

Assessment of Fetal Lung Maturity by Lamellar Body Count (LBC) in Comparison to the Fluorescence Polarization TDx FLM-II and L/S Ratio Methods

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Abstract

Respiratory distress syndrome causes morbidity and mortality of the new-born and is most often associated with premature birth. Laboratory testing can be helpful in establishing fetal lung maturity (FLM) status in situations when premature delivery may be necessary. In addition, the rising incidence of late-preterm births at >34 and <38 weeks gestation and the associated increases in cost of preterm infant care indicate a need for better preterm delivery FLM assessment. Over the years, the laboratory's primary amniotic fluid FLM screening method included lung surfactant 1) using the lecithin/sphingomyelin ratio (L/S ratio) by thin layer chromatography and 2) the TDx FLM-II method, the latter method being automated and hence available in a timely manner on a 24/7 basis. The lamellar body count (LBC) on amniotic fluid has been shown in studies to be an alternative for FLM assessment however the level of agreement and associated diagnostic cut-offs with LBC with these other classic lung surfactant methods have not been firmly established on our hematology analyzer. We found our LBC method compared favourably with the TDx FLM-II method ($LBC=0.990 \times TDx\ FLM-II-3.01$; $R^2=0.501$) as well as with the classic "gold standard" L/S ratio ($LBC=15.2 \times L/S-3.36$; $R^2=0.762$) leading us to conclude that our LBC was an acceptable surrogate for the well-established and no longer available fluorescent polarization TDx FLM-II method for rapid FLM screening. In addition, based on these comparison studies, LBC cut-offs were as follows: Immature, $\leq 20\ k/mm^3$; Indeterminate, 21 to 49 k/mm^3 ; and mature, $\geq 50\ k/mm^3$.

Keywords: Fetal lung maturity; Respiratory distress syndrome; Lamellar body count; L/S ratio; Late-preterm birth

Abbreviations

FLM: Fetal Lung Maturity; LBC: Lamellar Body Count; L/S: Lecithin Sphingomyelin Ratio

Introduction

Respiratory distress syndrome is a significant cause of morbidity and mortality of the new-born, especially those delivered prematurely. The incidence of RDS correlates with immaturity, ranging from approximately 0.3 percent of infants >38 weeks gestation to more than 10 percent of late preterm infants defined as >34th weeks of gestation but <36-6/7 weeks [1]. In the past FLM assessment was extensively utilized in screening deliveries <38 weeks gestation, however more recent literature demonstrates that these births are at increased risk of adverse outcome regardless of FLM testing status [2]. Thus, universal testing is no longer recommended. However, as the incidence of late-preterm birth continues to rise along with the high cost of NO and ECMO therapy, the need for better pre-delivery assessment is essential, especially in the late-preterm group [3,4]. Also, the cost of care for persistence of morbidity in these late-preterm infants throughout the first year of life is three times higher compared to term infants [4]. Therefore, it is important to maintain a laboratory FLM assessment test to support obstetric service in pregnancies that may involve a semi-elective, medically indicated delivery or uncertain gestational age.

Historically, a number of methods have been used to assess FLM. One of the earliest methods and also considered the "gold standard" is the thin layer chromatographic lecithin/sphingomyelin (L/S) ratio test described in 1971 by Gluck et al. [5]. Several years later, another chromatographic assay for the presence of phosphatidyl-glycerol (PG) was introduced with the advantages that it can be used for vaginal pool samples and in specimens contaminated with maternal blood and meconium [6]. However, these chromatographic methods require a highly skilled technologist and several hours to perform, making the

assay rarely available on a timely basis. Several permutations of rapid and easy surfactant screening tests have been introduced over the years including the Shake test, Tap test and Foam Stability test [7,8]. While these tests can be useful in identifying infants unlikely to develop RDS, the poor positive predictive value limited the usefulness of these tests as a rapid, 24/7 screening test. Accordingly, the latter surfactant assessment screening tests have fallen out of favor and are rarely performed in most laboratories. Until recently, the most popular and reliable assay was the automated Abbott TDx FLM-II (Abbott Laboratories, Chicago, IL), which measures the surfactant (phospholipid) to albumin ratio [9]. However, the TDx FLM-II was recently discontinued necessitating a need for a replacement method with at least a similar level of accuracy and automation. One option available was the lamellar body count, an assay that measures a stored form of surfactant in the amniotic fluid using a standard hematology analyser [10-14].

While our laboratory used the TDx FLM-II assay for screening, we maintained the legacy L/S ratio test as a reflex test to be used only when the TDx FLM-II gave an indeterminate result. Therefore, 1) since the Abbott TDx FLM-II automated method has since become no longer available and 2) the non-automated L/S ratio was not an acceptable 24/7 FLM screening option, we elected to use the LBC assay as the replacement method for the expiring TDx FLM-II while maintain the L/S ratio as a reflex protocol test when this LBC method yielded

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indeterminate results. While many studies have shown that LBC correlates with clinical outcomes, specific cutoffs relative to the TDx FLM-II and the L/S ratio test had not been firmly established. Hence, the present study's focus is to 1) establish a high degree of correlation of the LBC to our previously established FLM methodologies, i.e., the TDx FLM-II and L/S ratio, then 2) establish specific cut-offs for maturity, immaturity, and the indeterminate range, the latter range requiring reflex testing by L/S ratio in accordance with our established protocol.

Methods

For L/S ratio determination, our laboratory uses the Helena Fetal Tek 200 assay (Helena, Beaumont, TX). This involves an extraction step with a methanol-chloroform mixture followed by separation of lecithin and sphingomyelin by thin layer chromatography. The phospholipid bands are visualized using cupric acetate-phosphoric acid reagents with quantization via integration of peak area of the densitometric scan. An L/S ratio greater than or equal to 2.0 indicates maturity while a value less than or equal to 1.5 indicates immaturity. A ratio between 1.5 and 2.0 is considered indeterminate.

The FLM-II assay is performed on the Abbott TDx according to the manufacturer's instructions. The TDx utilizes a homogeneous fluorescence polarization method to determine the surfactant to albumin ratio. Our confirmed cut offs are maturity greater than or equal to 55 mg/g; immaturity was confirmed at less than or equal to 40 mg/g. Indeterminate FLM values, ranging from 40 to 55 mg/g, require testing using the L/S ratio to establish maturity or immaturity.

The LBC in this study was performed on the BCI Coulter LH 780 (Beckman Coulter Inc., Brea, CA) haematology analyzer. This method uses electrical impedance for counting particles passing between two electrodes. Normally on the BCI LH 780, formed elements ranging in size from two to twenty fL are counted as platelets. Lamellar bodies in amniotic fluid are of similar size and hence the BCI LH 780 platelet channel can be used for the LBC.

Historical data from 134 amniotic fluid samples, tested for maturity by L/S ratio and the TDx FLM-II as part of a validation of the TDx method, are included for reference. Our current study examines 51 amniotic fluid samples tested by the TDx FLM-II and BCI LH 780 LBC methods to establish concordance of the two methods. Samples found indeterminate by LBC were reflex tested for maturity using the L/S ratio.

Results

Correlation between the L/S ratio and TDx FLM-II showed acceptable linearity as depicted in Figure 1, especially considering that different methodologies and internal references were being utilized. Regression analysis yielded the following: $\text{TDx FLM-II} = 14.7 \times \text{L/S} + 1.89$; $R^2 = 0.640$. Figure 1 illustrates the methods correlates more closely around the clinical decision points for maturity, i.e., $\text{L/S} \geq 2.0$ and $\text{TDx FLM-II} \geq 55 \text{ mg/g}$. Indeed, while immature test results by the TDx FLM-II would occasionally be labelled indeterminate or mature by the L/S ratio, every mature result by the TDx FLM-II method was confirmed as mature by the L/S ratio. We felt it is critical to avoid falsely mature results in the rapid screening method since such results could potentially lead to adverse clinical outcomes.

Correlation studies comparing LBC to the TDx FLM-II yielded the linear regression equation with a regression line slope equal to unity ($\text{LBC} = 0.990 \times \text{TDx FLM-II} - 3.01$; $R^2 = 0.501$), as seen in Figure 2. Although the correlation coefficient between methods was not optimal, the methods do agree acceptably in the clinically relevant ranges (TDx

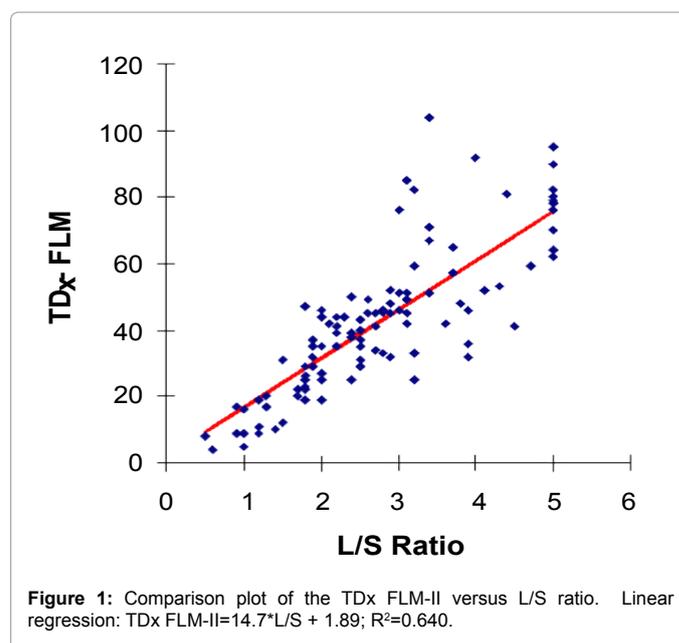
FLM-II, <40 and $>55 \text{ mg/g}$). Based on our LBC correlation studies with these two reference methods, we set conservative cut off LBC values of less than or equal to 20 k/mm^3 for immaturity and greater than or equal to 50 k/mm^3 for maturity. Following our legacy protocol, indeterminate values ranging between 20 to 50 k/mm^3 were reflexed to the L/S ratio test. Table 1 compares the clinical endpoints of the chosen cut offs for LBC with those of the TDx FLM-II. There was diagnostic agreement in the majority of cases (i.e., LBC/TDx: 11/15 immaturity vs. 22/26 maturity) with the TDx method giving better diagnostic lung maturity discrimination. The LBC also had more values falling into the indeterminate diagnostic range (LBC/TDx: 18/9). The overall diagnostic agreement was 66.7 percent between LBC and TDx FLM-II and there was no statistically significant diagnostic difference between these methods. (Chi Square; $p = 0.119$).

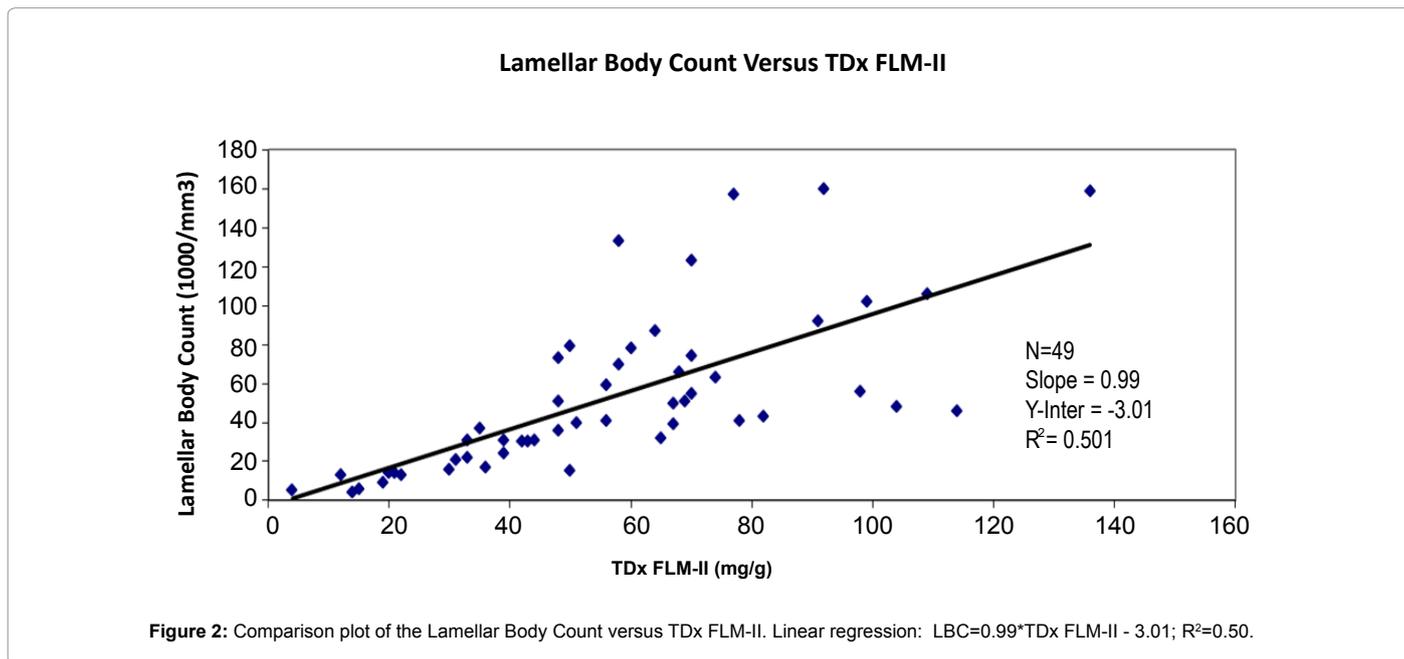
Sixteen samples found to be indeterminate by LBC were reflexed to the L/S ratio test. On these samples where both LBC and L/S were performed, there was acceptable agreement as depicted in Figure 3 and linear regression yielded the following: $\text{LBC} = 15.2 \times \text{L/S} - 3.36$; $R^2 = 0.762$. When comparing clinical endpoints of the methods, there were no instances of discordance between the LBC and L/S methods. Most importantly, there were no instances where a mature result by LBC was not confirmed by the L/S ratio of 2.0 or greater.

Analytical sensitivity of the LBC was experimentally observed to be $1,000/\text{mm}^3$. Regarding interferences, blood and meconium can interfere with the LBC⁹; these also interfere with the TDx FLM-II and L/S ratio.

Conclusion

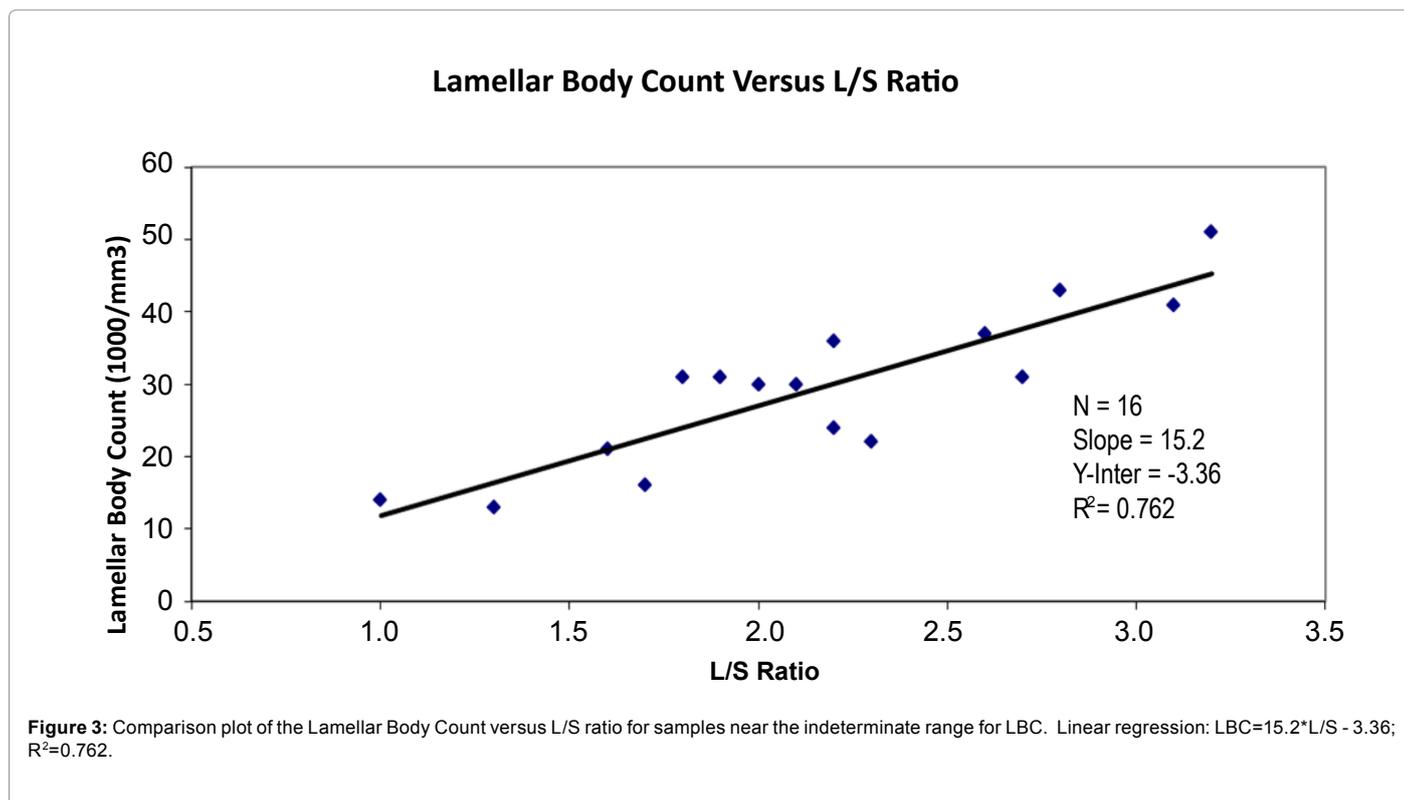
We observe acceptable diagnostic concordance between LBC and TDx FLM-II and L/S ratio methods. However, it did appear that the older, and no longer commercially available TDx surfactant based method gave better diagnostic discrimination than LBC between mature and immature with the LBC showing more patient results falling into the indeterminate ranges. Note that for LBC and TDx FLM-II respectively, 21 to 49 k/mm^3 and 41 to 54 mg/g were set as indeterminate ranges based, in part, by L/S ratio comparison data. We





		TDx FLM-II		
		Immature <40 mg/g	Intermediate 41-54 mg/g	Mature >55 mg/g
LBC	Immature 20 k/mm ³	10 (91%)	1 (9%)	0 (0%)
	Intermediate 21-49 k/mm ³	5 (28%)	6 (33%)	7 (39%)
	Mature >50 k/mm ³	0	3 (14%)	19 (86%)

Table 1: Concordance of fetal lung maturity assessment based on lamellar body count and the TDx FLM-II.



therefore conclude the LBC is clinically acceptable when compared to the older fluorescence polarization TDx FLM-II assay for rapid 24/7 fetal lung maturity assessment. Moreover, we demonstrate that the LBC method shows good diagnostic correlation with the legacy chromatographic L/S ratio test.

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