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The Physiological Variation in Plasma Presepsin Levels During the Early Neonatal Period

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Neonatal sepsis continues to be a global problem with significant morbidity and mortality, because of the difficulty in predicting its onset with clinical symptoms alone. Thus, the presence of biomarkers is useful for the diagnosis of neonatal sepsis. Presepsin is a 13-kDa truncated form of soluble CD14 that is produced through proteolytic cleavage on activated monocytes. Presepsin, consisting of 64 amino acid residues, has been proposed as a reliable biomarker for the early diagnosis of sepsis in neonates. However, some biomarkers for the diagnosis of sepsis are elevated during the early neonatal period due to physiological variation, whereas such variation in presepsin levels is uncertain. The objective of this study is to investigate the physiological variation in plasma presepsin levels during the early neonatal period. This prospective study included 30 full-term healthy neonates, including 15 neonates delivered by cesarean section. Plasma presepsin levels were examined at birth and on the first day and the fifth day of life in neonates, and the levels on the 5th day of life were lower than those at any other points ($P < 0.001$). Moreover, there was no significant difference of plasma presepsin levels between neonates delivered vaginally and by cesarean section. The physiological variation in plasma presepsin levels was observed during the early neonatal period. Attention needs to be paid when measuring plasma presespsin levels for the screening of sepsis during the early neonatal period.

Keywords: biomarker; early neonatal period; neonatal sepsis; physiological variation; presepsin
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Introduction

Neonatal sepsis continues to be a global problem with significant morbidity and mortality. The evaluation of some biomarkers is useful for the diagnosis of sepsis because of the difficulty in predicting its onset by clinical symptoms alone. Peripheral white blood cell (WBC) counts and serum levels of C-reactive protein (CRP) and procalcitonin, the representative biomarkers for the diagnosis of bacterial infection, are elevated in the early neonatal period due to physiological variations and can peak at approximately 24 hours after birth (van Rossum et al. 2004; Hofer et al. 2011; Miyake et al. 2016). Therefore, attention should be paid to the interpretation of the elevation of these biomarkers during the early neonatal period.

Presepsin, which is a 13-kDa truncated form of soluble CD14 and consists of 64 amino acid residues, is released into circulation through proteolytic cleavage on activated monocytes (Yaegashi et al. 2005). In common with other biomarkers, plasma presepsin levels are also proposed as a reliable biomarker for the early diagnosis of sepsis in neonates (Bellos et al. 2018). However, the physiological variation of plasma presepsin levels during the early neonatal period is uncertain.

We herein performed a prospective study to investigate the physiological variation in plasma presepsin levels during the early neonatal period. In addition, we investigated the relationship between modes of delivery and plasma presepsin levels.

Materials and Methods

Subjects

The present prospective study included 30 neonates born at the Hospital of the University of Occupational and Environmental Health, Japan, from September 1, 2015, to July 31, 2017, of whom each 15 were delivered vaginally and by cesarean section beyond 37 weeks of

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gestational age. None of the neonates had neonatal diseases, including respiratory disorders and asphyxia, and was born to mother with premature rupture of membranes. Maternal underlying diseases included diabetes (n = 4), hypothyroidism (n = 2), systemic lupus erythematosus (SLE, n = 2), epilepsy (n = 1), schizophrenia (n = 1), hyperthyroidism (n = 1), hypertension (n = 1) and ovarian teratoma (n = 1). All neonates were discharged on the 5th day of life without any postnatal complications. Peripheral WBC counts, serum CRP and procalcitonin levels, and plasma presepsin levels were examined at birth and on the 1st day and 5th day of life. In addition, the expression level of neutrophil CD64 (nCD64), a reliable marker for the diagnosis of severe bacterial infection (Ng et al. 2004), was examined on the same days. All eligible neonates were born during the day time (11 am to 4 pm), and the first blood sample was collected as soon as possible after birth. The sampling on 1st day and 5th day of life was performed between 9 to 11 am. The measurements of WBC counts and serum CRP and procalcitonin levels were routinely performed in the laboratory of the Hospital of the University of Occupational and Environmental Health, Japan. The expression level of nCD64 was measured by staining whole blood cells with QuantiBrite CD64-phycoerythrin (PE)/CD45-peridinin chlorophyll protein (PerCP) (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA). The samples were analyzed by flowcytometry using a Coulter Epics-XL-MCL flow cytometer (Beckman Coulter, Brea, CA, USA). For quantification, we created a standard curve using Quanti-Brite PE beads (Becton Dickinson Biosciences). Finally, we measured the mean fluorescence intensity of CD64PE, and calculated the mean numbers of PE molecules on neutrophils (Miyake et al. 2016). Informed consent was obtained from the parents of all patients. Our study was approved by the Institutional Review Board of the University of Occupational and Environmental Health, Japan.

Measurement of presepsin levels

Presepsin levels were measured by the PATHFAST® immunoassay analytical system (PROGEN Biotechnik GmbH, Germany; Mitsubishi Chemical Medience Corporation, Japan). According to the instruction manual, plasma or whole blood, but not serum, was suitable for the measurement of it. We tried to measure plasma presepsin levels. The coefficient of variance of the method in plasma is between 3.8% and 5.0%. Blood samples were collected using sodium citrate tubes, and were centrifuged at 1,000 × g at 4°C for 15 minutes for the separation of plasma within 30 minutes. Separated plasma was frozen and stored at −80°C until the measurement.

Statistical analysis

The results were analyzed using the SPSS statistical software program (SPSS Inc., Chicago, IL, USA). Serial changes in WBC counts, serum CRP and procalcitonin levels, nCD64 expression and plasma presepsin levels were compared using the Kruskal-Wallis test. P values < 0.05 were considered to be statistically significant. The comparisons of these biomarkers between 2 of the 3 time points were performed by Mann-Whitney U test with the Bonferroni correction. P values < 0.017 were considered to be statistically significant. The comparisons of the demographic profiles and the levels of biomarkers between neonates delivered vaginally and by cesarean section were performed by the Mann-Whitney U test. P values < 0.05 were considered to be statistically significant.

Results

The demographic profiles of 30 neonates are summarized in Table 1. Plasma presepsin levels on the 5th day of life (median, 180.5 pg/mL; range, 89.5-421.5 pg/mL) were lower than those at any other points (at birth, median, 318.5 pg/mL; range, 99.2-1180.0 pg/mL, $P < 0.001$ and 1st day of life, median, 343.8 pg/mL; range, 129.0-655.0 pg/mL, $P < 0.001$) (Fig. 1 and Table 2). By contrast, WBC counts and serum CRP and procalcitonin levels were the highest on the 1st day of life, while there was no significant difference in the serial expression levels of nCD64 in the eligible neonates (Table 2).

The serial changes of all 5 biomarkers in 21 neonates born to mothers without underlying diseases showed almost same tendency as those in all eligible neonates (Table 3). However, among those neonates, there were two neonates whose plasma presepsin levels were higher than the 75th percentile; in one neonate, the plasma presepsin level was elevated at birth, while in another neonate, the levels were elevated on 1st day and 5th day of life. On the other hand, in two neonates born to mothers with SLE, plasma presepsin levels on 5th day of life were higher than the 75th percentile.

Table 1. Demographic characteristics of enrolled neonates.

	All neonates (n = 30)	Neonates delivered by cesarean section (n = 15)	Neonates delivered by vaginal delivery (n = 15)	P value
Sex, the number of male (%)	12 (40)	7 (47)	5 (33)	0.367
Gestational age, weeks, median (range)	38.5 (37.0-40.9)	38.3 (37.0-38.6)	39.9 (37.6-40.9)	0.001
Birth-weight, g, median (range)	2,973 (2,500-4,352)	2,874 (2,500-4,352)	3,052 (2,640-3,584)	0.250
Apgar score at 1 min, median (range)	8 (8-9)	8 (8-9)	8 (8-9)	0.217
Apgar score at 5 mins, median (range)	9 (8-9)	9	9 (8-9)	0.775
Physiological weight loss, %, median (range)	6.8 (2.3-12.2)	7.0 (4.3-12.2)	6.3 (2.3-9.3)	0.202
Neonates born to mothers with underlying diseases, n (%)[*1]	9 (30)	2 (13)	7 (47)	0.108

*[1]Five and four mothers had one and two underlying diseases, respectively.

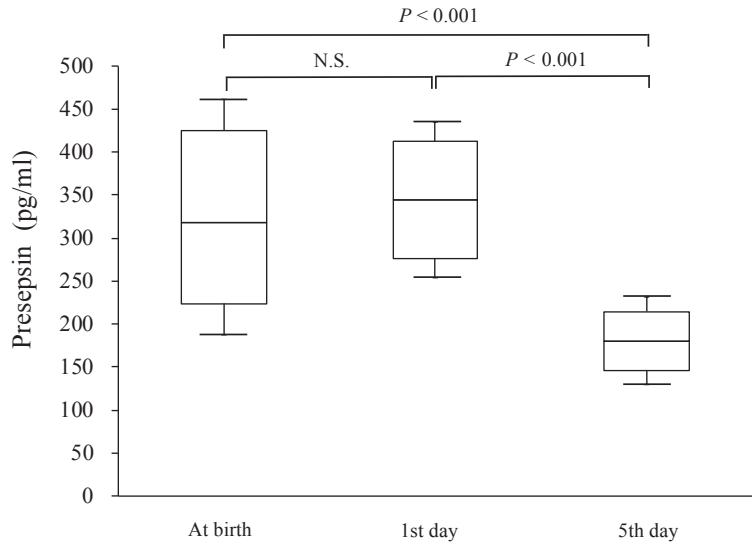

Fig. 1. Comparison of plasma presepsin levels during the early neonatal period.
Plasma presepsin levels were measured at birth and on the 1st day and the 5th day after birth. The bottom and the top of the line correspond to 25th and 75th percentile points, respectively. The line within the box represents median.
N.S., not significant.

Table 2. Serial changes of plasma presepsin levels, WBC counts, serum CRP and serum procalcitonin levels and nCD64 expression.

	At birth	Day 1	Day 5
Presepsin, pg/mL, median	318.5	343.8$^{\#}$	180.5$^{\#*}$
(range)	(99.2-1180.0)	(129.0-655.0)	(89.5-421.5)
WBC, cells/μL, median	10,950	17,645$^{\#}$	10,020*
(range)	(5,590-27,530)	(3,130-29,760)	(5,070-30,130)
CRP, mg/dL, median	0.00	0.20$^{\#}$	0.04$^{\#*}$
(range)	(0.00-0.27)	(0.07-7.74)	(0.0-0.48)
Procalcitonin, ng/mL, median	0.10	3.39$^{\#}$	0.11*
(range)	(0.02-36.14)	(0.47-100)	(0.06-0.56)
nCD64, molecules/cell, median	1,817	1,829	1,835
(range)	(1,762-1,908)	(1,775-1,872)	(1,750-2,087)

Normal ranges of presepsin levels, WBC counts, CRP and procalcitonin levels and nCD64 expression are < 320 pg/mL, 3,000-9,000/μL, < 0.20 mg/dL, < 0.25 ng/mL and 1,762-1,908, respectively. Normal ranges in our hospital were used as references for WBC counts and CRP and procalcitonin levels and that described in the kit manual for presepisin. The range of nCD64 expression is referred to the results of our previous study (Miyake et al. 2016).
There were significant differences in WBC counts, serum CRP and procalcitonin levels and plasma presepsin level among the 3 groups by Kruskal-Wallis test ($P < 0.001$).
$^{\#}P < 0.017$ compared with 'at birth'; $^{*}P < 0.017$ compared with 'Day 1.'
WBC, white blood cell; CRP, C-reactive protein; nCD64, neutrophil CD64.

We also compared the levels of the above 5 biomarkers between neonates delivered vaginally and by cesarean section (see Table 1). Only gestational age showed a significant difference between the two groups ($P = 0.001$). There was no significant difference in plasma presepsin levels, WBC counts and serum procalcitonin levels on each day of life between the two groups (Table 4). The same trend of plasma presepsin levels was observed even in the population excluding neonates born to mothers with underlying diseases (data not shown). On the other hand, serum CRP levels on the 1st day and 5th day of life were higher in neonates delivered vaginally than in those delivered by cesarean section. Furthermore, the expression of nCD64 on the 1st day of life was higher in neonates delivered vaginally.

Discussion

In the present study, the physiological variations in WBC counts and serum levels of CRP and procalcitonin were observed during the early neonatal period. As with these biomarkers, there was the physiological variation in plasma presepsin levels during the early neonatal period. Although plasma presepsin levels in healthy term neonates were not as high as those in neonates with sepsis, which was shown in the previous studies (Pugni et al. 2015; Bellos et al. 2018), attention needs to be paid when using it as a marker for the diagnosis of sepsis during the early neonatal

Table 3. Serial changes of plasma presepsin level, WBC counts, serum CRP and serum procalcitonin levels and nCD64 expression in neonates born to mothers without underlying diseases.

	At birth	Day 1	Day 5
Presepsin, pg/ml, median (range)	320.5 (99.2-1180.0)	360.5 (129.0-655.0)	172.0$^{\#*}$ (89.5-421.5)
WBC, cells/μl, median (range)	10,910 (5,590-27,530)	17,370$^{\#}$ (3,130-29,760)	9,120* (5,070-30,130)
CRP, mg/dl, median (range)	0.00 (0.00-0.27)	0.17$^{\#}$ (0.07-7.74)	0.03$^{\#*}$ (0.0-0.43)
Procalcitonin, ng/ml, median (range)	0.12 (0.02-36.14)	3.54$^{\#}$ (0.47-100)	0.11* (0.06-0.56)
nCD64, molecules/cell, median (range)	1,823 (1,762-1,908)	1,823 (1,775-1,872)	1,823 (1,775-2,028)

Of the eligible 30 neonates, 21 were included in this group.
Normal ranges of presepsin level, WBC counts, CRP and procalcitonin levels and nCD64 expression are < 320 pg/mL, 3,000-9,000/μL, < 0.20 mg/dL, < 0.25 ng/mL and 1,762-1,908, respectively. The ranges of WBC counts and serum CRP and procalcitonin levels are presented those in our hospital. The range of presepsin is presented those described in the manuals of its kit. The range of nCD64 expression is referred to the results of our previous study (Miyake et al. 2016).
There were significant differences in WBC counts, serum CRP and procalcitonin levels and plasma presepsin level among the 3 groups by Kruskal-Wallis test ($P < 0.001$).
$^{\#}P < 0.017$ compared with 'at birth'; $^{*}P < 0.017$ compared with 'Day 1.'
WBC, white blood cell; CRP, C-reactive protein; nCD64, neutrophil CD64.

Table 4. The variations of biomarkers by the difference of modes of delivery.

	Neonates born by cesarean delivery (n = 15)	Neonates born by vaginal delivery (n = 15)	*P* value
Presepsin, pg/mL			
At birth	324.0 (152.5-1180)	262.5 (99.20-669.5)	0.412
1st day of life	376.5 (213.0-655.0)	296.5 (129.0-534.0)	0.389
5 th day of life	180.5 (102.3-421.5)	180.5 (89.5-446.5)	0.902
WBC counts, cells/μL			
At birth	10,910 (5,590-15,640)	12,380 (6,320-27,530)	0.137
1st day of life	17,370 (10,460-29,760)	18,630 (3,130-28,430)	0.902
5 th day of life	8,660 (5,070-12,460)	10,360 (5,950-30,130)	0.108
CRP, mg/dL			
At birth	0.00 (0.00-0.02)	0.00 (0.00-0.27)	0.233
1st day of life	0.13 (0.03-0.95)	0.72 (0.08-7.74)	0.006
5 th day of life	0.03 (0.00-0.26)	0.09 (0.01-0.48)	0.004
Procalcitonin, ng/mL			
At birth	0.10 (0.06-0.19)	0.10 (0.02-36.14)	0.806
1st day of life	3.54 (0.47-15.51)	1.98 (0.83-100.00)	0.744
5 th day of life	0.11 (0.06-0.19)	0.11 (0.08-0.56)	0.539
nCD64, molecules/cell			
At birth	1,811 (1,775-1,908)	1,823 (1,762-1,884)	1.000
1st day of life	1,811 (1,775-1,860)	1,835 (1,775-1,872)	0.032
5 th day of life	1,835 (1,775-2,028)	1,835 (1,750-2,087)	0.935

WBC, white blood cell; CRP, C-reactive protein; nCD64, neutrophil CD64.

period.

Some biomarkers change considerably during the early neonatal period. These changes are attributable to direct stress on neonates during the perinatal period or maladaptation to the extra-uterine environment, causing the increase of certain substances (van Lente and Pippenger 1987). Serum CRP levels can be affected by the timing of membrane rupture, mode of delivery, and birth weight (Ishibashi et al. 2002). Serum procalcitonin levels during the early neonatal period may also be affected by the timing of membrane rupture and gestational age (Turner et al. 2006). The mechanism of the physiological variation of plasma presepsin levels during the early neonatal period is unknown. Transient renal dysfunction may be associated with the elevation of presepsin levels during the early neonatal period. Presepsin levels were elevated in adult patients with renal

dysfunction (Nagata et al. 2015). Renal function is low at birth, and glomerular filtration rate rapidly increases during the neonatal period (Arant 1978), suggesting that the low renal function at birth may lead to the transient elevation of plasma presepsin levels. The other possibility is the effect of monocyte activation during the early neonatal period. Activated monocytes are the main source of presepsin in humans (Arai et al. 2015). The levels of granulocyte-macrophage-colony stimulating factor, an important molecule for monocyte activation, were elevated in cord blood, and the number of monocytes increased during the perinatal period (Ishii et al. 1995; Takahashi et al. 2010). Further biological analysis is warranted to investigate the cause of the physiological variation of plasma presepsin levels.

As with the result of a previous study (Ishibashi et al. 2002), serum CRP levels were higher in neonates delivered vaginally than the levels in those delivered by cesarean section. In addition, nCD64 expression was also higher in neonates delivered vaginally. These results imply that the physical stress on neonates during delivery may be related to the magnitude of the elevation of inflammatory biomarkers. Although there was no significant difference, plasma presepsin levels were higher in neonates delivered by cesarean section than those delivered vaginally. Presepsin is secreted from activated monocytes (Arai et al. 2015). The decreased phagocytic activity was found in neonates delivered vaginally (Muniz-Junqueira et al. 2003), possibly leading to reduced secretion of presepsin. A further large scale study is desired to investigate the difference of the secretion of presepsin according to the mode of delivery.

The present study has some limitations. First, study population was relatively small; this could have affected the accuracy of the statistical analysis. Second, the physiological variation according to gestational age could not be analyzed because the present study did not include preterm infants. Finally, some eligible neonates were born to mothers with underlying diseases. Two of the four neonates whose plasma presepsin levels were higher than the 75th percentile were born to mothers with SLE. In these neonates, plasma prespsin levels on 5th day of life, but not at birth, were higher than the 75th percentile. We speculated that the marked elevation might be affected by some neonatal factors rather than those by maternal diseases. Although the physical stress induced by intrauterine condition may affect the values of biomarkers, further study is needed to investigate the influence of maternal disease to the elevation of presepsin.

In conclusion, we are able to show the physiological variation in plasma presepsin levels during the early neonatal period. Attention needs to be paid when measuring plasma presespsin level for the screening of sepsis during the early neonatal period.

Conflict of Interest

The authors declare no conflict of interest.

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