fig. 1. Receiver operating characteristic curves of multivariable logistic regression. The solid line shows the multivariable logistic regression of Model 3 (0.803; 95% CI, 0.701–0.905), the dotted-dashed line shows that of Model 1 (0.634; 95% CI, 0.511–0.758), and the dotted line shows that of Model 2 (0.785; 95% CI, 0.684–0.887).

4. Discussion

The availability of amniotic fluid biomarkers is important for improving the prediction of HC. Early detection of HC may contribute to neonatal morbidity and mortality [9,11]. In this study, we demonstrated that the combination of mWBC, mCRP, aGlucose, aCell, and aLDH (Model 3) can improve the predictive accuracy for HC. We also noted that the c-statistic of Model 3 was high (> 0.80). Therefore, Model 3 (c-statistic, 0.803) can be used to improve the predictive diagnostic accuracy of HC. By adding aLDH and aCell to Model 2, we obtained a c-statistic of 0.785. Some reports have suggested that the predictive value of aLDH is a useful biomarker in predicting HC [15]. Our results support these findings. Moreover, aGlucose was a significant predictor of HC independent of mWBC and mCRP in Model 2. Thus, aCell was the only significant predictor of HC independent of mWBC, mCRP, aGlucose, and aLDH in Model 3. Unexpectedly, not aGlucose but aCell was the only independent predictor of HC among the five factors of Model 3; even the c-statistic of Model 3 (integrative analysis of five factors) showed a higher prediction ratio for HC than the other two models. The difference in these statistical findings could be explained by the diversity of the pathogenic microorganisms involved in HC. Not only bacteria but also some viruses and other microorganisms such as *Ureaplasma* sp. and *Mycoplasma* sp. are known causes of chorioamnionitis, intrauterine infection, and sequential preterm labor. These microorganisms are categorized as intracellular parasites and do not require amniotic glucose because they can survive only in intracellular conditions [16–18]. Our findings suggest that aGlucose analysis cannot be used to predict HC derived from intracellular parasitic infection. Taken together, these findings indicate that the combined analysis of five factors, especially the addition of aCell and aLDH to Model 2, can be used to predict HC caused by a broad range of microorganisms.

Table 3
Receiver operating characteristics of histologic chorioamnionitis.

	c-statistics (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Model 1 (combination of mWBC and mCRP)	0.634 (0.511–0.758)	58.1	69.4	54.5	72.3
Model 2 (combination of Model 1 and aGlucose)	0.785 (0.684–0.887)	83.9	71.4	65.0	87.5
Model 3 (combination of Model 2, aCell and aLDH)	0.803 (0.701–0.905)	87.5	74.0	80.8	91.4

mWBC, maternal white blood cell count; mCRP, maternal C-reactive protein level; aCell, amniotic white blood cell count; aGlucose, amniotic glucose level; aLDH, amniotic lactate dehydrogenase level; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Amniocentesis is performed to detect early intrauterine infection during pregnancy, and several reports have focused on candidate biomarkers in amniotic fluid and amniotic fluid culture tests for detection of intrauterine infection [19,20]. Romero et al. [21] reported that the measurement of interleukin (IL)-6 in amniotic fluid was an indicator of intrauterine infection according to amniotic fluid culture results (area under the curve, 0.927). However, amniotic fluid culture results also have high false-negative rates in the detection of intrauterine infection [20,22]. Several other reports have shown that measurement of neutrophil elastase, IL-6, IL-8, matrix metalloproteinase-9, and TNF-α in amniotic fluid are associated with HC [15,23]. We previously reported absolute quantification and sequencing of 16S rDNA copies for prediction of HC [16]. However, facilities capable of such special inspections are limited, and inspection costs are high. In contrast, the amniotic fluid markers aGlucose, aCell, and aLDH are measurable within a relatively short time at hospital biochemical laboratories. Moreover, these measurements are affordable for most patients. Thus, their measurement can be a useful clinical tool.

We could not evaluate the relationship between HC and neonatal infection, which is a limitation of the study. Therefore, we can make no conclusions about the delivery mode after early detection of HC. Although some studies have shown that a single course of an antenatal steroid is clinically safe in the presence of HC [11], further studies are needed to establish the relationship between HC and neonatal infection. We evaluated inflammatory cell infiltration into the placental tissue using hematoxylin-eosin staining only. Therefore, further studies should evaluate bacterial infection in the placenta using Gram staining or alternative staining to identify the appropriate inflammatory biomarker.

In conclusion, we found that the combination of maternal blood and amniotic fluid biomarkers can be a reliable predictor of HC. Therefore, our data provide relevant information to support counseling with regard to improving the predictive accuracy of HC in patients with suspected intrauterine infection.

Conflicts of interest

The authors declare no conflicts of interest or competing financial interests related to this work.

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