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Letter to the Editor

Validation of the L/S ratio determined by digital scanning densitometry ☆

Dear Editor,

Pulmonary surfactant is a complex mixture of phospholipids (~90%) and proteins (~10%) that functions to prevent atelectasis by reducing surface tension at the alveolar air–liquid interface. Synthesized by type II pneumocytes and packaged into lamellar bodies, surfactant is secreted into the developing fetal airways and starts increasing in amniotic fluid at about 32 weeks of gestation, with a 10-fold increase observed during the third trimester. Respiratory distress syndrome (RDS) is the most common cause of respiratory failure in newborns, and is nearly always associated with premature birth. Prevention of premature birth is the best approach to avoid RDS, although administration of steroids to the mother effectively accelerates surfactant production in-utero [1,2].

Measurement of amniotic fluid surfactants accurately estimates the amount of surfactants in fetal lungs. The concentration of lecithin, the major pulmonary surfactant, rises sharply after 35 weeks of gestation until birth, while the concentration of sphingomyelin remains relatively constant. The lecithin/sphingomyelin (L/S) ratio in amniotic fluid increases with gestational age and represents an estimate of fetal lung maturity (FLM) [2,3].

Thin layer chromatography (TLC) is used to separate lecithin and sphingomyelin, and densitometric quantification of the phospholipid bands determines their relative abundance [4]. The objective of the present study was to validate the quantification and clinical interpretation of the L/S ratio using flat-bed digital scanning densitometry as compared to conventional optical densitometry.

The present study was performed using 100 amniotic fluid specimens of unknown gestational age received at ARUP Laboratories between November 2007 and March 2008. Specimens were received frozen and analyzed within 24 h. The commercially available Helena Fetal-Tek 200® method (Helena Laboratories, Beaumont, TX) was utilized to separate the phospholipids in the amniotic fluid by TLC. Phospholipids were extracted with a chloroform–methanol mixture (1:1), streaked onto a TLC plate, separated using a solvent system, and made visible with phosphoric acid-cupric acetate reagent, followed by charring [5]. The lecithin and sphingomyelin bands were quantified by densitometry using an Appraise® optical densitometer (Beckman Coulter, Fullerton, CA) at 525 nm, and the relative intensities of the 2 bands were expressed as a ratio. Additionally, the same 2 bands were quantified within 1–3 days using an Epson Expression® 1680 flat-bed digital scanner and the resulting image analyzed by the Phoresis Imaging System (Sebia Electrophoresis, Norcross, GA), which determined relative amounts of the lecithin and sphingomyelin bands based on an integration system. For both optical and digital densitometry, L/S ratios of ≤1.5, 1.6–2.4, and ≥2.5 were interpreted as immature, transitional, and mature fetal lungs, respectively. Specimens were deidentified using University of Utah Institutional Review Board approved protocols (IRB #7275).

L/S ratio results from immature, transitional, and mature quality control material were not significantly different when determined by optical or digital densitometry (data not shown). Table 1 summarizes the L/S ratio results of the 100 patient specimens tested. L/S ratios were not significantly different when determined by either method for immature specimens, but transitional specimens were significantly lower, and mature specimens were significantly higher when determined by digital compared to optical densitometry (Table 1). Overall, the clinical interpretations were consistent between the 2 methods (Table 2). Of the 11 immature specimens by optical densitometry, 1 was interpreted as transitional and none as mature by digital densitometry. Similarly, of the 61 mature specimens by optical densitometry, 1 was interpreted as immature and 4 as transitional by digital densitometry. By contrast, there were 28 transitional specimens by optical densitometry, 3 of which were interpreted as immature and 13 of which were interpreted as mature by digital densitometry. Without patient outcome data, the clinical impact of these 13 transitional-to-mature results is unknown. However, we noted that the L/S ratios (as determined by optical densitometry) for these specimens were ≥2.0 [mean (SD), 2.3 (0.14); range, 2.0–2.4)], a result that is commonly used to indicate maturity [2]. Furthermore, given the well-documented poor precision (CV, 26%) of L/S ratio results >2.0 [2] we calculated that the 95% confidence interval for these 13 ratios was 2.0–2.6, results that could be interpreted as transitional or mature.

Table 1
Statistical analysis and comparison of L/S ratios determined by optical vs. digital densitometry

Clinical interpretation	Densitometry method	*N*	Mean (SD)	*p*
Immature	Optical	11	1.3 (0.17)	0.08
	Digital	14	1.1 (0.26)	
Transitional	Optical	28	2.2 (0.14)	0.04
	Digital	17	2.1 (0.20)	
Mature	Optical	61	3.0 (0.32)	<0.0001
	Digital	69	4.3 (1.38)	

Table 2
Clinical interpretation of the L/S ratio determined in 100 amniotic fluid specimens by digital densitometry compared to optical densitometry

		Optical Densitometry		
		Immature	Transitional	Mature
Digital densitometry	Immature	10	3	1
	Transitional	1	12	4
	Mature	0	13	56

☆ These data were presented at the 2008 annual meeting of the AACC, Washington, DC.

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We conclude that digital densitometry is an acceptable replacement for traditional optical densitometry for the determination of the L/S ratio. Although digital densitometry produces lower L/S ratios for transitional specimens and higher L/S ratios for mature specimens, the clinical interpretations remain largely unaffected.

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