

Prediction of histological chorioamnionitis and neonatal and infantile outcomes using procalcitonin in the umbilical cord blood and amniotic fluid at birth

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Abstract

Aim: We aimed to clarify the usefulness of procalcitonin (PCT) in the evaluation of histological chorioamnionitis (CAM) and in the prediction of neonatal and infantile outcomes as a reference of interleukin-6 (IL-6).

Methods: Subjects were 36 singleton pregnant women delivered at 22–37 weeks' gestation due to threatened premature delivery and/or preterm premature rupture of membranes. Cases were classified into the CAM and non-CAM groups, according to Blanc's criteria. Comparisons were made on umbilical venous and amniotic fluid PCT levels among the groups. The relations between umbilical venous PCT and IL-6 levels and neonatal and infantile outcomes were also analyzed.

Results: The umbilical venous PCT level in the CAM group (240.2 pg/mL, 125.4–350.3 pg/mL: median, first quartile–third quartile) was higher than that in the non-CAM group (105.1, 50.2–137.5 pg/mL; $P = 0.0006$). There were no differences in the amniotic fluid PCT levels between the groups. There was a strong correlation between umbilical venous PCT and IL-6 levels (correlation coefficient: 0.793). Among 10 cases with an umbilical venous PCT level of ≥ 170.0 pg/mL and six cases with $IL-6 \geq 11.0$ pg/mL, six (60.0%) and five cases (83.3%), respectively, had adverse neonatal and infantile outcomes. Among seven cases with adverse neonatal and infantile outcomes, six (85.7%) and five (71.4%) cases showed umbilical venous PCT levels of ≥ 170.0 pg/mL and IL-6 levels of ≥ 11.0 pg/mL, respectively.

Conclusion: Similar to IL-6, the umbilical venous PCT level is a promising parameter for predicting histological CAM and adverse neonatal and infantile outcomes related to *in utero* inflammatory status.

Key words: chorioamnionitis, interleukin-6, neonatal and infantile outcomes, procalcitonin, umbilical venous blood.

Introduction

Preterm birth is one of the most significant factors for morbidity and mortality in infants among developed countries, including Japan. The prediction and prevention of preterm birth is a crucial issue to be solved in the current perinatal clinical setting. Chorioamnionitis (CAM) is the leading cause of preterm birth, and fetal inflammatory response associated with CAM

leads to devastating outcomes after birth, including periventricular leukomalacia and chronic lung diseases.^{1–6}

CAM is diagnosed by pathological examination of fetal membranes, which keep the integrity of the fetus. The severity of histological CAM is determined by Blanc's criteria, based on the degree of inflammatory cell infiltration, extending from the chorion to the amnion, and further invading into the amniotic fluid.⁷

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The more inflammatory cell infiltration advances, the more fetal hypercytokinemia is likely to be indicated. There is a strong correlation between the presence of CAM, and neonatal morbidity and mortality. Evaluating CAM at birth is, therefore, essential to predicting neonatal outcome. Histological examination, however, is time-consuming.

Procalcitonin (PCT) is a peptide precursor of calcitonin composed of 116 amino acids, and PCT levels rise in response to a proinflammatory stimulus, especially of bacterial origin. PCT observation has been clinically available on an in-house test basis, as one of the biomarkers for the diagnosis of sepsis, with high accuracy and an ability to determine prognosis and therapeutic effects. However, to date, few studies have been done using PCT evaluation for the diagnosis of CAM. The aim of this study was to clarify the usefulness of PCT in diagnosing and evaluating of histological CAM, and predicting neonatal and infantile outcome, while referencing interleukin-6 (IL-6), the standard biomarker of fetal inflammatory response.

Methods

The subjects were 36 singleton pregnant women with no fetal complications, who delivered at 22–37 weeks' gestation at Kurume University Hospital due to threatened premature delivery and/or preterm premature rupture of membranes (pPROM) between April 2012 and March 2016. This study was conducted with the approval of the Institutional Review Board of our hospital (#12008) and all patients gave written informed consent to participate in this study.

For each case, an umbilical venous blood sample was collected at delivery for measurements of white blood cell (WBC) count, C-reactive protein (CRP), immunoglobulin M (IgM), PCT, and IL-6 levels. Amniotic fluid was also taken at delivery for measurement of the PCT level. Except for WBC count, collected samples were centrifuged at 1,670 g for 15 min and plasma from the umbilical venous blood and supernatant from the amniotic fluid were collected and cryopreserved at -80°C until analysis. PCT and IL-6 were assayed using the Ray Bio, Inc. ELISA kit and R&D System ELISA kit, respectively, and quantified using the Bio Rad 680 microplate reader. The lower limits for detection of PCT and IL-6 were 30.0 and 0.7 pg/mL, respectively. When the values were less than the detection limit, the concentrations of PCT and IL-6 were represented as 30.0 and

0.7 pg/mL, respectively. The placenta was histologically diagnosed by qualified pathologists.

CAM was diagnosed using Blanc's criteria as stages I to III. Cases were classified into two groups – CAM and non-CAM – as cases with CAM stage II or more and cases with CAM stage I or negative. There were 16 and 20 cases in the CAM and non-CAM groups, respectively. As for maternal background, there were no significant differences except in the percentages of cases with cesarean delivery (Table 1). As for neonatal background, there were no differences except for 1-min Apgar score and percentage of cases with chronic lung disease. Parameters of clinical chorioamnionitis, including maternal body temperature, maternal WBC counts, CRP values, and fetal heart rate patterns on cardiotocogram, were carefully monitored. Pregnancies were terminated for reasons including tocolysis failure, abnormal fetal heart rate monitoring, and exacerbation of infection.

Comparisons were made on WBC count, CRP, IgM, and PCT and IL-6 levels in umbilical venous blood and amniotic fluid between the CAM and non-CAM groups. In addition, the relations between the umbilical venous PCT and IL-6 levels and between umbilical venous PCT/IL-6 levels and neonatal and infantile outcomes, including death up to 28 days/ 1 year after birth, chronic lung disease, periventricular leukomalacia, and necrotizing enterocolitis, were analyzed.

The Mann–Whitney *U*-test and Fisher's exact test were used for inter-group comparisons. The Spearman's rank-order correlation was used for correlation analysis. The significance was set at $P < 0.05$. All analyses were conducted using JMP 10.0 (SAS Institute Inc.).

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent was obtained from all patients included in the study.

Results

There were no differences in WBC count, CRP, or IgM levels in the umbilical venous blood between the groups (Table 2). The umbilical venous PCT levels in the CAM group (240.2 pg/mL, 125.4–350.3 pg/mL: median, first quartile–third quartile) were higher than those in the non-CAM group (105.1 pg/mL, 50.2–137.5 pg/mL; $P = 0.0006$; Fig. 1). The umbilical

Table 1 Perinatal characteristics of the CAM and non-CAM groups

	CAM (<i>n</i> = 16)	Non-CAM (<i>n</i> = 20)	<i>P</i> -value
Maternal			
Age (years) [†]	31.0 ± 1.2	31.0 ± 1.2	NS
Primipara	11 (68.8%)	18 (90.0%)	NS
Cesarean delivery	8 (50.0%)	16 (80.0%)	0.0379
Reasons for delivery			
Tocolysis failure	14 (87.5%)	17 (85.0%)	NS
Non-reassuring fetal status	0 (0.0%)	1 (5.0%)	NS
Exacerbation of infection	2 (12.5%)	0 (0.0%)	NS
Pre-eclampsia	0 (0.0%)	2 (10.0%)	NS
Neonatal/infantile			
Gestational age at delivery (weeks) [†]	33.3 ± 2.6	33.3 ± 0.7	NS
Birthweight (g) [†]	1653 ± 140	1881 ± 126	NS
Sex (male/female)	7/9	11/9	NS
1-min Apgar score [†]	5.5 ± 2.0	7.4 ± 1.7	0.0063
5-min Apgar score [†]	7.8 ± 1.4	8.6 ± 0.3	NS
Umbilical arterial pH [†]	7.36 ± 0.07	7.32 ± 0.02	NS
Duration of NICU admission (days) [†]	7.3 ± 3.3	3.8 ± 2.6	NS
Duration of respiratory support [‡] (days) [†]	8.1 ± 2.6	7.4 ± 1.7	NS
Adverse neonatal/infantile outcomes			
Death up to and including at 28 days	0 (0.0%)	0 (0.0%)	NS
Death up to 1 year after birth	0 (0.0%)	0 (0.0%)	NS
Chronic lung disease	5 (31.3%)	1 (5.0%)	0.0357
Periventricular leukomalacia	1 (6.2%)	0 (0.0%)	NS
Necrotizing enterocolitis	1 (6.2%)	0 (0.0%)	NS

[†]Data are shown as mean ± standard deviation; [‡]Mechanical ventilation, including intermittent mandatory ventilation, nasal continuous airway pressure, and nasal directional airway pressure. CAM, chorioamnionitis; NICU, neonatal intensive care unit; NS, not significant.

venous IL-6 levels in the CAM group (7.0 pg/mL, 1.25–37.5 pg/mL) were higher than those in the non-CAM group (0.7 pg/mL, 0.7–2.1 pg/mL; *P* = 0.0006; Fig. 2).

Amniotic fluid PCT levels of the CAM group (40.3 pg/mL, 30.1–67.5 pg/mL) were not different from those of the non-CAM group (65.8 pg/mL, 35.3–92.5 pg/mL; *P* > 0.05; Fig. 3).

There was a strong correlation between umbilical venous PCT and IL-6 levels with a correlation coefficient of 0.793 (Fig. 4). Moreover, among the 16 cases in the CAM group, PCT levels in the umbilical vein were highly correlated with IL-6 levels (correlation coefficient: 0.883). The receiver–operator curve for discrimination between the CAM and non-CAM groups

revealed the cut-off PCT value of 170.0 pg/mL (area under curve: 0.836). Ten (62.5%) out of 16 cases in the CAM group showed a PCT level of ≥170.0 pg/mL (Fig. 5). On the other hand, none of the 20 cases in the non-CAM group indicated either PCT level of <170.0 pg/mL, or IL-6 level of <11.0 pg/mL, which is the criteria for fetal inflammatory response syndrome (data not shown).

We further examined seven cases with adverse neonatal and infantile outcomes. There were six cases and one case in the CAM and non-CAM groups, respectively (Table 3). Five cases in the CAM group (cases 1–5) demonstrated high umbilical PCT levels of ≥170.0 pg/mL and high IL-6 levels of ≥11.0 pg/mL. One case in the CAM group (case 6) showed a high

Table 2 Inflammatory markers in umbilical venous blood in the CAM and non-CAM groups

	CAM (<i>n</i> = 16)	Non-CAM (<i>n</i> = 20)	<i>P</i> -value
White blood cell (/μL)	10 750 (9175–14 450)	8850 (7600–10 350)	NS
C-reactive protein (mg/dL)	0.04 (0.04–0.09)	0.04 (0.04–0.04)	NS
IgM (mg/mL)	6.00 (4.00–7.75)	6.50 (4.75–9.00)	NS

Data are shown as median (first quartile–third quartile). CAM, chorioamnionitis; IgM, immunoglobulin M; NS, not significant.

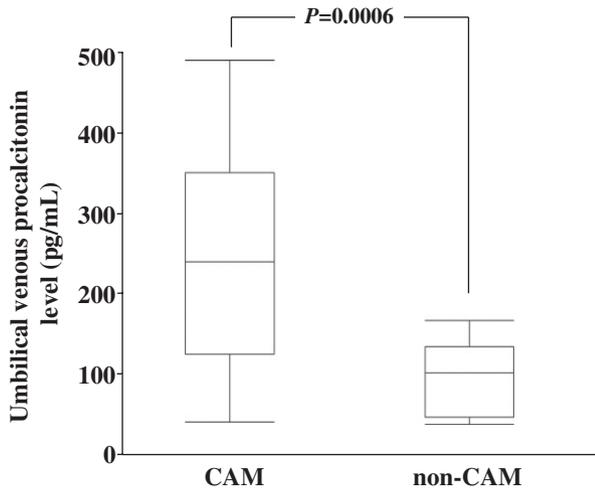


Figure 1 Comparisons of umbilical venous procalcitonin levels in the chorioamnionitis (CAM) and non-CAM groups. Boxes represent interquartile range (IQR), interior lines median, whiskers are maxim and minima within $1.5 \times$ IQR.

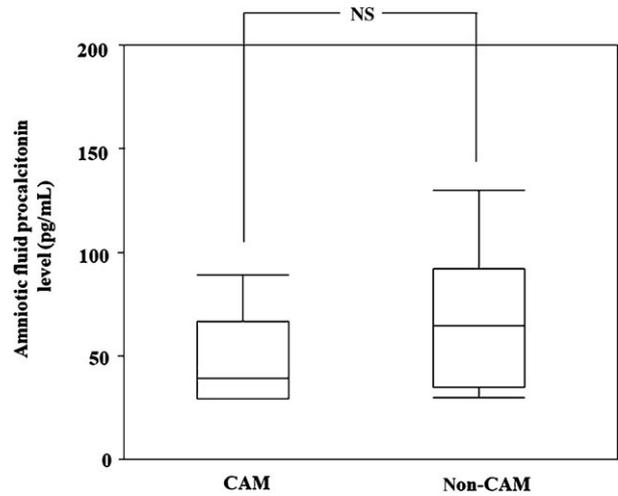


Figure 3 Comparisons of amniotic fluid procalcitonin levels in the chorioamnionitis (CAM) and non-CAM groups. Boxes represent interquartile range (IQR), interior lines median, whiskers are maxim and minima within $1.5 \times$ IQR. NS, not significant.

umbilical venous PCT level but low IL-6 level. One case in the non-CAM group (case 7) had low umbilical venous PCT and IL-6 levels. Among the 10 cases with an umbilical venous PCT level of ≥ 170.0 pg/mL, six (60.0%) had adverse neonatal and infantile outcomes (Fig. 5), whilst among the six cases with umbilical venous IL-6 level of ≥ 11.0 pg/mL, five cases (83.3%) had adverse neonatal and infantile outcomes.

On the other hand, among 26 cases with umbilical venous PCT levels of < 170.0 pg/mL, only one case at 26 weeks' gestation (3.8%) had an adverse neonatal and infantile outcome, while among 29 cases with umbilical venous IL-6 level of < 11.0 pg/mL, two cases (6.9%) had adverse neonatal and infantile outcomes.

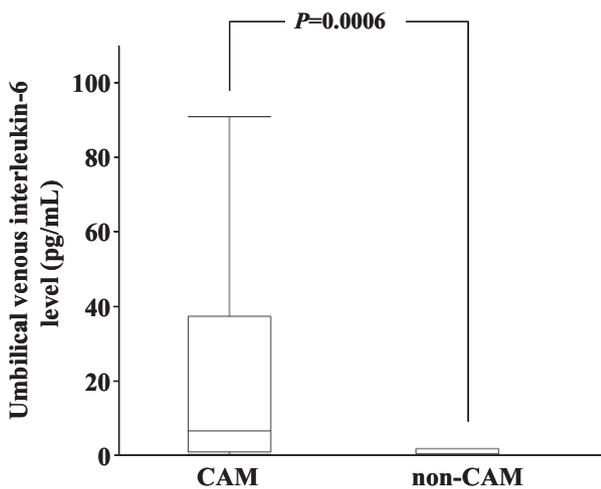


Figure 2 Comparisons of umbilical venous interleukin-6 levels in the chorioamnionitis (CAM) and non-CAM groups. Boxes represent interquartile range (IQR), interior lines median, whiskers are maxim and minima within $1.5 \times$ IQR.

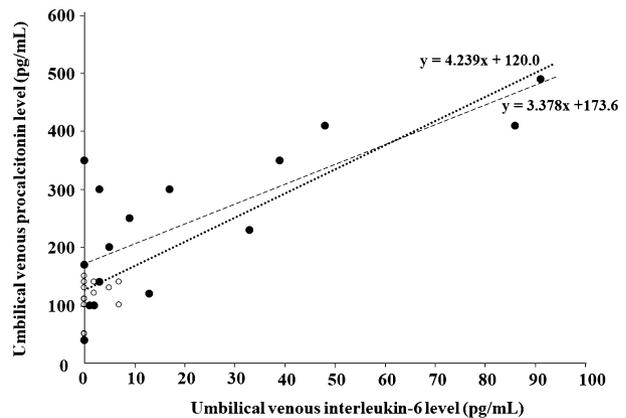


Figure 4 Correlation of umbilical venous blood between procalcitonin and interleukin-6 levels in the (closed circle) chorioamnionitis (CAM) and (open circle) non-CAM groups. Dotted and dashed lines indicate the regression lines between umbilical venous procalcitonin and interleukin-6 levels overall and the CAM group, respectively. Dotted line indicates the cut-off procalcitonin value of 170.0 pg/mL.

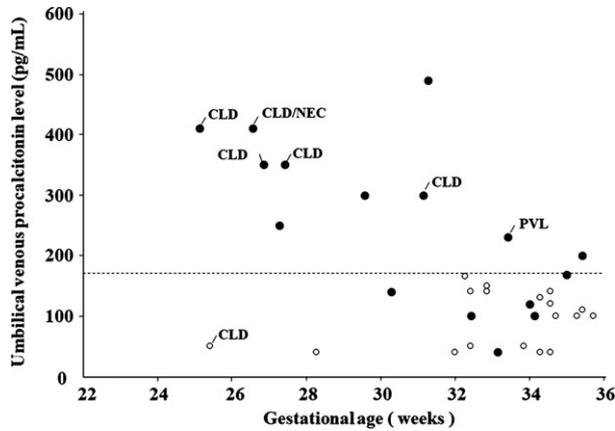


Figure 5 Relation between gestational age at delivery and umbilical venous procalcitonin levels in the (closed circle) chorioamnionitis (CAM) and (open circle) non-CAM groups. CLD, chronic lung disease; NEC, necrotizing enterocolitis; PVL, periventricular leukomalacia.

Discussion

Sepsis and CAM are among the most devastating causes of neonatal morbidity and mortality, and the prediction and early diagnosis of neonatal sepsis and CAM are very important for neonatal care.^{8–10} To date, several reports have been made on the usefulness of umbilical venous PCT, mainly for two purposes: the prediction of neonatal sepsis^{11–13} and the early diagnosis of CAM.^{14,15} Oludag *et al.* reported that sensitivity and specificity of the umbilical venous PCT level to predict CAM in cases with pPROM were 92.3% and 68.4%, respectively, in preterm neonates.¹⁴ Janota *et al.* reported that the umbilical venous PCT level had a relation with CAM and neonatal sepsis.¹⁵ Moreover, umbilical venous PCT level had higher sensitivity than umbilical venous CRP level. However, no reports have been made regarding the

relation between umbilical venous PCT and severity of histological CAM, or between PCT and IL-6 level, one of the most representative biomarkers for fetal inflammatory response.

In this study, we demonstrated that umbilical venous PCT levels increased along with histological CAM. PCT, having a molecular weight of 11.4–11.6 kDa, cannot cross the placenta. PCT detected in the umbilical venous blood is considered to have originated in the fetus. The gestational age at delivery in the CAM group was earlier than that in the non-CAM group. Guibourdenche *et al.* reported that in infants born prematurely without intrauterine infection, umbilical cord venous PCT levels were unrelated to gestational age at delivery.¹⁶ Therefore, elevated umbilical cord PCT levels in the CAM group are not considered as being influenced by gestational age, but rather reflecting the status of intrauterine infection and inflammation.

There have been few reports regarding the usefulness of amniotic fluid PCT in predicting intrauterine inflammation. Dulay *et al.* reported that they were unable to diagnose intrauterine inflammation *in utero* with amniotic fluid PCT obtained by amniocentesis.¹⁷ Torbé *et al.* stated that PCT in vaginal discharge in cases with pPROM could not predict CAM or intrauterine infection.^{18,19} In this study, we also found that amniotic fluid PCT was unrelated to the presence of histological CAM. As amniotic fluid is mainly produced by fetal urine, it was assumed that the amniotic fluid PCT level parallels the umbilical venous PCT level; however, this was not the case.^{20,21} We speculate that in cases with CAM, fetal renal function is impaired, which may result in a decrease of PCT excretion into fetal urine and amniotic fluid.

In this study, a bacterial culture of the amniotic fluid obtained by sterile methods was not performed in every case for detecting the presence of intrauterine infection of bacterial origin. Although fetal inflammatory

Table 3 Clinical characteristics of cases with adverse neonatal/infantile outcomes and umbilical procalcitonin and interleukin-6 levels in the CAM and non-CAM groups

Case no.	Adverse neonatal/infantile outcome	Gestational age at delivery (weeks ⁺ days)	Birthweight (g)	Umbilical venous procalcitonin level (pg/mL)	Umbilical venous interleukin-6 level (pg/mL)	Group
1	PVL	33 ⁺³	1926	233.1	33.5	CAM
2	CLD	31 ⁺¹	1750	301.4	17.1	CAM
3	CLD	27 ⁺³	1428	353.1	39.1	CAM
4	CLD/NEC	26 ⁺⁴	851	412.2	48.6	CAM
5	CLD	25 ⁺¹	830	411.3	86.2	CAM
6	CLD	26 ⁺⁶	1192	303.2	0.7	CAM
7	CLD	25 ⁺³	682	50.3	0.7	Non-CAM

CAM, chorioamnionitis; CLD, chronic lung disease; NEC, necrotizing enterocolitis; PVL, periventricular leukomalacia.

response is highly associated with chorioamnionitis, bacterial infections to the fetuses and neonates are not always evident. One of the reasons is that mycoplasma/ureaplasma, which are common pathogens related to preterm delivery (i.e. chorioamnionitis), are not detected in a routine in-house bacterial culture and recently bacterial genomic analysis has revealed that there are many pathogens other than mycoplasma/ureaplasma that can be associated with preterm delivery.^{22,23} We believe 'sub-clinical' intra-amniotic infection may play a role, in part, in the elevation of umbilical venous PCT.

We showed that the umbilical venous PCT level had a strong correlation with the IL-6 level. All cases with umbilical cord PCT levels of ≥ 170.0 pg/mL and IL-6 levels of ≥ 11.0 pg/mL were in the CAM group. Among 10 cases with umbilical venous PCT levels of ≥ 170.0 pg/mL and six cases with IL-6 levels of ≥ 11.0 pg/mL, six cases (60.0%) and five cases (83.3%), respectively, had various adverse neonatal and infantile outcomes, including chronic lung disease. This suggests that similar to IL-6, the umbilical venous PCT level can be a strong candidate for not only the prediction of histological CAM, but also for the prediction of adverse neonatal and infantile outcomes related to *in utero* inflammation. Among seven cases with neonatal and infantile adverse outcomes, one case was included in the non-CAM group with low PCT and IL-6 levels. The adverse neonatal and infantile outcomes, including chronic lung diseases, necrotizing enterocolitis, and periventricular leukomalacia, are attributed largely to prenatal inflammation, but in part, to inflammation during resuscitation, and postnatal inflammation because of prematurity and circulatory instability after birth. Therefore, *in utero* biomarkers, such as umbilical venous PCT and IL-6 levels, cannot be used to make predictions of adverse neonatal and infantile outcomes in cases without any prenatal inflammation.

The limitation of this study was that it was retrospective, and the number of subjects was quite limited. However, we demonstrated that umbilical venous PCT is a possible promising biomarker for predicting adverse neonatal and infantile outcomes. The selective treatment strategy based on inflammatory status at birth using parameters including umbilical venous PCT is expected to lead to an improvement in neonatal/infantile outcome. This study is only preliminary, and further prospective studies are necessary to show the clinical usefulness of umbilical venous PCT at birth.

In conclusion, in this study we found that the umbilical venous PCT level was predictive of

histological CAM. Furthermore, the umbilical venous PCT level suggested the possibility of predicting adverse neonatal and infantile outcomes related to the *in utero* inflammatory status.

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Disclosure

There are no financial or other issues that could lead to a conflict of interest.

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