

Association between lamellar body count and respiratory distress in neonates

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ABSTRACT

Objectives: Assessment of fetal lung maturity by a simple and rapid test has a pivotal role in obstetric managements. Lack of modern laboratory techniques in our country made us investigate whether lamellar body count (LBC) can be applied efficiently in the evaluation of fetal lung maturity.

Methods: Lamellar body count was assessed in 104 unspun amniotic fluid samples taken from pregnant women admitted at Akbar Abadi Hospital, Tehran, Iran between May 2003 and November 2003 whose fetuses were at risk for respiratory distress syndrome (RDS). Cut-off points for LBC were determined to evaluate the risk of RDS. Standard clinical and radiographic criteria were used to diagnose RDS.

Results: An LBC of less than 10,000 was 99.1% specific for lung immaturity (positive predictive value = 99.1%, negative predictive value = 83.5%). The LBCs of greater than 45,000 eliminates RDS (negative predictive value = 98.9%).

Conclusion: Lamellar body count is an easy, rapid and cost-effective test to assess fetal lung maturity in high-risk fetuses. Using the cut-off points of 10,000 and 45,000, LBC can serve as the first screening test of fetal lung maturity.

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One important element in obstetric managements is fetal lung maturity (FLM), since respiratory distress syndrome (RDS) is a dangerous threat to newborn with immature lungs. Among the standard tests for determining lung maturity, only shake test (foam stability test) is available in Iran. Putting together the gestational age estimated birth weight and the results of shake test when necessary, the gynecologists have assessed lung maturity for years. So, there is a certain need to develop a rapid, inexpensive and efficient method to solve the problem. Lamellar body is a surfactant-containing lamellar structure secreted by type II pneumocytes. As pregnancy goes on, lamellar bodies are secreted increasingly into the amniotic fluid, thereby reflecting the progress of lung maturation. Lamellar body count (LBC), first introduced by Dubin,¹ is a

simple, rapid and noninvasive test by which lamellar bodies in a sample of amniotic fluid are counted using widely available platelet counters due to size similarity between platelets and lamellar bodies.

The LBC proved to be promising in a number of studies with different cut-off points to predict the risk of newborn RDS.²⁻⁸ To satisfy the need described above and due to the relatively high false negative rate sometimes observed in shake test, we evaluated LBC as a method to predict RDS in newborns. The purpose of this study was to determine whether LBC could be applied as the first test of lung maturity in Iran as a developing country and also to evaluate the cut-off points for LBC in assessing the risk of RDS.

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Methods. Amniotic fluid samples were obtained from 104 pregnant women between 26th week of gestational age to term who referred to Akbar Abadi Hospital, Tehran, Iran between May 2003 and November 2003 and were at risk of preterm labor or anticipating elective delivery. Written informed consent was obtained from all patients and the study was approved by the Ethical Committee of Iran University of Medical Sciences. Samples were taken from vaginal amniotic flow (in premature rupture of membranes) or at cesarian section. Just 3 samples were taken by amniocentesis. Grossly bloody or meconium contaminated samples were discarded. We performed shake test on each sample. The LBC was quantified as well using Sysmex K.800 (Toa Medical, Japan). Sysmex K.800 analyzes whole blood sample for 8 hematology parameters (white blood cell, red blood cell, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelet). We excluded the samples with hematocrit $< 1\%$. To further simplify testing, we used unspun samples.

The diagnosis of RDS was based on the documented presence of tachypnea, retraction, nasal flaring, supplemental oxygen requirement of more than 24 hours and reticulonodular pattern in chest x-rays. Newborns were followed for 7 days to detect probable RDS. Sensitivity, specificity, negative and positive predictive values were calculated.

Results. A total of 104 clear samples, one sample from each patient, were evaluated. The RDS was diagnosed in 23 (22%) of the infants. The LBC ranged from 8,000-533,000. The LBC cut-off points of less than 10,000 and greater than 45,000 were selected to maximize the positive and negative predictive values. When LBC $< 10,000$, the likelihood ratio was 11.5, indicating an 11.5-fold risk of RDS for the cases with low LBCs. The sensitivity, specificity, and positive and negative predictive values of the test in terms of prediction of RDS are shown in **Table 1**. To make sure that low gestational age has no independent effect on the test, we considered the cases before the 34th week of gestation. Of the 27 patients in this group, RDS occurred in 18 (66.6%). Using the cut-off points introduced above, we could predict all the cases with or without RDS with 99.1% accuracy. The LBC was significantly lower in the groups with distress than in the group without it (LBC mean of 17,000 versus 78,111, p -value <0.001).

Discussion. Management of pregnancies at risk for the development of neonatal respiratory distress would be enhanced by a rapid, accurate and objective test for FLM.^{2,4,8,9} The most important aspect of such a test is to predict accurately the

Table 1 - Performance of lamellar body count to predict respiratory distress.

LBC	Sensitivity	Specificity	PPV	NPV
$< 10,000$	(30.4)	(99.1)	(99.1)	(83.5)
$< 45,000$	(98.7)	(98.35)	(76.6)	(98.6)

PPV - positive predictive value
NPV - negative predictive value, LBC - lamellar body count.

absence of RDS. A test that allows clinicians to predict FLM confidently, will enable the establishment of appropriate management plans based on the test results.

The incidence of RDS in our study was 22%, which is considerable in comparison with 12% reported by Dalence et al,⁴ 14% by Fakhoury et al⁶ and 11% by Ashwood et al.⁷ Our data indicated that using the cut-off point of 45,000, LBC showed 98.7% sensitivity and 98.6% negative predictive value. In this study LBC $< 10,000$ made fetal lung immaturity almost sure, whereas LBC $> 45,000$ virtually guaranteed FLM. Based on these associations, we recommend the use of LBC as a screening test for FLM in Iran, prior to the use of other expensive and time-consuming assays. However, there are now several accepted tests to assess FLM, the most accurate of which is lecithin/sphingomyelin (L/S) ratio.^{10,11} Other tests are phosphatidylglycerol determination, foam stability test (shake test), fluorescence polarization and optical density at 650 nm. The most complex and expensive tests are L/S ratio and phosphatidylglycerol determination, while the simplest test is optical density measurement.¹²

The predictive values for RDS of immature test results range from approximately 30-60%.¹² Other characteristics, including cost, ease of test performance, availability and reproducibility, are important factors in selecting maturity tests. With this large armamentarium of tests, some authors have proposed a 3-armed testing approach that included no further testing for LBC $< 8,000$ and LBC $> 32,000$.² They recommended assaying L/S ratio for LBCs between 9,000 and 32,000.

In some developing countries, however, modern laboratory facilities in maternity hospitals are limited and the risk of RDS due to imprecise estimation of gestational age is higher than in western societies. Therefore, in the present study we considered cut-off points of 10,000 and 45,000 to make sure on the presence of FLM. It means that our range is wider than that in Pamela et al² study (35,000 versus 24,000), although using unspun specimens and Sysmex counter is similar in both studies.

Shake test is the only other available test for evaluation of FLM in our country, and the risk of RDS is higher in pregnancies before 34th week.¹³ Thus, we performed shake test for all the above specimens to evaluate its usefulness in this group. There were 27 samples in this group and the incidence of RDS was 66%, while shake test was positive in just 15%. Therefore, considerable false negative rates accompanying shake test prevents us from using it as a diagnostic test in our country. The problem of decision making in pregnancies with transitional zone LBCs remains unsolved in our country. One possibility would be, for example, to start with LBC due to its simplicity and then if results are borderline, continue with L/S ratio as a more differentiated method.³ As fluorescence polarization and optical density at 650 nm are among the least expensive and simple tests,¹² we propose to launch another study using these 2 techniques for transitional zone LBCs to evaluate the efficacy of this approach to assess FLM in developing countries with limited health investments.

Regardless of which method of FLM assessment is chosen, no mature result from one or a group of tests can completely eliminate the risk of RDS or other neonatal complication.¹³ The risk of adverse outcome associated with delivery on the basis of lung maturity assessment must be weighed against the potential risk of untoward outcome by permitting the pregnancy to continue in uterus.

In conclusion, LBC is a rapid, easy and cost-effective test for the assessment of fetal lung maturity. While other assays except shake test are not available in developing countries and shake test is accompanied by considerable false negative rates, LBC seems to be able to provide acceptable evaluation of RDS risk in infants. Using the cut-off points of 10,000 and 45,000, LBC can serve as the first screening test of fetal lung maturity.

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