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# Lamellar Body Count (LBC) in amniotic fluid for prediction of fetal lung maturity

Ahmed Mohamed Fahmy Selim<sup>1</sup>, Sherif Fathi El Mekkawi<sup>2</sup>,  
Ghada Mahmoud Mansour<sup>2</sup>, Sherif Hanafi Hussain<sup>2</sup>

<sup>1</sup> Obstetrics & Gynecology Department, Kafr El Zayyat General Hospital, Kafr El Zayyat, Egypt

<sup>2</sup> Obstetrics & Gynecology Department, Faculty of Medicine - Ain Shams University, Cairo, Egypt

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## Correspondence

Ahmed Mohamed Fahmy Selim, Obstetrics & Gynecology Department, Kafr El Zayyat General Hospital, Kafr El Zayyat, Egypt

## Abstract

**Background:** Neonatal respiratory distress syndrome (RDS) is a disorder due to pulmonary immaturity with a high mortality characterized by low levels of pulmonary surfactant. RDS is one of the important causes of mortality in neonates.

**Objective:** The aim of the study is to determine the lamellar body count (LBC) cutoff value for foetal lung maturity and to evaluate the clinical usefulness of LBC in predicting the neonatal respiratory distress syndrome (RDS) and to find if the Measurement of LBC can replace the conventional Lecithin/Sphingomyelin ratio.

**Methods:** This prospective observational study was performed on total 47 pregnant women divided as 19 patients with premature labor and 28 patients with healthy full-term singleton pregnancy as controls, with gestational age between 28 – 41 weeks at Ain Shams university maternity hospital in period between April 2014 and January 2015 with inclusion and exclusion criteria.

**Results:** RDS was diagnosed in 20 neonates, two neonates in the 1st group (7.1%) While 18 neonates in 2nd (94.7%) showed development of RDS. Mean lamellar body count in newborns with RDS:  $39,200 \pm 12,438$  with median 37,500 and p-value was  $<0.0001$ . ROC (receiver operator characteristics) curve showed that by using  $51000/\mu\text{L}$  as a cut-off point for LBC it is a good predictor for fetal lung maturity with sensitivity 95% and specificity 96.3%. There is a direct relationship between the gestational age and the lamellar body count as the lamellar body count increases as long as the gestational age proceeds with a highly significant positive correlation (p value  $<0.0001$ ).

**Conclusion:** As evident from the current study, LBC count now replacing the conventional Lecithin/Sphingomyelin ratio because the test can be performed with equipment found in most clinical analysis laboratories and is reliable in predicting foetal lung maturity. This study suggests that LBC cutoff value of  $\geq 51,000/\mu\text{L}$  can predict pulmonary maturity and reduce the risk of neonatal respiratory distress syndrome.

**Key words:** Lamellar body count (LBC), Fetal lung maturity.

## Introduction

Neonatal respiratory distress syndrome (RDS) is a disorder due to pulmonary immaturity with a high mortality characterized by low levels of pulmonary surfactant. Gestational age determines risk based on concentration of pulmonary surfactant, i.e., as gestation progresses the concentration of pulmonary surfactant increases. As a result, newborns delivered at less than 28 weeks have more than 60% risk of RDS, whereas those delivered at more than 34 weeks have less than 5% risk of RDS. In situations where gestational age alone is not sufficient to determine RDS risk and preterm delivery is medically needed, amniotic fluid analysis can be performed to determine pulmonary surfactant concentration<sup>1</sup>.

Prenatal analysis of the amniotic fluid can provide data giving insight into the fetal lung maturity, which enables planning of the further outcome of high-risk pregnancies. Surfactant prevents atelectasis by forming a layer rich in phospholipids between the air and liquid phase in alveoli thus leading to increased surface tension in them, which is a precondition for a good lung function after birth<sup>2</sup>.

There are common methods as the lecithin-sphingomyelin ratio, phosphatidylglycerol measurement and surfactant-albumin ratio. All of these tests have excellent negative predictive values but poor positive predictive values, i.e., they are great at confirming maturity but poor at confirming immaturity<sup>1</sup>.

Surfactant is stored in the form of lamellar bodies. They are secreted into alveolar space and passed into amniotic fluid where they can be found. The similarity of lamellar body size to platelet size permits the use of a standard automated hematologic cell counter to estimate the number of lamellar bodies in amniotic fluid<sup>2</sup>

Lamellar bodies are essentially small packages of lung surfactant which are found in intracellular storage granules in lung cells or pneumocytes. The

lamellar bodies are released (exocytosed) and unfold to form a surfactant monolayer in the alveolar space. Surfactant and lamellar bodies are released into amniotic fluid due to fetal breath movements beginning around 28 to 32 weeks of fetal development, with levels increasing exponentially as the fetus matures. The risk of respiratory distress syndrome due to insufficient surfactant levels is significant during gestational weeks 32 to 36, and more accurate assessment of that risk is facilitated by measurement of surfactant phospholipid ratios or, as has recently been shown, by lamellar body counts. Cases with LBC < 15,000 are considered to have lung immaturity while cases with LBC > 45,000 in centrifuged AF samples or >55,000 in uncentrifuged AF samples are likely to have mature lungs<sup>3</sup>.

Still, there are no clearly established protocols and cutoff values for LBC that predict RDS. The current study was designed to assess the role of the amniotic fluid lamellar body counting in predicting fetal lung maturity<sup>4</sup>.

This study aims to determine the lamellar body count (LBC) cutoff value for foetal lung maturity and to evaluate the clinical usefulness of LBC in predicting the neonatal respiratory distress syndrome (RDS) and to find if the Measurement of LBC can replace the conventional Lecithin/Sphingomyelin ratio.

## Patients and methods

After ethical committee approval and written consents from the patients, this prospective observational study was performed on total 47 pregnant women divided as 19 patients with premature labor and 28 patients with healthy full-term singleton pregnancy as controls, with gestational age between 28 – 41 weeks at Ain Shams university maternity hospital in period between April 2014 and January 2015.

Table 1. Characteristics of patients giving birth to babies with or without RDS

Variable	No RDS (n=27)	RDS (n=20)	p-value
Age (years)	25.0 (4.4)	26.0 (4.2)	0.415†
Parity			0.881‡
P0	6 (22.2%)	5 (25.0%)	
P1	10 (37.0%)	6 (30.0%)	
P2	9 (33.3%)	7 (35.0%)	
P3	2 (7.4%)	2 (10.0%)	
Number of previous abortions			0.005‡
No previous abortion	16 (59.3%)	5 (25.0%)	
One abortion	9 (33.3%)	8 (40.0%)	
Two abortions	2 (7.4%)	5 (25.0%)	
Three abortions	0 (.0%)	2 (10.0%)	
Gestational age (weeks)	38.0(1.3)	33.4(1.8)	<0.0001†
LBC (LB/μl)	154,926 (84,284)	39,200 (12,438)	<0.0001†

Data are presented as mean (SD) or number (%). †Unpaired t test. ‡Chi-squared test for trend

**Study population:** Pregnant women carrying singleton fetuses with gestational age [28– 41 weeks] at Ain Shams university maternity hospital with the following inclusion criteria:

**Inclusion criteria:** Women during child bearing period (18-35 years old), Singleton pregnancies, Gestational age from 28-41 weeks, Inevitable delivery, Elective cesarean deliveries by puncturing visible embryonic membranes.

**Exclusion criteria:** Any gestational pathology that can influence fetal lung maturity as Premature rupture of membranes, Oligohydramnios, Infants with major congenital or chromosomal abnormalities, Amniotic fluid samples containing blood or meconium, Other maternal risk factors as pre-eclampsia or eclampsia, etc .

**Study Procedures:** All participants were submitted to the following:

Counseling about all the steps of the study and had the procedure fully explained.

**History:** including Careful history taking regarding personal, menstrual, obstetric, medical and surgical histories. Gestational age was calculated from the last menstrual period and confirmed by ultrasound measurement during the first 20 weeks of pregnancy.

**Examination:** including Complete physical examination to exclude any disorders may interfere with the results.

**Investigations:** such as U/S to ensure that they comply with the inclusion and exclusion criteria.

Amniotic fluid samples were obtained from the vaginal pool after rupture of membranes, trans-vaginal amniotomy, or amniotomy during Cesarean delivery. Samples containing 5 mL of amniotic fluid were immediately transported to the clinical laboratory in a capped plastic syringe and analyzed according to an established protocol. LBC was estimated in uncentrifugated amniotic fluid samples using The Sysmex K-800 hematological analyzer and its platelet channel.

Table 2. Lamellar body count in patients giving birth to babies with or without RDS

Variable	No RDS (n=27)	RDS (n=20)	p-value
LBC (LB per μl)	142,000 (78,000 – 199,250)	37,500 (30,500 – 47,000)	<0.0001†

Data are presented as median (interquartile range). LBC, Lamellar body count. †Mann Whitney test

New Consensus of lamellar body count (LBC) Protocol<sup>5</sup>.

1. Mix the amniotic fluid by inverting the capped sample container five times.
2. Transfer the fluid to a clear tube.
3. Inspect the specimen. Fluids containing obvious mucus or meconium should not be processed for a lamellar body count.
4. Place the test tube on a tube rocker for 2 min.
5. Flush the platelet channel; analyze the instrument's diluent buffer until zero is obtained in two consecutive analyses.
6. Process the specimen through the cell counter and record the platelet channel as the lamellar body count.
7. Notify the physician if the associated hematocrit exceeds 1%. The hematocrit is obtained from the hematocrit channel of the cell counter.

**Outcome measures:** The neonatal respiratory status and existence of RDS were reviewed by attending neonatologists, who had not been informed about the concentration of LBs as follows:

- a) APGAR score at 1 and 5 min.
- b) Development of respiratory distress syndrome (RDS): need for incubation, continuous posi-

tive airway pressure or surfactant.

- c) Neonatal mortality.

**Ethical Considerations:** The patient data were anonymous. Data presentation was not by the patient's name but by diagnosis and patient confidentiality was protected. An informed consent was taken from all participants, it was in Arabic language and confirmed by date and time. confidentiality was preserved by assigning a number to patients initials and only the investigator knew it

**Conflict of interest:** the candidate declared that there is no conflict of interest and the cost of the study was paid by the candidate.

**Statistical analysis:** Analysis is to be performed using SPSS for windows v20.0, Data to be presented in terms of range, mean and standard deviation (for numeric parametric variables); range, median and inter-quartile range (for numeric non-parametric variables); or number and percentage (for categorical variables). Difference between two independent groups is to be analyzed using independent student's t-test as well as the mean difference and its 95% CI (for numeric parametric variables); or chi-squared test as well as the risk ratio and its 95% CI (for categorical variables). Binary logistic regression analysis is to be performed for estimating the association between good/poor response and the measured variables ROC curves are to be constructed for estimating the validity of measured variables as predictors of good or poor response validity is to be presented in terms of sensitivity, specificity, positive and negative predictive values and their corresponding 95% Cis significance level is set at 0.05.

Table 3. Receiver-operating characteristic (ROC) curve analysis for prediction of RDS using the LBC.

ROC curve index	Estimate
Area under the ROC curve (AUC)	0.986
95% CI for AUC	0.899 to 1.000
p-value (AUC=0.5)	<0.0001
Youden (J) index	0.913
Associated criterion (cut-off value), (LB/ $\mu$ l)	$\leq$ 51,000
Sensitivity, %	95
95% CI for sensitivity	75.1 - 99.9
Specificity, %	96.3
95% CI for specificity	81.0 - 99.9
Positive predictive value (PPV), %	95
95% CI for PPV	75.1 - 99.9
Negative predictive value (NPV), %	96.3
95% CI for NPV	81.0 - 99.9

## Results

During this study, 60 patients were assessed for eligibility and 47 patients were included in the study. Of all eligible patients, 13 patients were lost to follow up and excluded from the study. Ultimately, the

Table 4. Correlation between the gestational age and the lamellar body count in the whole study population, in those giving birth to babies with RDS, and those giving birth to babies without RDS.

LBC	Spearman rho ( $\rho$ )	Gestational age		
		All patients(n=47)	No RDS (n=27)	RDS (n=20)
	p-value	0.894	0.614	0.751
		<b>&lt;0.0001</b>	<b>0.0007</b>	<b>0.0001</b>

LBC, lamellar body count.

analysis was based on the data of 47 patients. RDS was diagnosed in 20 neonates, two neonates in the 1st group (7.1%) While 18 neonates in 2nd (94.7%) showed development of RDS. Mean lamellar body count in newborns with RDS:  $39,200 \pm 12,438$  with median 37,500 and p-value was  $<0.0001$ . ROC (receiver operator characteristics) curve showed that by using  $51000/\mu\text{L}$  as a cut-off point for LBC it is a good predictor for fetal lung maturity with sensitivity 95% and specificity 96.3%.

There is a direct relationship between the gestational age and the lamellar body count as the lamellar body count increases as long as the gestational age proceeds with a highly significant positive correlation (p value  $<0.0001$ ).

## Discussion

In the current study the lamellar body count (LBC) was used in prediction of fetal lung maturity. The study included 47 pregnant women with singleton normal pregnancies between 28 and 40 completed weeks of gestation. During cesarean section or normal vaginal delivery, amniotic fluid sample was collected for each woman for performance of lamellar body count.

After delivery, neonates were assessed clinically for evidence of respiratory distress syndrome RDS. RDS was diagnosed in 20 neonates. These neonates with diagnosis of prenatal lung immaturity had significantly younger gestational age in cases 28-35 weeks subgroup compared to 36-40 subgroup.

Using ROC curve analysis, gestational age  $< 35$  weeks predicts lung immaturity with a sensitivity 95% and specificity of 96.3%. The mean gestational weeks was significantly lower ( $p < 0.001$ ) in the RDS versus non-RDS group ( $33.4 \pm 1.8$  vs.  $38 \pm 1.3$ ).

Similarly, gestational age was a good predictor of RDS in a previous study by Serizawa M and Maeda K.<sup>6</sup> They found that the mean gestational weeks was significantly less in the RDS group ( $28.8 \pm 2.4$ ) than the non-RDS group ( $31.5 \pm 2.2$ ). Caryn ST et al.<sup>7</sup> found that gestational age and L/S ratio are predictors of RDS. They have used multivariate logistic regression with L/S ratio and gestational age as predictor of RDS. The relationship between RDS and gestational age is in fact well-known<sup>8</sup>.

In the current study, the lamellar body count (LBC) in neonates who develop RDS was significantly lower than normal neonates ( $p > 0.001$ ). It was  $39,200 \pm 12,438 /\mu\text{L}$  versus  $154,000 \pm 84,000 /\mu\text{L}$ , respectively.  $\text{LBC} > 51000 /\mu\text{L}$  was predictive of RDS with a sensitivity of 95% and specificity of 96.3%. Taking a lower cut-off point of  $51000 /\mu\text{L}$ .

Investigators have recommended different LBC cutoffs obtained from BC hematology analyzers to rule out RDS. Zarean et al.<sup>4</sup> had conducted a study with One hundred and twenty-eight amniotic samples and 128 infants were evaluated. The means of maternal and gestational ages were  $28.12 \pm 3.84$  years and  $32.56 \pm 2.72$  weeks, respectively. The mean of lamellar body was  $63081 \pm 16966 \mu\text{L}$  in matured lung infants compared to  $31266 \pm 15831 \mu\text{L}$  in immature lung infants ( $p < 0.001$ ). The optimal cut-

off point was evaluated as 47500  $\mu\text{L}$  in predicted pulmonary maturity with sensitivity of 85.1%, specificity of 91.2%, positive predictive value of 92.6% and negative predictive value of 82.5%<sup>4</sup>.

Ruiz-Hernandez and colleagues conducted a prospective, blinded study to measure LBCs on 264 patients using amniotic fluid collected at the time of caesarean section or delivery. The rate of RDS in this population was 14.8%. A cutoff of  $>79,000/\mu\text{L}$  resulted in 100% sensitivity and 43% specificity. Although this cutoff of  $>79,000/\mu\text{L}$  is greater than the consensus protocol recommendation of  $50,000/\mu\text{L}$ , it is supported by Szallasi's comparison of the Cell-Dyn with the Beckman Coulter Gen-S analyzer. When compared with the Beckman Coulter Gen-S on a Bland-Altman plot, the Cell-Dyn 3500 had a positive slope (0.76; 95% CI, 0.66 to 0.87;  $P<0.001$ ),<sup>23</sup> and an LBC cutoff of  $50,000/\mu\text{L}$  on the Gen-S analyzer corresponded to  $\sim 80,000/\mu\text{L}$  on the Cell-Dyn 3500<sup>9</sup>.

Ashwood,<sup>7</sup> used a maturity cutoff of  $< 55,000/\mu\text{L}$  which obtained a 100% sensitivity and 59% specificity, as the decision threshold with no cases of RDS above 48000/ml. Similarly, Lee et al. (11) reported 100% sensitivity and 73% specificity using a maturity cutoff of LBC  $<50,000/\mu\text{L}$ . These two studies both had large patient populations (247 and 170, respectively), and both used centrifuged amniocentesis and vaginal pool specimens. Greenspoon et al.<sup>12</sup> used uncentrifuged amniocentesis specimens from 62 patients and reported 100% sensitivity and 89% specificity using a maturity cutoff of  $46,000/\mu\text{L}$ .

A recent study of 313 patients with singleton pregnancies 24-41 weeks, a cutoff value of  $<20000/\mu\text{L}$  had sensitivity, specificity, and positive and negative predictive values in determining mature fetal lungs of 96%, 88%, 45.6%, and 99.5% respectively<sup>13</sup>.

The incidence of RDS in this study was 42.5%

which is comparable to the 14% reported by Fakhoury<sup>14</sup> this difference was due to the high percentage of the preterm cases in this study which was about 40.4%. In the critical function of predicting fetal lung maturity, our data demonstrate that when using a cut-off point ( $50000/\mu\text{L}$ ), the LBC showed 85% sensitivity (64 - 95%) and 93% negative predictive value.

KulKarni and Jayamma,<sup>15</sup> found that Among 50 cases, LBC was  $<30,000/\mu\text{L}$  in 15 cases between  $30,000-35,000/\mu\text{L}$  in five cases and  $>35,000/\mu\text{L}$  in 30 cases. Those who developed RDS had LBC between  $3,000-28,000/\mu\text{L}$ . Sensitivity and specificity of LBC to predict RDS with cut-off values of  $30,000/\mu\text{L}$  was 100% and 97.2% respectively. Dalence et al.<sup>16</sup> reported a lower cut-off point for pulmonary maturity ( $30000/\mu\text{L}$ ). These differences are likely due to centrifugation protocols and different Coulter apparatuses used in these studies.

Wijnberger et al.,<sup>17</sup> reported that a LBC  $32000/\mu\text{L}$  guaranteed fetal lung maturity. They showed that the performance of the LBC in the prediction of RDS equal to the L/S ratio. In their Meta-analysis they concluded the LBC may be considered as the test of first choice in the assessment of fetal lung maturity. Neerhof et al.,<sup>18</sup> reported that centrifugation is not a necessary step; maturity is suggested by count of  $50000/\mu\text{L}$  or greater and immaturity is suggested by a count of  $15000/\mu\text{L}$  or lower. The different methodologies for specimen preparation result in differences in maturity cutoffs used. Lamellar body counts between these 2 values were considered indeterminate and further testing, using alternative biochemical methods, was recommended<sup>18</sup>.

LBC predicted the absence of RDS within 72 hours of delivery equally as well as L/S ratio or the presence of PG concentrations. Therefore, it is necessary to determine the L/S ratio and do the PG test on patients with either the mature or immature categories of biochemical FLM status and LBC testing

is superior to them<sup>5</sup>.

In the current study, we found the value of 51000/ml to be a good predictive value for fetal lung maturity. LBC is fast, inexpensive and requires smaller amount of sample than do phospholipid analysis, and it is not invalidated by the presence of lysed blood or meconium. In addition, the apparatus required for LBC is almost universally available, allowing it to be performed in laboratories where traditional phospholipid analysis is not available.

The incidence of RDS in this study was 42.5% (20 cases), which is higher than that recorded by Dalence et al.,<sup>16</sup> (10%) and Ashwood et al.<sup>10</sup> (11%) This difference most probably reflects the high-risk characteristics of our patients and the lack of antenatal care and high rate of premature labor in our area, which is in agreement with a report by Kaplan et al.,<sup>19</sup> The authors consider gestational age as a very important clinical predictor in the interpretation of the fluorescence polarization FLM assay.

To predict the lung maturity of a newborn, we can use certain tests (LBC, fluorescence polarization FLM assay, and FSI). The result values of these tests will be lower in the amniotic fluid of the newborns expected to have RDS.

In the current study, it was found that LBC results was significantly lower in cases of RDS compared with controls, which is in agreement with Dalence et al.,<sup>16</sup> and Sher and Statland<sup>20</sup>.

No cases with RDS were detected with a cutoff value of 51x10<sup>3</sup>/μL or greater, and the lowest value at which newborns were found to have RDS was 23 x10<sup>3</sup>/ μL or less.

DeRoche et al.<sup>21</sup> set a lamellar body count of 37,000/microL was found to have a sensitivity of 80% and a specificity of 100% in the prediction of fetal lung maturity by standardized methods of phospholipid analysis. There were no cases of neonatal respiratory distress syndrome in this study population.

The recommended cut-off value for LBC is 30,000/ml according to Dalence et al.,<sup>16</sup> Fakhoury,<sup>14</sup> studies; 35,000/ml according to Ashwood et al.,<sup>10</sup> 26,000/ml according to Dubin.,<sup>22</sup> and 37,000/ml according to De Roche study<sup>21</sup>.

## Conclusion

Lamellar body count (LBC) is an effective, safe, easy, cost-effective method to detect fetal lung maturity (FLM). It does not need highly equipped laboratory or specially trained personnel; it just needs the conventional blood count analyzer. Measurement of LBC is now replacing the conventional Lecithin/Sphingomyelin L/S ratio. LBC cut-off value of 51000/ml as a cut-off point for LBC it is a good predictor for fetal lung maturity with sensitivity 95% and specificity 96.3%.

Lamellar body count (LBC) has taken its place in many laboratories and fetomaternal centers worldwide to wide confirm fetal lung maturity (FLM) in high and low risk cases. Further studies are needed with a larger number of patients to determine accurately the best cut-off value for LBC to be significant.

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