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Amniotic lamellar body count: predicting and distinguishing neonatal respiratory complications in twin pregnancies

Hiroyuki Tsuda [a,b,*], Tomomi Kotani [a], Seiji Sumigama [a], Yukio Mano [a], Ichiro Kawabata [b], Yuichiro Takahashi [b], Shigenori Iwagaki [b], Akihiro Hirakawa [c], Fumitaka Kikkawa [a]

[a] Department of Obstetrics and Gynecology, Nagoya University, School of Medicine, Nagoya, Japan
[b] Department of Fetal and Maternal Medicine, Nagara Medical Center, Gifu City, Japan
[c] Biostatics Laboratory, Nagoya University, School of Medicine, Nagoya, Japan

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ABSTRACT

Background: Twin pregnancies have a higher rate of preterm births, making precise prediction of neonatal respiratory disorders essential. We herein examined the amniotic lamellar body count (LBC) and found it to be an accurate predictor of respiratory disorders in twin pregnancies.
Methods: Five hundred fourteen amniotic fluid samples, comprising 132 dichorionic twin (DCT) and 125 monochorionic twin (MCT) gestations, were obtained at cesarean section performed at 29 to 38 gestational weeks. Samples were analyzed immediately without centrifugation.
Results: There were 26 neonates (5.1%) with respiratory distress syndrome (RDS) and 43 (8.4%) with transient tachypnea of the newborn (TTN). The LBC in neonates with TTN (5.12×10^4/μl) was between the counts in RDS (1.26×10^4/μl) and controls (10.6×10^4/μl), which differed significantly. Twin concordance rates were significantly higher for TTN in MCT gestations than DCT gestations ($p = 0.003$) and delta LBC value was significantly smaller in MCT ($3.15 \pm 0.4 \times 10^4$/μl) than DCT ($5.17 \pm 0.5 \times 10^4$/μl) gestations ($p = 0.003$).
Conclusions: The amniotic LBC is useful for predicting respiratory disorders, including RDS and TTN, in twin pregnancies. The data in this study may indicate a genetic predisposition to TTN among MCTs.

1. Introduction

Neonatal respiratory complications associated with immature fetal lungs, resulting from delivery before the 37th week of gestation, are a serious concern [1]. More than 40% of preterm deliveries result in transient tachypnea of the newborn (TTN) due to residual fluid in the lungs. Respiratory distress syndrome (RDS) constitutes the most common long-term respiratory complication of preterm delivery, affecting more than one-third of infants delivered before the 34th week of gestation. These neonates develop bronchopulmonary dysplasia, chronic oxygen dependence and are prone to asthmatic episodes [2]. The situation is more dramatic in twin pregnancies, which carry 6 times the risk of prematurity than singletons [3]. The precise timing of delivery is particularly critical in the management of multiple pregnancies.

Over the past two decades, the most commonly used fetal lung maturity test has been the measurement of the lecithin/sphingomyelin (L/S) ratio in amniotic fluid [4–6]. Lung maturity, defined as a L/S ratio 2, is generally reached at 36 weeks of gestation in singleton pregnancies. In contrast, Leveno et al. [7] reported that twin fetuses to reach this threshold at 32 weeks of gestation. However, a recent retrospective study reported that a mature result with this test does not guarantee the absence of lung morbidity [8]. Furthermore, there is a global trend toward a reduction in the use of the L/S ratio because it is time-consuming and costly [9]. Cost-effective alternatives are urgently needed to monitor fetal lung maturity, especially in multiple gestations.

The amniotic lamellar body count (LBC) is an accurate predictor of fetal lung maturity in singleton pregnancies [10–12]. The LBC can be determined quickly and inexpensively, making it a more cost-effective predictor of RDS than the L/S ratio [12,13]. We recently reported that the LBC in neonates with TTN was significantly lower than that in non-RDS/TTN infants, and was significantly higher than in neonates with RDS [14]. Therefore, the LBC is potentially useful for predicting and distinguishing between RDS and TTN in singleton pregnancies. We also reported the validity of the LBC for the assessment of fetal lung maturity in twin pregnancies [15]. The LBC cutoff value for predicting RDS was 2.95×10^4/μl, the same value as was seen in singleton pregnancies [16]. In the present study, we evaluated the LBC in twin gestations in order to address the following questions: 1) Is the LBC in twin neonates with TTN significantly lower than that in non-RDS/TTN twin infants? 2) Are the respiratory outcomes different between

* Corresponding author at: Department of Obstetrics and Gynecology, Nagoya University, School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, 466–8550, Japan. Tel.: +81 52 744 2261; fax: +81 52 744 2268.
E-mail address: hiro-t@med.nagoya-u.ac.jp (H. Tsuda).

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dichorionic twin (DCT) and monochorionic twin (MCT) gestations? 3) Does twin birth order alter the LBC value or the rate of RDS and/or TTN? 4) Does the twin concordance rate for RDS and/or TTN differ between DCT and MCT gestations? If so, can this be confirmed by the LBC?

2 . Materials and methods

2.1. Subjects

This prospective study on twin pregnancies was conducted between April 2006 and December 2012 at the Nagara Medical Center, Gifu City, Japan. All mothers provided written informed consent for this study, which was approved by the Ethics Committee of Nagara Medical Center. All patients were delivered by cesarean without labor. Chorionicity was determined by early ultrasound findings and placental pathology following delivery. We excluded gestations in which amniotic fluid was not collected for at least one twin, or in which amniotic fluid samples were contaminated with blood and/or meconium. We also excluded all gestations associated with congenital abnormalities affecting respiratory function, neonatal death or twin–twin transfusion syndrome. We analyzed the data from 514 registered neonates (257 twin pairs), comprising 132 DCT and 125 MCT gestations.

2.2. Measuring LBC

Amniotic fluid samples were obtained from each amniotic sac at the time of cesarean section performed between 29 and 38 gestational weeks. Samples were analyzed immediately after arrival at the laboratory, without centrifugation, according to a standardized methodology for the LBC reported by Neerhof et al. [11]. The LBC was determined using the platelet channel on an automated hematology analyzer (Sysmex SF-3000, Sysmex Corporation, Kobe, Japan); the procedure took less than 30 min. At least 1 ml of amniotic fluid was needed to measure the LBC.

2.3. Characteristics and diagnosis of RDS/TTN

Neonatal data were extracted from the patients' records, including the gestational age at delivery, birth weight and respiratory outcomes. Maternal clinical characteristics such as preeclampsia and preexisting or gestational diabetes mellitus [17] were documented in order to assess factors that may influence the risk of neonatal respiratory complications. The diagnoses of RDS and TTN were established by at least two neonatologists based on a combination of the clinical signs, clinical course and chest radiography findings [14]; the neonatologists were blinded to the LBC data. The cohort of study neonates with neither RDS nor TTN was used as the control population.

2.4. Statistical analyses

The data were entered on a spreadsheet. The Statistical Package for the Social Sciences (SPSS) software program was used for the data analysis (ver. 9.0, SPSS). The normality of the data was assessed with the Shapiro–Wilk test. The Mann–Whitney *U* test was used to compare continuous variables between two groups, with Student's *t*-test used as required. The χ^2 test or Fisher's exact test were used for the comparison of categorical variables. The Kruskal–Wallis test was used to compare continuous variables among three groups as a nonparametric post-hoc test. The Wilcoxon rank test was used to compare continuous variables among twin pairs as a nonparametric test. We calculated the crude rates of neonatal RDS/TTN among neonates in the DCT and MCT, and expressed the association between the twin chorionicity and RDS/TTN in term of odds ratios (ORs) and 95% confidence intervals (CIs). Adjusted ORs were derived from logistic regression models, after controlling for the influences of gestational age. A receiver-operating characteristic curve was generated to characterize the ability of the LBC to predict RDS and/or TTN; the area under the curve was a measure of diagnostic accuracy. A $p < 0.05$ was considered significant.

3 . Results

3.1. Maternal characteristics

The pregnant females included in this study were separated into 2 groups based on the type of twin gestation: DCT or MCT. The maternal and fetal characteristics of each group are shown in Table 1. All infants were delivered by cesarean section (CS) without labor. Maternal characteristics, including the age and the rates of preterm rupture of membranes, pregnancy-induced hypertension and maternal diabetes mellitus did not differ significantly between the 2 groups. Fetal characteristics, including the gestational age at delivery and the rates of male gender and fetal growth restriction also did not differ significantly between the two groups. The indications for CS were considered to include the following: planned delivery after 37 weeks (including patient's desire and malpresentation), pregnancy-induced hypertension (PIH), FGR, preterm rupture of the membranes (PROM), placenta previa and an abnormal fetal heart rate pattern. As a result, the rate of preterm birth before 37 weeks of gestation was 54.1%. Next, we examined the maternal and fetal characteristics stratified by the RDS, TTN and control groups. We found that the gestational age at delivery was the only significant factor (31.0 ± 1.6 weeks in the RDS, 34.1 ± 2.1 weeks in the TTN and 35.9 ± 2.6 weeks in controls; $p < 0.001$).

3.2. Effect of chorionicity

We examined the LBCs and neonatal respiratory outcomes, including RDS and TTN, in twin pregnancies. There were 26 neonates (5.1%) with RDS and 43 (8.4%) with TTN. The LBCs in DCT gestations were significantly higher (10.4×10^4/μl vs 8.90×10^4/μl; $p = 0.019$) and the rates of respiratory disorders were significantly lower (3.4% vs 6.8% in RDS; $p = 0.025$ and 6.3% vs 11.6% in TTN; $p = 0.025$, respectively) than in MCT gestations (Table 2). Next, we calculated the OR for neonatal RDS/TTN. The odds ratio for neonatal RDS/TTN was 0.49 (95% CI: 0.29–0.83) among DCTs, compared with MCTs. The adjusted odds ratio for neonatal RDS/TTN was 0.69 (95% CI: 0.61–0.78) among DCTs, compared with MCTs. We calculated the heritability, and found that it was 0.58 in the LBC value and was 0.85 for RDS/TTN.

3.3. Disease specificity of the LBC

The amniotic fluid LBC test accurately identified neonates diagnosed with TTN or RDS. Fig. 1 shows that the LBC values were 2.1-fold lower in neonates with TTN than in control neonates ($p < 0.001$). The difference was even more remarkable for neonates with RDS; their LBC values were 8.4-fold lower than those of the controls ($p < 0.001$). More

Table 1
The maternal and neonatal characteristics in DCT and MCT.

	DCT (132 pairs)	MCT (125 pairs)	*p* value
Maternal age; years (range)[a]	30.8 (22–46)	30.2 (21–40)	NS
Nulliparity[b]	75/132 (56.8%)	74/125 (59.2%)	NS
Male neonate[b]	139/264 (52.7%)	123/250 (49.2%)	NS
GAD; weeks (mean ± SD)[c]	35.6 (±3.2)	35.3 (±2.2)	NS
Fetal growth restriction[b]	68/264 (25.8%)	78/250 (31.2%)	NS
Preterm delivery < 34 weeks[b]	19/132 (14.4%)	24/125 (19.2%)	NS
Preterm rupture of membranes[b]	6/132 (4.5%)	10/125 (8%)	NS
Pregnancy-induced hypertension[b]	20/132 (15.2%)	20/125 (16%)	NS
Diabetes mellitus[b]	4/132 (3.0%)	2/125 (1.6%)	NS

DCT, dichorionic twins; MCT, monochorionic twins; GAD, gestational age at delivery.
[a] Student's *t*-test was used.
[b] The χ^2 test or Fisher's exact test was used.
[c] The Mann–Whitney *U* test was used.