

Thrombomodulin Levels in Umbilical Cord Blood-derived Plasma between Light-for-Date and Appropriate-for-Date Infants

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Thrombomodulin, a thrombin receptor on the surface of endothelial cells, plays an important role not only in maintaining normal hemostatic balance but also in in utero development. Plasma thrombomodulin has been used in the clinical setting as a marker of endothelial damage in adults; however, the clinical values for neonates have not yet been determined. Intrauterine growth restriction has deleterious effects on mortality and morbidity in both term and preterm infants. The purpose of the present study was to examine whether or not the association between thrombomodulin and gestational age differed between appropriate-for-date (AFD) and light-for-date (LFD) infants. We measured the thrombomodulin levels in the umbilical cord blood-derived plasma of 388 neonates divided into LFD and AFD infants and analyzed their association with gestational age, birth weight, umbilical cord blood pH, and Apgar scores. The plasma thrombomodulin levels were higher in neonates than in adults. There was an inverse correlation between the gestational age and thrombomodulin levels in AFD infants; however, LFD infants presented high plasma thrombomodulin levels throughout the gestational ages examined. While plasma thrombomodulin levels correlated with gestational age and differed between LFD and AFD infants, no clear correlations were seen with Apgar scores, birth weight, cord blood pH or base excess in a multivariate analysis. While such high plasma thrombomodulin levels in preterm infants may reflect their normal physiological background, the difference between the AFD and LFD infants may be caused by pathological processes other than asphyxiation events in LFD infants.

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Introduction

Thrombomodulin, a glycoprotein that is present on the endothelial cell surface and in plasma, binds with thrombin, and the thrombin-thrombomodulin complex converts protein C into the activated form (1). Although the plasma levels of the soluble form of thrombomodulin have been associated with vascular endothelial damage in adults (2, 3), few studies have investigated the plasma thrombomodulin levels in neonates.

A previous study found that the plasma thrombomodulin levels increases with the gestational age (GA), reaching a peak between the 23rd and 26th weeks and thereafter gradually decreasing during the prenatal and postnatal periods to reach the adult level at 5-15 years of age. The elevated plasma thrombomodulin levels in fetuses and neonates therefore seem to reflect the normal developmental process (4).

Intrauterine growth restriction (IUGR) has deleterious

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effects on mortality and morbidity in both term and preterm infants (5). Among various fetal and maternal etiologies that may cause IUGR, placental insufficiency accounts for the majority of cases (6). Previous studies have revealed an important role of thrombomodulin in the maintenance of the fluidity of the blood in the uteroplacental circulation and the increased expression of endothelial thrombomodulin in IUGR placentae (7, 8). Furthermore, animal studies have revealed thrombomodulin expression during early organogenesis in both vascular and extravascular tissues and embryonic lethality in mice with thrombomodulin deficiency before the development of a functional cardiovascular system (9-11).

Coagulation proteins do not efficiently cross the placental barrier and are independently synthesized by the fetus. The development of the human hemostatic system begins *in utero* and continues until after birth. By 10 weeks of gestation, most coagulation factors reach measurable levels in the plasma, ranging from 10% of the normal adult level for factor IX to approximately 30% for most of the other coagulation factors. In general, they continue to increase gradually with GA. Like many procoagulant factors, the functional levels of most coagulation inhibitors are significantly lower in fetuses and neonates than in adults, reaching adult levels of activity by six months of age (12).

Thrombomodulin works as a key component of the protein C anticoagulant pathway, which controls the balance between coagulation and anticoagulation. However, protein C and prothrombin exhibit completely different expression patterns from that of thrombomodulin during fetal development, suggesting that thrombomodulin acts independently of protein C and thrombin in the fetus (13). Thrombomodulin is present in gestational myometrium and fetal membranes, and the high expression of thrombomodulin in the lung bud, developing gonads, leptomeninges, and the parietal endoderm has been reported in mouse embryos (7, 9). Although analyses of knock-out mouse models have shown that almost all factors involved in the hemostatic system are indispensable for the embryonic and/or perinatal survival, the disruption of fetal genes encoding protein C and antithrombin results in lethal thrombophilia in the fetal circulation, while the placental function and development appear to be normal.

In contrast, the disruption of thrombomodulin leads to mid-gestational growth arrest around embryonic day 8.5. This occurs before the development of the cardiovascular system in the embryo (9, 11, 14). Thrombomodulin expression in the non-vascular cells during murine development as well as the early death of thrombomodulin-deficient embryos suggest that thrombomodulin plays critical roles in fetal development aside from its involvement in anticoagulant activity.

We therefore hypothesized that the thrombomodulin levels in umbilical cord blood-derived plasma might differ between light-for-date (LFD) and appropriate-for-date (AFD) infants according to their GA.

Subjects and Methods

Subjects

We collected umbilical arterial cord blood samples and information on the birth parameters of 477 neonates born at participating hospitals from April 2009 to December 2011. A total of 89 of the 477 cases were excluded from our study because of multiple pregnancies (n=40), insufficient sample volumes (n=15), insufficient data (n=30), or the presence of a major congenital anomaly (n=4). We measured the thrombomodulin levels in umbilical cord blood-derived plasma samples obtained from the remaining 388 neonates (Figure 1) and analyzed their relationship with the GA, birth weight, umbilical cord blood pH and base excess, and Apgar score. LFD infants were defined as infants with a birth weight that was below the 10th percentile for their corresponding GA (15).

Informed consent was obtained from the parents of the study participants. This study was approved by the Nagasaki University Ethics Committee for Human Investigation.

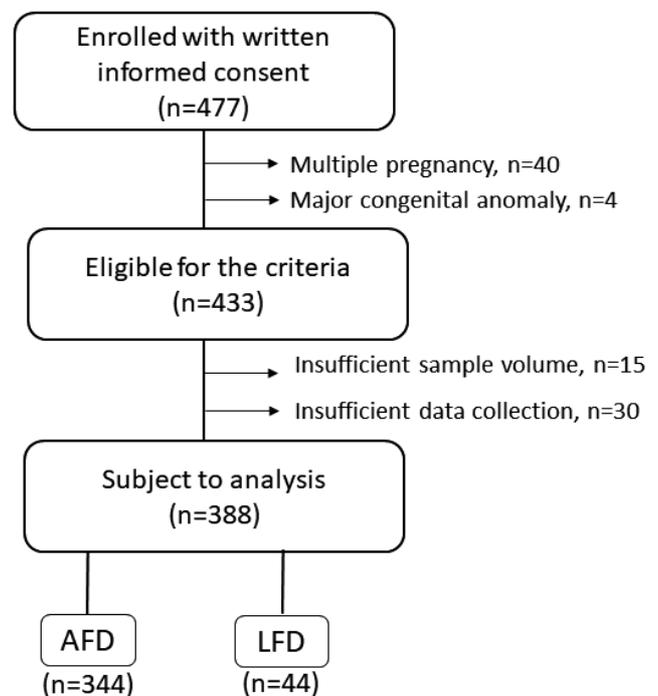


Figure 1: The study profile.

AFD, appropriate-for-date infants; LFD, light-for-date infants.

Methods of Immunoassay

The umbilical arterial cord blood samples were obtained soon after birth. The plasma thrombomodulin levels were measured at SRL, Inc. (Tokyo, Japan) by an enzyme immunoassay using human TM assay kit (TM panacela plate: FUJIREBIO Inc., Tokyo, Japan), which is the one-step sandwich enzyme Immunoassay method using two monoclonal antibodies (16).

Statistical methods

The statistical analyses were performed using the JMP® Pro software program (SAS Institute Japan, Tokyo, Japan). The group data were expressed as the median and range or as the mean \pm standard deviation. Pearson's correlation coefficients were used to measure the strength of the relationship between the plasma thrombomodulin level and the GA, birth weight, cord blood pH and Apgar scores. A multivariate analysis was also performed using an analysis of covariance to determine the factors contributing to plasma thrombomodulin levels.

Results

The study subjects were divided into LFD (n = 44) and AFD (n = 344) groups, and their characteristics, including GA, birth weight, Apgar score, and cord blood pH and base excess, were compared. Figure 2 shows the distribution of their GAs. The majority of term infants were AFD; the ratio of LFD to AFD was higher in preterm infants (LFD n=31: AFD n=96) than in term infants (LFD n=13: AFD n=248).

We measured the plasma thrombomodulin levels in both preterm and term infants and compared them with the

reference values in healthy adults according to the manufacturer's data (16). The neonatal levels were higher than those in adults, and those in preterm infants were higher than those in term neonates (Table 1). Next, we evaluated the association of the plasma thrombomodulin levels with the GA and birth weight. As shown in Figure 3A, there was an inverse correlation between the GA and the plasma thrombomodulin level (coefficient of correlation -0.61, 95% CI -0.66/-0.55). Although the birth weight also correlated with the plasma thrombomodulin level (coefficient of correlation -0.59, 95% CI -0.64/-0.53), this correlation decreased as the birth weight increased (Figure 3B).

To evaluate the possible effects of perinatal asphyxia on the plasma thrombomodulin level, we examined the association between plasma thrombomodulin levels and the Apgar scores or umbilical cord blood pH; however, no significant correlation was detected with the Apgar score (Figure 3C: coefficient of correlation -0.19, 95% CI -0.27/-0.10) and cord blood pH (Figure 3D: coefficient of correlation -0.23, 95% CI -0.31/-0.14).

The relationship between the GA and plasma thrombomodulin levels was reanalyzed separately for AFD and LFD infants. As shown in Figure 4, an inverse correlation between the GA and plasma thrombomodulin levels was detected in AFD infants (coefficient of correlation -0.64, 95% CI -0.70/-0.57) but not in LFD infants (coefficient of correlation -0.06, 95% CI -0.35/0.24). To determine factors influencing the plasma thrombomodulin levels, a multivariate analysis was performed on the following variables: GA, birth weight, Apgar scores, cord blood pH and base excess, and AFD vs. LFD among subjects (Table 2). Although the plasma thrombomodulin levels were significantly associated with the GA and differed between AFD and LFD infants, there was no apparent association with the birth weight, Apgar score, or cord blood pH or base excess.

Table 1. The plasma thrombomodulin concentrations

	Thrombomodulin (FU/mL; mean \pm SD)	95% CI
Term infants (n=261)	4.87 \pm 1.30	4.56-5.18
Preterm infants (n=127)	6.99 \pm 1.67	6.52-7.46
Male adults (n=289)	3.01 \pm 0.52*	
Female adult (n=246)	2.72 \pm 0.55*	

*Provided by manufacturer (SRL, Inc.)

The thrombomodulin concentrations (mean \pm SD) in the cord blood-derived plasma of term and preterm infants are shown in comparison to those in adult plasma that were provided by the manufacturer (SRL, Inc., Tokyo, Japan).

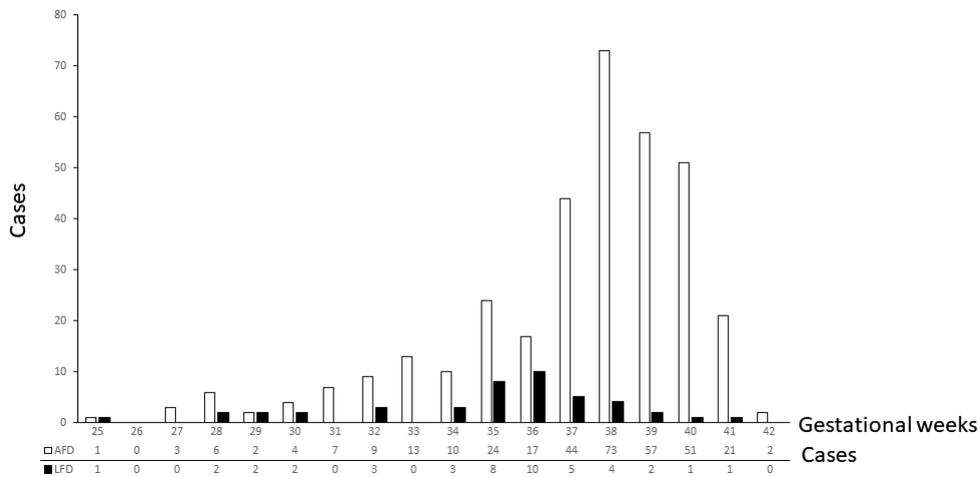


Figure 2: The distribution of the gestational ages of the study subjects. AFD (n=344) and LFD (n=44) infants are shown in open and closed bars, respectively.

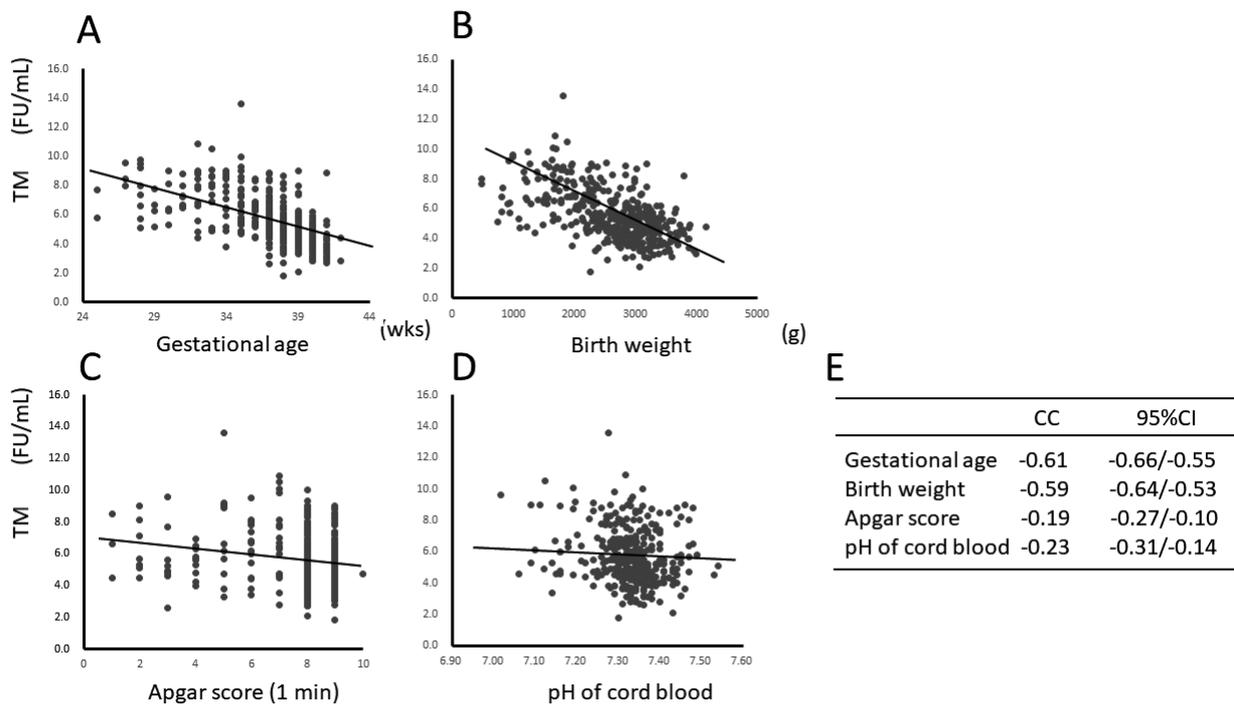


Figure 3: The correlation between the plasma thrombomodulin concentrations and the gestational factors. The thrombomodulin (TM) levels in cord blood-derived plasma are plotted in accordance with the GA (A), birth weight (B), Apgar score at one minute (C), and cord blood pH (D), respectively. The TM levels were correlated with the GA (coefficient of correlation -0.61) and birth weight (-0.59) but not the Apgar score or cord blood pH. Each correlation coefficient (CC) and its 95% confident interval (95% CI) is shown (E).

Table 2: The multivariate analysis of the gestational factors in AFD and LFD infants.

	Estimate	95% CI		<i>p</i> value
Gestational age (wks)	-0.144	-0.242	-0.047	0.0038
Birth weight (g)	-0.000	-0.001	0.000	0.1147
Apgar score/1min	-0.076	-0.017	0.170	0.1093
Cord blood				
pH	-0.604	-2.933	1.725	0.6103
Base excess	-0.036	-0.095	0.023	0.2330
AFD/LFD	-0.368	-0.656	-0.079	0.0127

Discussion

Only a few studies have reported on the plasma thrombomodulin levels of preterm and term neonates, and they all had small sample sizes. Menashi *et al.* determined the plasma thrombomodulin levels in fetuses at different stages of gestation, premature and term neonates, and children in different age groups. The plasma thrombomodulin levels in fetuses increased with the GA, reaching a peak between the 23rd and 26th weeks and thereafter decreasing gradually. The plasma thrombomodulin levels in neonates were still higher than in adults but gradually dropped to adult levels after five years of age (4). Uszynski *et al.* reported that the plasma thrombomodulin levels were more than two-fold higher in fetal blood than in maternal blood (17). Similar results were obtained in the present study. The plasma thrombomodulin levels were higher in neonates than in adults and were inversely correlated with the GA over the 25th gestational week. Given these findings, the higher levels of plasma thrombomodulin in fetuses and preterm infants seem to reflect the normal developmental process.

Plasma thrombomodulin has been used in the clinical setting as a marker of endothelial damage in adults; however, the clinical values for neonates have not yet been determined. Nako *et al.* reported that the thrombomodulin levels in very-low-birth-weight and asphyxiated infants were significantly higher than in normal full-term infants. They suspected that the high plasma thrombomodulin levels in these infants might reflect endothelial damage (18, 19). In the present study, however, our findings showed that the plasma thrombomodulin levels were not significantly associated with the Apgar score at one minute after birth or the umbilical cord blood pH or base excess, indicating that perinatal asphyxia made little to no contribution to the high plasma thrombomodulin levels. Why our study yielded such contradictory findings is unclear at present.

The present study found that plasma thrombomodulin levels were associated with the GA and differed between AFD and LFD infants (Table 2). In addition, an inverse correlation between the GA and plasma thrombomodulin levels was detected in AFD infants but not in LFD infants (Figure 4). Considering the association between the GA and plasma thrombomodulin levels in AFD infants, the immaturity of LFD infants seems to be the more likely reason for the difference. Alternatively, we also considered that the presence of vascular endothelial damage in the placenta might have led to the difference in the association of the thrombomodulin levels and the GA between AFD and LFD infants, as an increased endothelial expression of thrombomodulin was reported in the IUGR placenta (8). IUGR is usually classified as symmetrical and asymmetrical. Symmetrical growth restriction indicates a fetus whose entire body is proportionally small, implying IUGR during the first and early second trimesters of pregnancy, as seen in genetic disorders and congenital infections. Asymmetrical growth restriction spares cephalic size in comparison to body weight, usually resulting from placental insufficiency (20). To examine whether or not the placental dysfunction can cause the upregulation of thrombomodulin, we considered comparing the thrombomodulin levels between symmetrical and asymmetrical LFD infants. Unfortunately, however, the small sample size of LFD infants made such a precise comparison impossible.

Several limitations associated with the present study warrant mention. First, the small sample size, especially for LFD preterm infants, hinders us from drawing any definitive conclusions. Second, since cases complicated with disseminated intravascular coagulation were not precisely identified, they were not excluded from the analysis. Third, since the serum creatinine levels were not routinely measured, we could not evaluate the effects of renal dysfunction on the plasma thrombomodulin levels. Finally, neither the detailed clinical data nor the long-term prognoses of the enrolled infants were

available; therefore, it is unclear whether the high plasma thrombomodulin levels were a marker of a clinical condition or a prognostic factor in LFD infants.

Nevertheless, we believe that our data provide the reference values of the plasma thrombomodulin levels for neonates

and show their correlation with the GA. We showed that plasma thrombomodulin levels were significantly associated with the GA and differed between AFD and LFD infants. Further studies are warranted to clarify the underlying mechanism and the clinical significance of these findings.

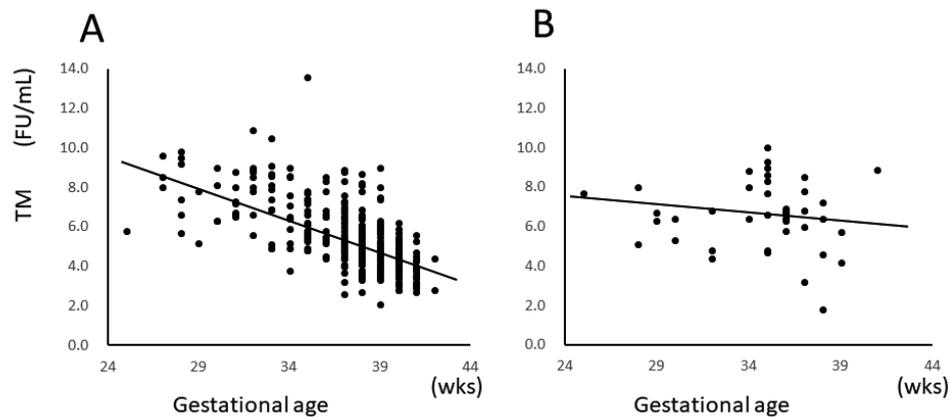


Figure 4: The relationship between plasma thrombomodulin concentrations and gestational age in AFD and LFD infants.

The thrombomodulin (TM) levels in cord blood-derived plasma were correlated with the GA in AFD infants (A: coefficient of correlation -0.64 , 95% CI $-0.70/-0.57$) but not in LFD infants (B: coefficient of correlation -0.06 , 95% CI $-0.35/0.24$).

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