Lamellar Body Counts for Fetal Lung Maturity Testing

Diana Ramačauskaitė[1]*
Gražina Stanislava Drąsutienė[2]

[1] *Department of Obstetrics,
Vilnius City University Hospital,
Antakalnio 57,
LT-2040 Vilnius, Lithuania*
[2] *Vilnius University, Clinic of
Obstetrics and Gynecology,
Antakalnio 57,
LT-2040 Vilnius, Lithuania*

The purpose of our study was to evaluate the lamellar body count (LBC) in amniotic fluid as a predictor of fetal lung maturity by using a Sysmex K-1000 hematology analyzer. The study was performed at Vilnius City University Hospital from May 2002 till June 2003. We conducted a prospective clinical outcome study in which 113 specimens associated with delivery within 72 h of collecting amniotic fluid were correlated with neonatal outcomes. The diagnosis of respiratory distress syndrome (RDS) was based on the results of the clinical examination by the attending neonatologist, the chest rentgenogram and supplementary oxygen requirement. All cases classified as transient tachypnea were considered as non-RDS cases. RDS was diagnosed in 18.6% of the infants (21 cases). The ability of LBC to predict fetal lung maturity was evaluated by calculating the sensitivity and specificity of each LBC value from 0 to 438,000 per µl. Receiver operating characteristic curves were then constructed to determine the LBC that would optimize the test's ability to predict fetal lung maturity. With a cut-off point of 28,000 per µl the diagnostic accuracy for LBC was as follows: sensitivity 95.2%, specificity 88%, positive predictive value 64.5%, negative predictive value 98.8%. The LBC is a quick, simple, universally available and accurate test for fetal lung maturity prediction.

Key words: fetal lung maturity (FLM), lamellar body count (LBC), respiratory distress syndrome (RDS)

INTRODUCTION

The annual rate of preterm deliveries in Lithuania is about 5%. Preterm birth is responsible for a significant mortality and morbidity. RDS was diagnosed in 11.2% of premature newborns in 2001. RDS is one of the most common causes of death in premature newborns in the pathology structure (11.1 per 1000 live births) (1). FLM tests are used to determine the optimal time of delivery – in cases of preterm labor, pregnancy-induced hypertension, diabetic pregnancy, intrauterine growth restriction and others (2, 3). In Lithuania, the foam stability test and absorbance at 650 nm test are used for FLM evaluation. These tests are time consuming, the absorbance at 650 nm is not universally available.

The LBC method was first described by Dubin (4). Lamellar bodies, concentrically layered "packages" of phospholipids that represent the storage form of surfactant, can be counted in the platelet channel of most electronic cell counters (5, 6). Because lamellar bodies and platelets are indistinguishable to cell counters, the LBC is obtained by analyzing an amniotic fluid sample with a cell counter and recording the platelet count. Many investigators used Coulter instruments, which have a 50 µm aperture (4, 7–9). A Sysmex counter with an 80 µm aperture was used only in two studies (7, 9). A larger aperture may allow multiple lamellar bodies to pass through the channel simultaneously but only be counted as one lamellar body, leading to lower counts. The hematology analyzer in our hospital, Sysmex K-1000, has an aperture of 70 µm. To our knowledge, this is the first report on LBC testing by using a counter with a 70 µm aperture. The LBC test for predicting FLM was for the first time used in Lithuania.

Several studies (7, 9–12) have shown LBC to be accurate predictors of FLM and have demonstrated a high correlation among the LBC and other methods used in FLM testing (10–13).

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* Author for correspondence.

OBJECTIVE

The purpose of our study was to evaluate the LBC in amniotic fluid as a predictor of FLM by using a Sysmex K-1000 hematology analyzer.

MATERIALS AND METHODS

Amniotic fluid specimens were obtained between May 2002 and June 2003 by transabdominal amniocentesis, vaginal pool method and during *sectio caesarea* operation on patients undergoing FLM evaluation. Indications for assesment of FLM included preterm labor, premature rupture of membranes, pregnancy-induced hypertension, preeclampsia, intrauterine growth retardation, Rh isoimmunisation, polyhydramnios. The laboratory received 139 specimens for LBC testing from patients seen at Vilnius City University hospital and Clinic of Obstetrics and Gynaecology of Vilnius University. All specimens containing obvious mucus, meconium, blood were excluded from the study. The study group included amniotic fluid samples from 113 women who delivered within 72 h following amniotic fluid collection. Amniotic fluid was collected and placed in a plastic tube. All samples were analyzed immediately on arrival in the laboratory.

LBC was quantified using the platelet channel of an automated Sysmex K-1000 hematology analyzer (Toa Medical Electronics Co, Japan). The plastic tube with amniotic fluid was capped and placed on a tube rocker for 2 min. While the specimens were being mixed on the rocker, the aspiration tip of the cell counter was wiped clean. A balanced electrolyte solution was aspirated at least once into the instrument to prime it and to purge any remaining blood that might have been left on the tip from previous analyses. The well mixed amniotic fluid specimens were aspirated into the Sysmex counter. The platelet channel measures the number and size of particles that are 2–70 μm in volume.

The respiratory status of each newborn was evaluated. The diagnosis of RDS was based on the presence of all three of the following: physical signs (nasal flaring, grunting, retractions and tachypnea), supplementary oxygen requirement longer than 24 h and typical radiographic findings of hyaline membrane disease. Those cases classified as transient tachypnea were considered as non RDS cases.

The statistical analyses were performed using the statistical programmes – SPSS for Windows (version 10.0) and MedCalc (version 7.1.01). The values of the parameters with standard deviations and 95% confidence intervals were calculated. The relation between LBC and gestational age was evaluated by the linear regression method, p values less

than 0.05 were considered statistically significant. The ability of LBC to predict fetal lung maturity was evaluated by calculating the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV). Receiver operating characteristic curves (ROC) were constructed to determine the LBC that would optimize the test's ability to assess FLM. The area under the ROC curve (AUC) measures discrimination, that is the ability of the test to correctly classify those with and without the disease. The area under the ROC is a convenient way of comparing the classifiers. A random classifier has an area of 50, while and ideal one has an area of 100.

RESULTS AND DISCUSSION

A total of 139 clear samples was evaluated. The study group included amniotic fluid samples from 113 women who delivered within 72 h of amniotic fluid collection. The LBC values ranged from 0 to 438,000, with a mean ±SD of 70.9 ± 62.8.

Gestation age at delivery ranged from 25 to 42 weeks, with a mean ±SD of 36.4 ± 3.3 weeks. The gestational age distribution of the study population is shown in Fig. 1.

The LBC correlated positively with advancing gestational age ($r^2 = 0.26$, $p < 0.05$) (Fig. 2).

The birth weight varied from 470 to 4500 g with a mean ±SD of 2876.5 ± 838.0 g.

The prevalence of RDS in the study population was 18.6%. In total, 21 infants from 113 had RDS. The clinical characteristics of RDS are shown in Table 1.

All RDS cases showed the LBC ranging from 0 to 39,000, with a mean ±SD of 10.4 ± 10.2. The gestational age of the RDS group was 25 to 36
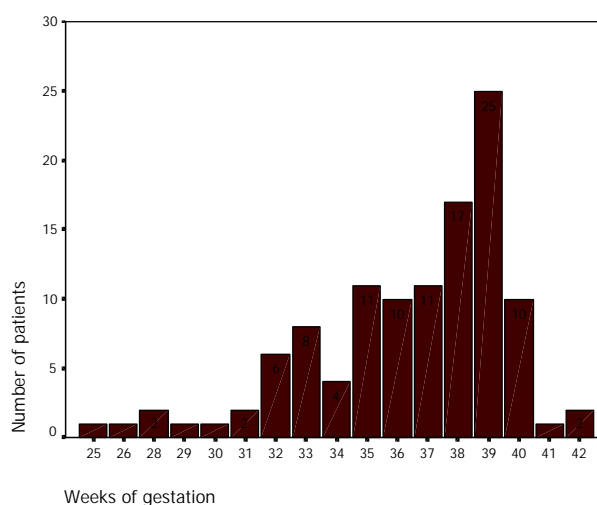

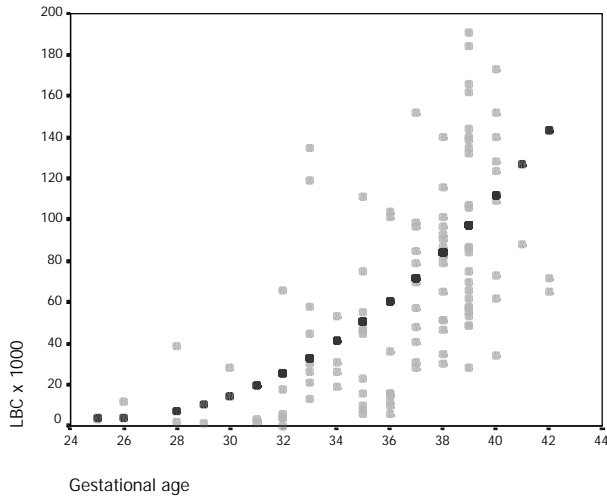Fig. 1. Gestational age distribution of the study population
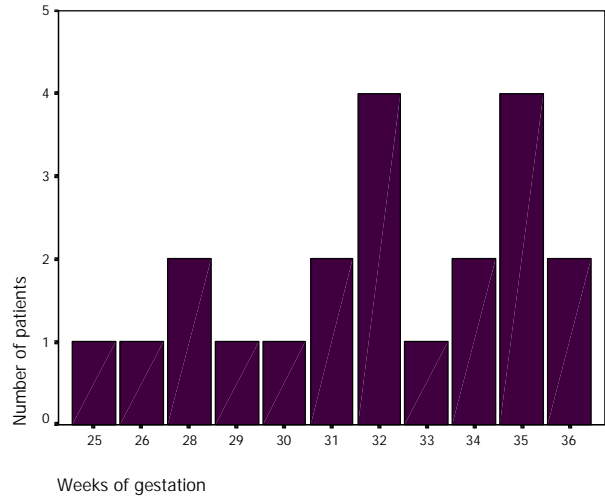
Fig. 2. LBC *vs* gestational age

Fig. 3. Gestational age distribution of the RDS population

| Table. **Clinical characteristics of RDS** | | | | |
N.	Gestational age (weeks)	Weight (g)	Sex	LBC ($\times$ 1000/μl)
1.	25	470	Female	3
2.	26	1250	Male	12
3.	28	820	Male	2
4.	28	1530	Male	39
5.	29	1200	Male	1
6.	30	1580	Female	28
7.	31	1750	Female	3
8.	31	2500	Female	2
9.	32	2500	Female	0
10.	32	1510	Male	6
11.	32	1550	Female	4
12.	32	1630	Female	4
13.	33	1550	Female	13
14.	34	2900	Male	19
15.	34	2400	Male	26
16.	35	2500	Female	10
17.	35	2480	Male	8
18.	35	1310	Male	6
19.	35	1320	Male	16
20.	36	1880	Female	10
21.	36	2450	Female	6

weeks, with a mean $\pm$ SD of 31.9 $\pm$ 3.2 weeks. The distribution of gestational age in the RDS cases is demonstrated in Fig. 3.

Using a value equal to and lower than 39,000 to indicate pulmonary immaturity, the LBC predicted all cases of RDS with no false-negative results (100% sensitivity and 100% negative predictive value). However, this 39,000 cutoff resulted in 18 false-positive results, rendering a relatively poor specificity 80.4% (95% CI 70.9–88.0) and positive predictive value (53.8%). By introducing a lower cutoff of 10,000 to predict pulmonary immaturity, the number of false-positive results decreased from 18 to 0. This resulted in an increase in specificity from 80.4% to 100% and in the PPV from 53.8% to 100%, though the sensitivity dropped from 100% to 66.7% (95% CI 43.0–85.4). Values between 10,000–39,000/μl indicated an intermediate risk (7 from 21, 33.3%) for developing RDS.

A receiver operator characteristic (ROC) curve was constructed (Fig. 4).

Using ROC analysis, the best cutoff of LBC was found to be 28,000. With a cut – off point of 28 000 the diagnostic accuracy for the LBC was: sensitivity 95.2% (95% CI 76.1–99.2); specificity 88% (95% CI 79.6–93.9); positive predictive value 64.5%; negative predictive value 98.8%. From the obtained ROC plot, an area under curve (AUC) value was calculated to be 97.5% (95% CI 92.7–99.5).

The incidence of RDS in our study was 18.6%, which is comparable to the 11% reported by Ashwood (8), 12% reported by Neerhof (5) and Dalen-
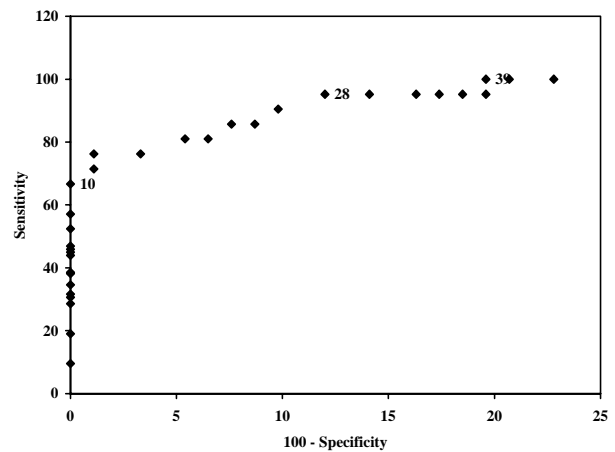

Fig. 4. ROC analysis of LBC

ce (9). The incidence of RDS in the Beinlich study was 7% (12).

By using the cut-off value of 28,000/µl, the LBC showed a sensitivity of 95.2% and a specificity of 88% in our study. The test's diagnostic accuracy in our study was similar to those reported by Anceschi (10) (sensitivity 92% and specificity 89%), Lewis (7) (sensitivity 85% and specificity 98%), Nerhof (5) (sensitivity for immature cut-off 62% and specificity 90%) and better than reported in Beinlich's study (12) (specificity 67% and sensitivity 83%).

Maturity is suggested by a count of 39,000/µl or greater and immaturity by a count of less than or equal to 10,000/µl by using a Sysmex K–1000 hematology analyzer. In the case of intermediate results, we recommend to re-evaluate the clinical situation and administer a course of dexamethasone for fetal lung maturation.

LBC, a rapid test available at all hospitals, would be very useful for the management of high-risk pregnancies and for delivery decisions needed at term.

ACKNOWLEDGEMENTS

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D. Ramaðauskaitë, G. S. Dràsutienë

LAMELARINIØ KÛNELIØ TYRIMAS VAISIAUS PLAUÈIØ BRANDUMUI ÁVERTINTI

S a n t r a u k a

Tyrimo tikslas – nustatyti lamelariniø kûneliø (LK) vaisiaus vandenyse prognostinæ vertæ vaisiaus plauèiø brandumui ir pirmà kartà Lietuvoje ðá testà pritaikyti praktiniame darbe.

Metodika. 2002–2003 m. Vilniaus miesto universitetinëje ligoninëje ir Vilniaus universiteto Akuðerijos ir ginekologijos klinikoje LK skaièius buvo nustatomas tiriant vaisiaus vandenis hematologiniu analizatoriumi Sysmex K-1000. Tyrimui atrinkti tie atvejai, kai nuo vaisiaus vandenø paëmimo iki naujagimio gimimo buvo praëjusios ne daugiau kaip 72 valandos. Vertinta priklausomybë tarp LK skaièiaus ir naujagimio kvëpavimo sutrikimo sindromo (KSS). Naujagimiui nustatytas KSS, esant KSS klinikai, bûdingiems pokyèiams plauèiø rentgenogramoje, kai reikëjo deguonies terapijos 24 valandas ar daugiau. Naujagimiai, sergantys praeinanèiàja tachipnëja, priskirti KSS neserganèiø grupei.

Rezultatai. Iðtirta 113 vaisiaus vandenø pavyzdþiø. LK skaièius svyravo nuo 0 iki 438 000/µl. 21 naujagimiui (18,6%) konstatuotas kvëpavimo sutrikimo sindromas. Naudojant ROC kreives nustatytas slenksèio taðkas – 28 000/ µl. Esant šiai reikšmei, tyrimo metodo jautrumas – 95,2% (95% CI 76,1–99,2), specifiškumas – 88% (95% CI 79,6–93,9).

Iðvada. LK skaièiaus nustatymas yra greitas, tikslus, pigus, naujø investicijø nereikalaujantis vaisiaus plauèiø brandumo ávertinimo metodas.

Raktaþodþiai: vaisiaus plauèiø brandumas, lamelariniø kûneliø (LK) skaièius, kvëpavimo sutrikimø sindromas