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Caryn M. St. Clair
Yale University

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By

Caryn M. St. Clair

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NEONATAL RESPIRATORY DISTRESS SYNDROME AS A FUNCTION OF GESTATIONAL AGE AND THE LECITHIN/SPHINGOMYELIN RATIO

Caryn M. St. Clair, Jessica L. Illuzzi, and Errol R. Norwitz. Section of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT.

This study was designed to derive predictive logistic regression equations to allow the risk of neonatal respiratory distress syndrome (RDS) to be defined as a function of both the lecithin/sphingomyelin (L/S) ratio and gestational age. We hypothesize that the optimal cutoff value will vary significantly depending on gestational age, and that our data will support the need to account for gestational age when interpreting test results.

Data was collected via a retrospective chart review. Women who underwent amniocentesis for the purpose of assessing fetal lung maturity at Yale-New Haven Hospital from 1998 to 2004 were identified and included if delivery of a liveborn, singleton, non-anomalous infant occurred within 72 hours of the lecithin/sphingomyelin ratio assay. Maternal and neonatal data were collected regarding demographics, pregnancy complications and neonatal outcomes, including respiratory distress syndrome.

A total of 210 mother-neonate pairs met criteria for analysis, with 8 cases of RDS. Both gestational age and L/S ratio were independent predictors of RDS. By modeling the odds of RDS using logistic regression, a probability of RDS approximating 10% was noted at an L/S cutoff of 3.6 at 32 weeks, 2.8 at 34 weeks, 1.8 at 36 weeks, and 1.4 at term. Under 32 weeks of gestation a probability as low as 10% was not observed by this model. We conclude that stratifying risk of neonatal RDS using both the L/S ratio and gestational age may aid in clinical decision-making concerning the timing of delivery.

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Introduction

Definition and Epidemiology of Neonatal Respiratory Distress Syndrome:

Respiratory distress syndrome (RDS), also known as hyaline membrane disease (HMD), refers to respiratory compromise presenting at or shortly after delivery due specifically to a deficiency of pulmonary surfactant. Though this deficiency was originally described by Avery and Mead in 1959 (*1)*, RDS remains a serious, frequently fatal, neonatal complication. Neonatal RDS affects approximately 1% of all live births (*2-4)*; however, not all infants are at equal risk. The pulmonary system is among the last of the fetal organ systems to become functionally mature. As such, RDS is primarily – although not exclusively – a disease of premature infants with an incidence and severity that is highly dependent upon gestational age (*2-5)*.

A recent epidemiological study estimated that there are approximately 80,000 cases of neonatal respiratory failure per year in the United States and roughly 8,500 deaths, with a hospital cost totaling $4.4 billion (*6)*. The 80,000 cases – representing 2% of all live births – were evenly distributed over three weight classes: very low birth weight (< 1,500 g), low birth weight (1,500 – 2,499 g), and normal birth weight (> 2,500 g). Thus, while prematurity and low birth weight have been associated with a higher risk for RDS, normal birth weight babies accounted for 1/3 of cases of neonatal respiratory failure. Neonates with very low birth weight, however, accounted for half of all deaths.

History of Neonatal Respiratory Distress Syndrome:

The documented history of respiratory distress syndrome dates back to the early twentieth century, when pulmonary hyaline membrane (PHM) was described and

considered by Hochheim to represent aspirated amniotic fluid. This theory was relatively accepted until the early 1950's when radiographic descriptions of the reticulogranular pattern in generalized neonatal atelectasis were distinguished from the radiographic appearance in cases of aspirated amniotic debris (*7).* Work at that time by Pattle, Clements and Brown had begun to show that a low surface tension in the lung was imperative for proper lung function. But it wasn't until Avery and Mead's publication in 1959 that the clinical relevance of these findings was fully understood.

In their paper *Surface Properties in Relation to Atelectasis and Hyaline Membrane Disease* (*1),* Avery and Mead provided evidence that the lungs of neonates with hyaline membrane disease lack the alveolar material – a "surface-active substance" – responsible for maintaining a low surface tension. Experimenting on lung extracts, they showed that the lowest surface tension values in neonates 1,100 to 1,200 grams were 20-30 dynes/cm, while the lowest tensions in heavier infants, older children and adults were all under 20 dynes/cm, and more commonly 5-7 dynes/cm. Notably, heavier infants with hyaline membrane disease were the only exception and fell into the former category with tensions of 20-30 dynes/cm, and generally above 30 dynes/cm.

Work by Adams and Fujiwara continued along this line. They were able to determine by thin-layer chromatography that the active components of the surface-active substance were predominantly that of lecithin and sphingomyelin. Further study showed that "without exception, lungs with poor surface activity contained considerably less total lipid, less total phospholipid, and a reduced percentage of lecithin" (*8).* This also indicated that the dysfunction in the lungs of premature infants and infants with HMD

was due to a decreased quantity of these active components rather than deactivation or decreased quality of the lipids.

Surfactant – Composition and Normal Physiology:

Research to elucidate the composition of surfactant – a term derived from "surface-active agent" – has been ongoing over the last several decades and has shown that surfactant is comprised mainly of lipids and proteins similar to a cell membrane (*7)*. Lipids make up approximately 90% of pulmonary surfactant, half of which is dipalmitoyl phosphatidylcholine (DPPC); this lipid portion conveys the surface-active properties of surfactant (*9)*. Phosphatidylcholines are the second most common lipid component, specifically phosphatidylglycerol, followed by cholesterol and anionic phospholipids.

The remaining 10% of pulmonary surfactant is comprised of proteins. While plasma proteins such as albumin and secretory IgA make up around half of the protein composition, apoproteins (SP-A, SP-B, SP-C and SP-D) make up the remainder (*9)*. SP-A and SP-D are large, water soluble proteins belonging to the collectin family that participate in both innate immunity and the metabolism of surfactant. The smaller apoproteins, SP-B and SP-C, are hydrophobic intrinsic membrane proteins that facilitate formation of the monolayer, speeding the rate at which surfactant moves into the air-water interface to form a stable film (*7, 9)*.

Synthesis and secretion of surfactant occurs predominantly within cuboidal type-II alveolar cells. Lipids enter these cells via the bloodstream, while the pneumocytes use the secretory pathway to synthesize the various apoproteins. Lamellar bodies act as the site for final assembly, and the resulting pulmonary surfactant is secreted from the cells

via constitutive exocytosis (*9)*. Surfactant levels are low until just prior to delivery, when increased synthesis is triggered by a surge in glucocorticoids, as well as other factors such as thyroid hormone, thyroid-releasing hormone, prolactin, and the growth-factor EGF. Cortisol acts by increasing the differentiation of type-II pneumocytes and the formation of lamellar bodies within the fetal lungs, stimulating surfactant production and thereby lung maturation (*9)*.

Physiologically, pulmonary surfactant serves three main functions. First and foremost, it increases the compliance of the lungs by decreasing surface tension. By decreasing the tendency for elastic recoil, it allows the neonate to expand the lungs without exerting extreme effort and prevents collapse of the alveoli at end expiration. Second, surfactant decreases the amount of fluid accumulation in the alveoli by preserving the tendency for fluid movement into the interstitium. Without surfactant, the increased surface tension collapses the alveolus, disturbs Starlings forces across the alveolar wall, and increases fluid movement into the alveoli, resulting in pulmonary edema. Finally, the presence of surfactant maintains the equality of alveolar size during the respiratory cycle, preserving uniform surface area and maximizing gas diffusion and ventilation throughout the pulmonary apparatus (*9)*.

Pathology & Pathophysiology of Neonatal Respiratory Distress Syndrome:

Though respiratory distress in the newborn can have many etiologies – for example, maternal sedation, fetal head injury during delivery, aspiration, or hypoxia due to a nuchal cord – the most common cause is respiratory distress syndrome (*10)*. The fundamental defect in this disorder is a deficiency of pulmonary surfactant, which leads

to increased surface tension in the alveoli as described above. The lungs are stiff and tend to collapse further with each breath, causing atelectasis; this effect is worsened by the soft thoracic wall that is pulled inward with inspiration. Resultant hypoventilation and poor perfusion cause the newborn to become hypoxemic and to retain carbon dioxide, leading to a respiratory acidosis. The pulmonary vasculature vasoconstricts, causing further hypoperfusion and ventilation/perfusion (V/Q) mismatch. Both endothelial and epithelial damage lead to leakage of a protein-rich, fibrin-rich exudate into the alveolar spaces; this exudate accumulates with the necrotic cells and forms hyaline membranes. These membranes act to increase the diffusion gradient across the alveoli, creating a barrier to gas exchange and worsening the acidosis and hypoxemia. These factors further impair surfactant synthesis and the cycle continues, severely impairing lung function in the neonate and increasing the likelihood of complete respiratory failure (*10)*.

On gross pathology, the lungs of infants with RDS may be of normal size, but are solid and airless; they generally sink when placed in water. They have a darker appearance than healthy lungs, commonly a reddish purple resembling the liver. The alveoli are poorly developed, and most are collapsed. Eosinophilic hyaline membranes composed of necrotic cellular debris, fibrinogen and fibrin, line the respiratory bronchioles, alveolar ducts and alveolar spaces. Inflammatory cells such as neutrophils are generally absent from these membranes; the cell debris is mostly comprised of necrotic type-II pneumocytes (*10)*.

Diagnosis of Neonatal Respiratory Distress Syndrome:

RDS is diagnosed based on clinical and radiological findings. The classic clinical signs of neonatal RDS include tachypnea, nasal flaring, chest retractions, and expiratory grunting (*11, 12).* These findings generally appear immediately after delivery or within the first few hours of life. Tachypnea is the newborn's attempt to increase minute ventilation to compensate for a decreased tidal volume. The relatively frequent finding of nasal flaring represents an effort to decrease the resistance in the upper airway, while retractions indicate an effort to increase negative intrapleural pressure to inflate the lungs. As the neonate tries to maintain end-expiratory lung volume, he may expire against a closed glottis, resulting in the audible grunting sounds characteristic of infants with RDS. More worrisome signs of neonatal respiratory distress include cyanosis, gasping, choking, and apnea (*11).* Physical examination often aids the diagnosis. Auscultation may reveal poor air exchange and/or fine rales, and observation may reveal the use of accessory muscles during inspiration as well as episodes of apnea (*12).*

Chest radiographs at birth and at regular intervals can be helpful in establishing this diagnosis as well and can often allow differentiation between transient tachypnea of the newborn (TTN) and respiratory distress syndrome (RDS). Unlike RDS, TTN is not the result of an underdeveloped pulmonary surfactant system; instead it represents delayed clearance of fetal lung fluid and a transient pulmonary edema (*11).* It presents clinically as a milder form of RDS, but it is generally self-limited and resolves more rapidly. The chest x-ray in TTN reveals increased pulmonary interstitial markings, fluid in the interlobar fissures, and occasionally a pleural effusion or alveolar edema (*11).* This differs from the chest radiograph in RDS, which shows decreased lung volumes, domed

diaphragms, air bronchograms, and a diffuse reticular-granular opacification or ground-glass appearance that may progress to total white-out of the lung fields (*12).* It is important to account for the degree of prematurity, as well as positive pressure ventilation or surfactant administration, which can alter the appearance and degree of pathology in the chest film.

Treatment of Neonatal Respiratory Distress Syndrome:

Treatment of neonatal RDS is based on interventions that occur in the prenatal, perinatal and postnatal periods. Given the close relationship between RDS and gestational age (*3-5),* the most effective way to prevent RDS is to prevent prematurity by delaying delivery, thereby prolonging gestation (*12).* Performing antenatal testing for fetal lung maturity and administering antenatal corticosteroids to women at risk for preterm delivery are additional means for reducing the risk of neonatal RDS prenatally. Options for antenatal fetal lung testing for prediction of RDS will be discussed separately.

Antenatal Corticosteroids:

The effect of steroids was first observed by Liggins in 1969 while experimenting with sheep given antenatal dexamethasone (*13)*; he noted that the lungs of their premature lambs – delivered at a point where the lungs would *not* be expected to be aerated – were partially inflated. Three years later Liggins and Howie published the first randomized control trial of antenatal administration of betamethasone in humans, showing that both respiratory distress syndrome and early neonatal mortality were significantly reduced in the treated group compared to controls (*14).* Subsequent studies have supported their

conclusions that antenatal steroid treatment improves overall pulmonary status, increases neonatal survival, and decreases the incidence and severity of RDS (*12, 15, 16).* A 2006 meta-analysis of twenty-one studies confirmed a significant reduction not only in neonatal death and RDS, but also in intraventricular hemorrhage, necrotizing enterocolitis, intensive care admissions, respiratory support, and systemic infections in the first 48 hours of life; the authors concluded that a single course of antenatal corticosteroids should be routine care in preterm deliveries with few exceptions (*17).*

The morbidity and mortality associated with neonatal RDS increase significantly with perinatal asphyxia (*12).* Therefore in the perinatal period, management of RDS is largely aimed at reducing the risk of asphyxiation and stress to the infant; an experienced resuscitation team led by a neonatologist or pediatric intensivist is highly recommended if the neonate is at high risk for respiratory distress.

Surfactant Replacement:

Though many interventions play a role in the immediate postnatal management of RDS – correction of acidosis, maintenance of a neutral thermal environment, correction of hypovolemia and hypotension, maintenance of electrolyte balance, continuous monitoring, and control of infection (*12)* – respiratory support and surfactant replacement remain the cornerstones of therapy. Once Avery and Mead determined that hyaline membrane disease was the result of a deficiency in surfactant (*1)*, efforts were undertaken to replace the surface-active agent. Initial attempts centered on administration of an aerosol form of the active phospholipid in natural surfactant, dipalmitoyl phosphatidylcholine (DPPC); unfortunately, this approach was not found to be effective

at treating RDS (*18)*. The first successful surfactant replacement took place in 1980 when Fujiwara et al. administered artificial surfactant from cow lungs endotracheally to infants with severe hyaline membrane disease; results showed an overall improvement in oxygenation, a decrease in respiratory support requirements, resolution of radiographic lung changes, and reversal of acidosis and systemic hypotension (*19)*. Prospective, randomized controlled trials followed, confirming that administration of artificial surfactant in the immediate postnatal period decreased the severity of respiratory distress in the first days of life (*20, 21)*.

Evidence has continued to demonstrate a decrease in morbidity, mortality, and use of resources with the advent of surfactant replacement (*22-24),* and attention has turned to comparison of timing, dosage, and type of preparation of the surfactant. Studies have indicated that animal-derived, natural preparations are more effective at treating RDS, and convey lower mortality than their synthetic counterparts lacking a protein component (*24, 25)*; however, recent randomized controlled trials of a new class of synthetic surfactants containing a peptide analog of SP-B have shown promising results with similar efficacy and mortality to the natural preparations (*26, 27).* Literature regarding the timing of surfactant administration indicates that early administration and shorter duration of mechanical ventilation is advantageous compared to delayed administration and a longer period of time spent on the ventilator (*28-31)*.

Prediction of Neonatal Respiratory Distress Syndrome:

Given the gravity of neonatal respiratory distress syndrome and its complications, prediction of fetal lung maturity or lack thereof prior to delivery has been a heavily

researched topic. To assist obstetric care providers in counseling pregnant women at risk of preterm delivery, a series of tests have been developed in an attempt to predict gestational age-related risk of developing RDS (*2)*. These can be divided into indirect, direct biochemical, and direct biophysical tests. Indirect tests involve determination of the gestational age or size of the fetus in order to infer maturity and pulmonary status; these include calculation of the last menstrual period, appearance of fetal heart tones, identification of the gestational sac on ultrasound, measurement of crown-rump length and biparietal diameter. Direct biochemical tests measure the concentration of various components of pulmonary surfactant secreted by the fetal lungs into the amniotic fluid, and include measurement of the lecithin/sphingomyelin (L/S) ratio and the phosphatidylglycerol (PG) band. Direct biophysical tests evaluate the surface-active properties of the phospholipids in pulmonary surfactant and include the foam stability index (FSI), fluorescence polarization (TDx-FLM), and the lamellar body count (LBC).

Lecithin/Sphingomyelin Ratio:

Introduced by Gluck and others at Yale University in 1971, the L/S ratio was the first widely accepted test available for assessment of fetal lung maturity (*32)*. This assay is based on the observation that pulmonary secretions from the lungs of the fetus flow into the amniotic fluid, affecting the phospholipid concentrations. Lecithin and sphingomyelin are present in relatively equal amounts until 32 to 33 weeks of gestation, at which point the lecithin level begins to rise appreciably. Gluck observed that neonatal respiratory distress syndrome was uncommon after the lecithin/sphingomyelin ratio had reached 2.0, which occurs around 35 weeks of gestation in uncomplicated pregnancies

(*32, 33)*. This value has remained an accepted threshold for the determination of fetal pulmonary status in nondiabetic women; however it is suggested that the value be correlated with clinical outcomes at individual centers, as the variation between laboratories can be considerable (*2)*.

One disadvantage of the L/S ratio is that it can be difficult to perform and interpret and is not readily available at all institutions (*34)*. The sample must be handled appropriately during transport and while awaiting analysis, and the test itself is labor intensive, taking 3-5 hours. Additionally, the presence of blood or meconium in the sample – common contaminants – affects the validity and utility of the result (*35)*. The L/S ratio has been studied against other assays for fetal lung maturity that have been developed over the years, and those comparisons are discussed in the following sections.

What is needed to improve obstetric care is not an innovation in biochemical assays and techniques, however, but rather an improved understanding of the probability of a fetus developing RDS for a given test value at a specific gestational age (*36, 37)*. Ghidini et al. reported significant morbidity in a study of preterm infants with mature lung indices, including a 12% incidence of neonatal RDS (*38)*. Gestational age-specific risk stratification cutoffs for RDS have been developed for other assays of fetal pulmonary maturity (*39-41)*; a similar approach must be taken for the L/S ratio, a test frequently used at many institutions despite newer alternatives.

Phosphatidylglycerol:

Phosphatidylglycerol is a minor constituent of surfactant; its concentration in the amniotic fluid tends to increase most appreciably several weeks after the rise in lecithin occurs (*42)*. Measured by thin-layer chromatography, presence of a PG band generally represents a more advanced state of fetal pulmonary status, as PG serves to enhance the spread of phospholipids along the alveolar surface (*2)*. The advantage to PG analysis is that it is not affected by common contaminants, such as blood or meconium (*2, 43)*; therefore, it is a good test in patients who have experienced rupture of membranes and can provide a vaginal pool sample. The disadvantage remains that the sensitivity of PG on thin-layer chromatography varies, so the significance of its absence also varies (*35)*. In other words, the PG band often becomes "positive" relatively late in pregnancy, and when used alone may suggest fetal lung immaturity at later gestational ages when the risk of neonatal RDS is low (*44)*.

Foam Stability Index:

The foam stability test, or "shake test," was described by Clements et al. in 1972 (*45)*. In this test, addition of ethanol to amniotic fluid eliminates any foam formed by non-surfactant substances (*2)*. If after shaking the tube a stable foam remains, then surface-active components of surfactant are present in the amniotic fluid; serial dilutions with ethanol allow quantification of the surfactant concentration. This test has since been modified and is now referred to as the foam stability index, or FSI (*46)*. The value of the index represents the highest ethanol volume fraction that permits the formation of

stable foam after vigorously shaking a mixture of ethanol and amniotic fluid; the accepted cutoff for fetal lung maturity is greater than or equal to 47.

Fluorescence Polarization:

One of the most widely used tests for fetal lung maturity is the TDx-FLM II surfactant-to-albumin assay (Abbott Laboratories, Abbott Park, IL), which uses fluorescence polarization to determine the relative concentrations of surfactant and albumin in amniotic fluid; results are given as mg of surfactant per 1 g of albumin. An elevated ratio greater than 55 mg/g has been used as a cutoff to indicate fetal lung maturity (*2, 47)*, although one study suggested that this threshold be lowered to 45 mg/g, maintaining a sensitivity of 100% and a specificity of 90% (*3)*. Again however, the dichotomous, "positive" or "negative" approach to biochemical assays for fetal lung maturity can be fundamentally misleading, and attempts have been made to account for gestational age when interpreting test results. A number of studies have used logistic regression to show the probability of RDS based on the TDx-FLM value as well as gestational age (*39-41)*. In general, a higher threshold value is required at lesser gestational age, while a lower, more borderline value may be acceptable at later gestational age.

The TDx-FLM surfactant-to-albumin ratio has performed similarly to the L/S and PG assays in terms of prediction of neonatal RDS (*48)*. Advantages of the TDx-FLM II assay are that it is a rapid test that is relatively easy to perform and highly reproducible. The disadvantage remains that blood and meconium contamination can interfere with interpretation of the results (*2)*.

Lamellar Body Count:

Lamellar body count (LBC) is a newer technique being utilized to assess fetal lung maturity. Final assembly and storage of surfactant occurs within lamellar bodies, which are extruded from type-II pneumocytes into the alveoli (*2, 9)*; lamellar body count is, therefore, a relatively direct measurement of surfactant production. While some studies have cited values of 30,000 per microliter as the lower limit for indication of fetal lung maturity (*2, 49)*, a recent consensus of protocol by Neerhof et al. reported that values over 50,000 per microliter were more suggestive of pulmonary maturity, while values under 15,000 per microliter were indicative of pulmonary immaturity (*50)*. Ventolini and colleagues studied neonatal outcomes before and after this change in threshold, and concluded that the higher cutoff value of 50,000 per microliter resulted in significantly decreased neonatal morbidity and complication rates (*51)*. Efforts have again been undertaken, however, to create gestational age-specific risk predictions for this assay to replace dichotomous cutoff values (*52)*.

Lamellar body count has been shown to perform as well or better than the PG, the L/S ratio, and the TDx-FLM assay (*52-54)*. LBC conveys many advantages; it is faster, more objective, less labor intensive, less technique dependent, and less expensive than both PG and L/S analysis (*53)*. Furthermore, the similar size of lamellar bodies and platelets allows the LBCs to be conducted on a standard Coulter counter, available in all hospital laboratories. Some authors suggest that lamellar body count replace the lecithin/sphingomyelin ratio for prediction of fetal lung maturity and risk of neonatal RDS given these considerations (*49, 54)*. However the L/S ratio remains the oldest, most studied index for fetal pulmonary status, and continues to be the test of choice in many

institutions. Given this, gestational age-specific L/S cutoff values to predict neonatal RDS are critical for improving the performance of this assay.

Statement of Purpose

This study was designed to derive predictive logistic regression equations to allow the risk of neonatal respiratory distress syndrome (RDS) to be defined as a function of both the lecithin/sphingomyelin (L/S) ratio and gestational age. We hypothesize that the optimal cutoff value for this assay's prediction of RDS will vary significantly depending on gestational age, and that our data will support the need to account for gestational age when interpreting these test results. Secondarily we will contribute data to describe the current incidence of neonatal RDS in the post-steroid, post-surfactant, era.

Materials and Methods

An application to conduct a medical record review for the purposes of this study was submitted to the hospital's Human Investigations Committee, and the study was approved. Women who underwent amniocentesis for fetal lung maturity screening with the lecithin/sphingomyelin (L/S) assay at Yale-New Haven Hospital in New Haven, Connecticut, between June 1998 and December 2004 were identified. Patients were included only if the lecithin/sphingomyelin ratio measurement was obtained within 72 hours of delivery, and the delivery resulted in a singleton, liveborn infant without major congenital anomalies. Maternal records from the above dates were requested and reviewed for gestational dating criteria, timing and results of the lecithin/sphingomyelin ratio testing, presence or absence of the phosphatidylglycerol (PG) band, date and time of delivery, type of delivery, maternal age and race, smoking status, presence or absence of diabetes, chronic hypertension, preeclampsia, and preterm premature rupture of membranes (PPROM), as well as antenatal corticosteroid status. Neonatal records provided information on infant sex, birth weight, apgar scores, resuscitation effort at delivery, and disposition to the well baby nursery or the newborn special care unit (NBICU). Those not meeting inclusion criteria were documented as excluded, and the cause for exclusion was noted; such mother-neonate pairs were not included in the final statistical analysis.

Infants sent to the well baby nursery were assumed not to have respiratory distress syndrome or other major complications as those clinical entities would not be managed in this setting, and no further data was collected. For those sent to the NBICU, data was collected regarding type and duration of oxygen requirement (intermittent mandatory

ventilation, nasal cannula, nasal continuous positive airway pressure), diagnoses of respiratory distress syndrome (RDS), transient tachypnea of the newborn (TTN), persistent pulmonary hypertension (PPH), bronchopulmonary dysplasia (BPD), pneumothorax (PTX), and other complications including retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), and sepsis at various points of admission. The length of stay in the NBICU, as well as discharge with or without oxygen, was also noted.

Gestational age was determined by last menstrual period confirmed by second-trimester ultrasound, first trimester ultrasound, or in vitro fertilization or artificial insemination dating, as available. Cases dated only by a second- or third-trimester ultrasound were excluded. Maternal race was self-described in the medical record. Maternal diabetes was described by the White classification as documented in the medical record; diabetes in the study population was defined by any insulin requirement, including both insulin-dependent pregestational diabetes and insulin-requiring gestational diabetes. Diet-controlled diabetes was noted separately. Preeclampsia was defined as the presence of new-onset persistent hypertension (blood pressure 140 mm Hg or more systolic and/or 90 mm Hg diastolic) and new-onset proteinuria (300 mg or more urinary protein per 24 hours) after 20 weeks of gestation. Chronic hypertension was defined by maternal antihypertensive use before pregnancy. Maternal smoking status was identified in the medical record.

Neonatal RDS, TTN, PPH, and BPD diagnoses were obtained via an existing database maintained by the Section of Perinatal Medicine in the Department of Pediatrics at Yale-New Haven Hospital. Neonatal RDS – our primary outcome – was diagnosed by

the presence of at least 2 of the following 3 criteria: 1) evidence of respiratory compromise (tachypnea, retractions, and/or nasal flaring) shortly after delivery and a persistent oxygen requirement for more than 24 hours, 2) administration of exogenous pulmonary surfactant, and/or 3) radiographic evidence of neonatal pulmonary hyaline membrane disease as diagnosed by an attending pediatric radiologist or neonatologist. Radiographic evidence of neonatal RDS included atelectasis, air bronchograms, and a diffuse reticulogranular infiltrate.

The data was analyzed using the SAS 9.1 statistical software package. Maternal and neonatal characteristics were compared in infants with and without neonatal respiratory distress syndrome using Chi-square analysis as well as Fisher's exact test. The gestational age at the time of amniocentesis and the lecithin/sphingomyelin ratios were analyzed using the Student's t-test; these values were compared in neonates with and without RDS by Pooled and Satterthwaite t-tests, after assessment of the equality of variances. Wald odds ratios with 95% confidence intervals were determined for predictors of neonatal RDS; these values were also calculated after adjustment for gestational age. Finally using multivariate logistic regression, a prediction equation for the probability of neonatal RDS was developed with both the lecithin/sphingomyelin ratio value and gestational age as the descriptive variables; step-wise backward elimination was used to eliminate other possible confounders, using the partial F test and testing for changes in parameter estimates of greater than 10%. This equation is discussed further within the results.

Results

A total of 443 maternal charts identified by billing codes designating amniocentesis for fetal lung maturity within the study period were identified and screened for this study; 210 mother-neonate pairs met criteria for analysis and were abstracted, while 233 were excluded. The most common cause for exclusion was a time interval from amniocentesis to delivery greater than 72 hours, resulting in 158 exclusions (68%). Nineteen subjects (8%) were eliminated based on absence of key data such as the lecithin/sphingomyelin ratio, while 20 subjects (9%) were excluded based on delivery at an outside hospital and lack of neonatal data. Amniocentesis for genetic purposes and not for assessment of fetal lung maturity ruled out 11 subjects (5%), and multiple gestations accounted for 15 exclusions (6%). Seven maternal-neonate pairs (3%) were excluded because the mother was already represented in the database. Finally three cases (1%) were excluded due to severe congenital malformations that could complicate the diagnosis of neonatal respiratory distress syndrome.

As indicated in Figure 1, sixty-five of the 210 included neonates (31%) went to the Newborn Intensive Care Unit (NBICU), while 145 neonates (69%) went to the well-baby nursery. Those sent to the nursery were assumed not to have neonatal RDS, as oxygen support is not administered outside of the NBICU. Of the included population of 210 pairs, 8 cases of neonatal RDS were diagnosed, for an incidence of 3.8%. An additional 8 neonates were noted to have transient tachypnea of the newborn (TTN), also with an incidence of 3.8%. One newborn had documented persistent pulmonary hypertension (PPH), for an incidence of 0.05%.

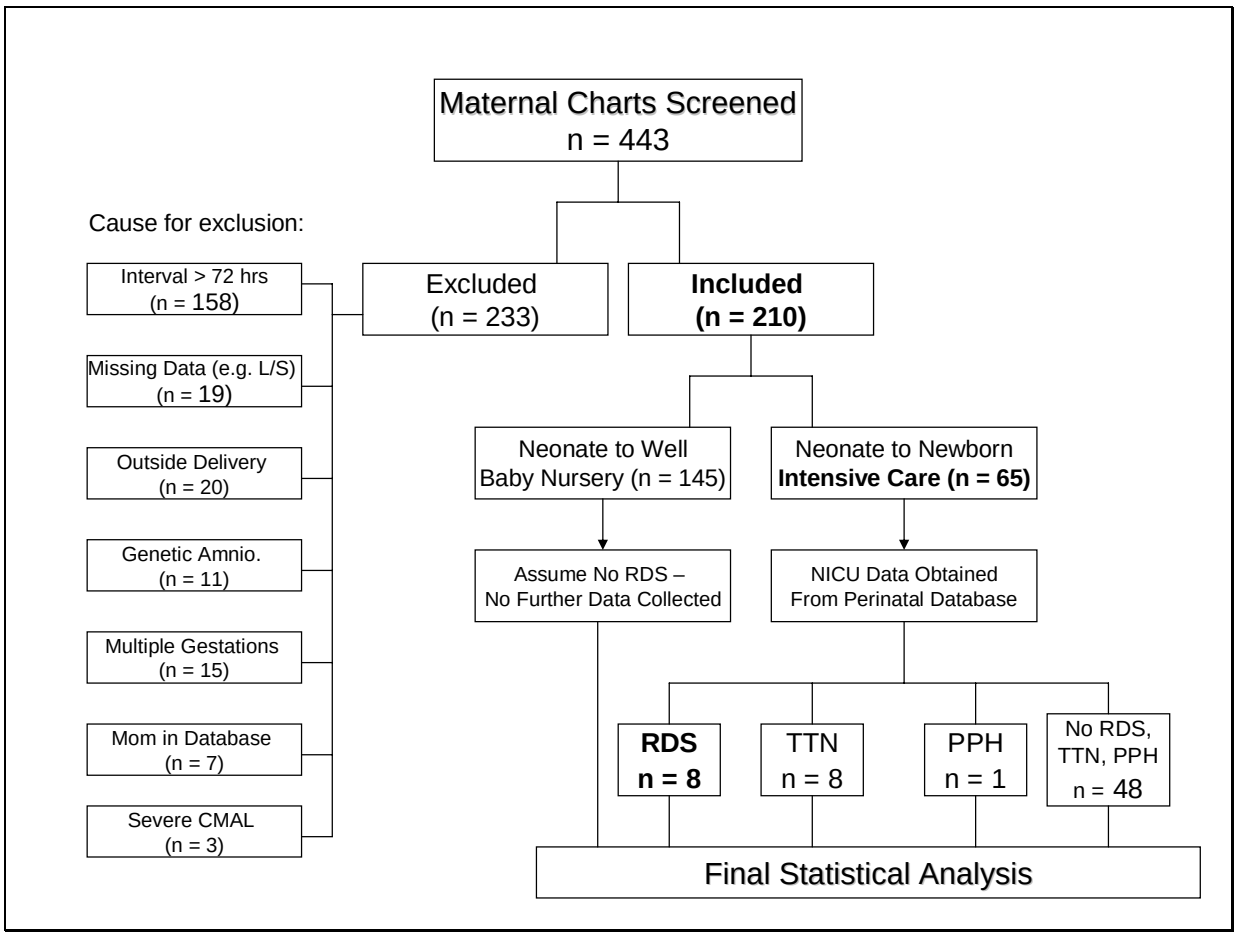


Figure 1: Study Population

Figure 2 displays the gestational age distribution of the patient population at the time of amniocentesis, which occurred within 72 hours of delivery. In the population without RDS (n = 202), the mean gestational age was 36.5 ± 1.2 weeks, with a median of 36.6 weeks (interquartile range: 35.9 - 37.3 weeks). Interquartile range represents the 25th through 75th percentiles. The lowest gestational age in the population without RDS was 32.9 weeks, while the highest was 39.1 weeks. In the population with RDS (n = 8), the mean gestational age was 32.6 ± 3.6 weeks, with a median of 33.1 weeks (interquartile range: 30.1 - 35.0 weeks). The lowest gestational age in the population with RDS was

26.0 weeks, while the highest in this population was 37.4 weeks. The difference in the mean gestational age between the two populations was statistically significant, $P = 0.02$.

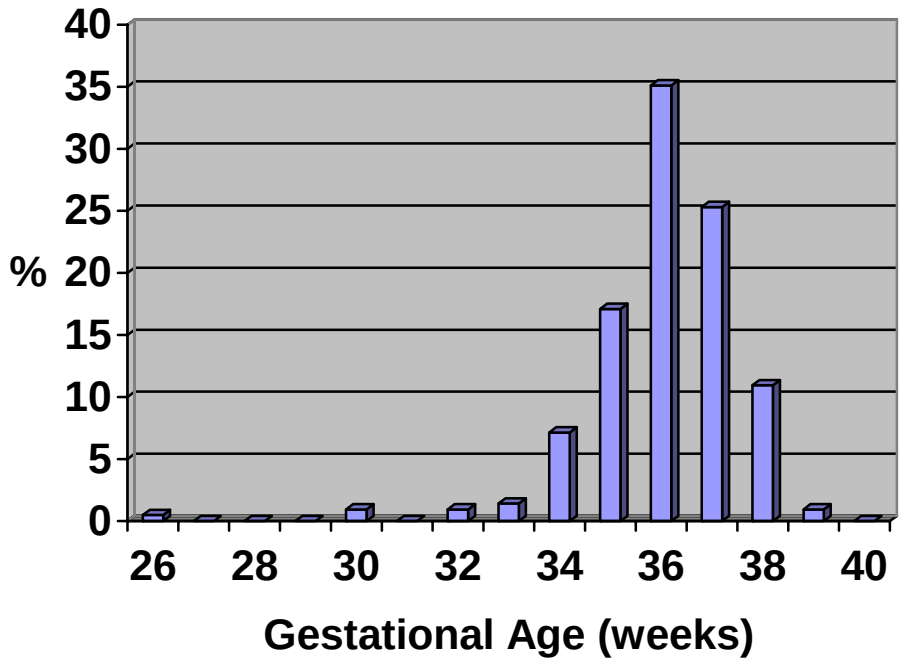


Figure #2: Gestational age distribution of the patient population. Data represent gestational age at amniocentesis for fetal lung maturity; all samples were obtained within 72 hours of delivery.

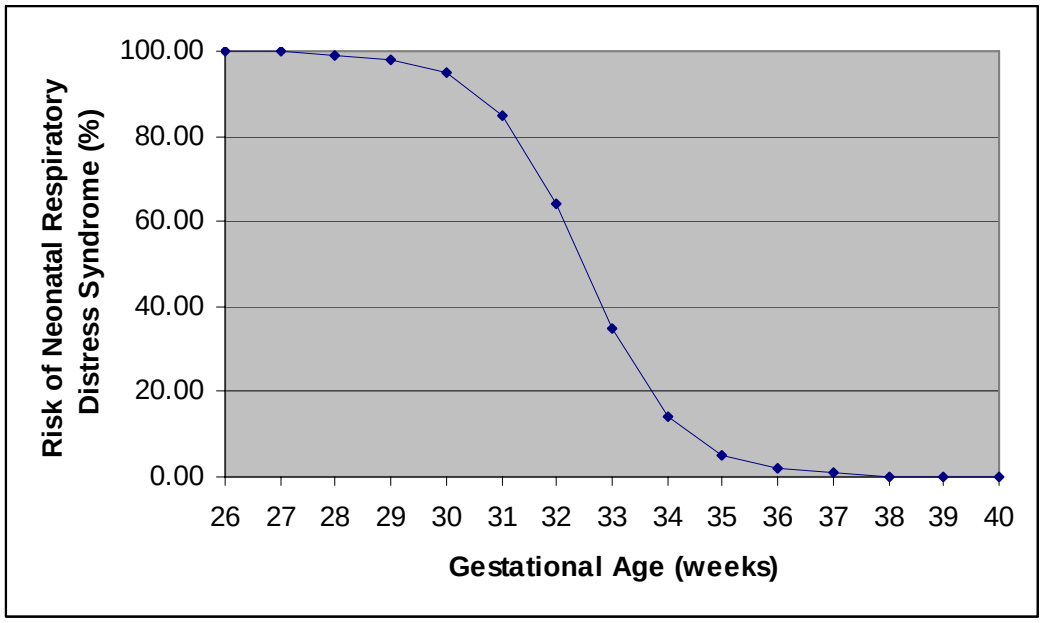
The incidence of RDS did decline with increasing gestational age. While the incidence in the total population was 3.8%, the incidence of RDS was 80% (4/5) for deliveries at 32 weeks or less of gestation, 2.4% (3/127) between 33 and 36 weeks of gestation, and 1.3% (1/78) for deliveries at or after 37 weeks of gestation. A univariate logistic regression equation was derived using this data, and the probability of neonatal RDS for each week of gestational age was determined. Equation 2 shows Equation 1 rearranged to solve for the probability of RDS:

(Eq. 1) $$\log_e\left(\frac{prob(RDS)}{1-prob(RDS)}\right) = 38.0220 - 1.1705(GA(wks))$$

(Eq. 2) $$prob(RDS) = \frac{e^{38.0220 - 1.1705(GA(wks))}}{1 + e^{38.0220 - 1.1705(GA(wks))}}$$

The results are shown in Figure 3a; similar studies of RDS and gestational age are plotted for comparison in Figure 3b. It should be noted, however, that these studies may plot incidence rather than probability.

a.



b.

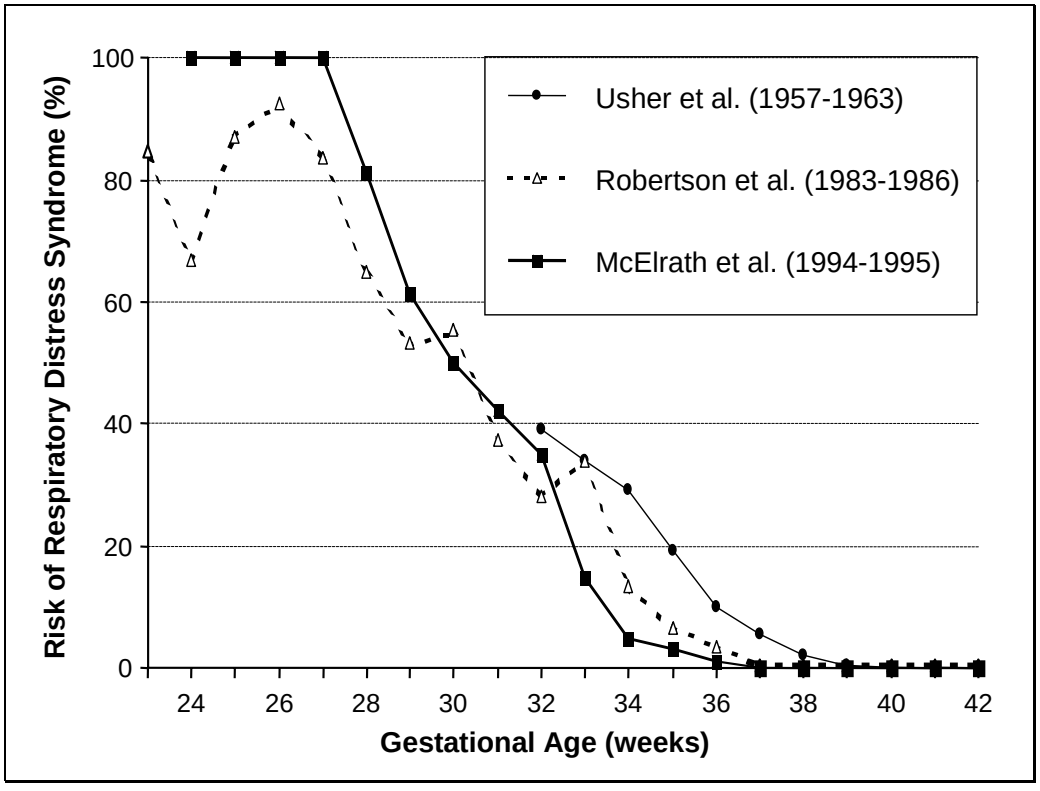


Figure 3: The risk of neonatal RDS as a function of gestational age in our population (a) compared to three previous studies (b).

In the population without RDS, the mean lecithin/sphingomyelin (L/S) ratio was 3.6 ± 1.1, with a median of 3.5 (interquartile range: 2.8 - 4.0). The lowest L/S value in the population without RDS was 1.4, while the highest was 8.5. In the population with RDS, the mean L/S ratio was 1.8 ± 0.7, with a median value of 1.8 (interquartile range: 1.2 - 2.3). The lowest L/S value in the population with RDS was 1.0, while the highest was 2.8. The difference in mean L/S ratio between the two populations was statistically significant, $P < 0.0001$

Table 1 presents the maternal clinical characteristics of the study population and their association with neonatal respiratory distress syndrome. Within the limits of our study population, the categorical clinical characteristics of race, tobacco use, diabetes status, and presence or absence of hypertension and preeclampsia did not differ among maternal-infant dyads who did and did not develop RDS.

Other variables were also studied. The mean maternal age for neonates without RDS was 31.0 years ± 6.0, while the mean maternal age for infants with RDS was 30.0 years ± 9.9; this was not a significant predictors of neonatal RDS, with $P = 0.78$. Only four cases of PPROM were documented in the study sample, one of those four cases resulting in a case of neonatal RDS; this was also found to be an insignificant predictor of RDS, with $P = 0.14$ by Fisher's exact test. Finally, neonatal sex was studied. There were an equal number of male and female infants in the study sample, with 105 each. Five females developed neonatal RDS, while three males developed the condition; this variable was also found not to be associated with RDS in our population, yielding a p-value of 0.72 by Fisher's exact test.

In our total study population of 210 mother-neonate pairs, 40 mothers (19.1%) received corticosteroids in the antenatal period while 170 (80.9%) did not. In the population without RDS (n = 202), 33 (16.3%) received steroids while 169 (83.7%) did not; in the population complicated by RDS (n = 8), seven (87.5%) received steroids while one (12.5%) did not. Alternatively, 17.5% (7 of 40) pregnancies in the study population exposed to antenatal steroids were diagnosed with neonatal RDS, compared with 0.59% (1 of 170) of those not exposed. This was a statistically significant result, with $P < 0.0001$ by Fisher's exact test. It is known, however, that administration of corticosteroids to women in premature labor (spontaneous or planned) has been shown to decrease the incidence of neonatal RDS, as well as intraventricular hemorrhage and necrotizing enterocolitis (*14, 55).* As administration of antenatal corticosteroids is only recommended for pregnancies threatening to deliver before 34 weeks of gestation (*2, 56),* and the average gestational age in our study population was 36 weeks, it is likely that antenatal corticosteroids in this study were a marker for early gestational age, and not that steroids increased the probability of developing neonatal RDS.

Table 1: Incidence of Neonatal RDS by Maternal & Neonatal Characteristics

Characteristic	Incidence		P value
	Subgroup without RDS (n = 202)	Subgroup with RDS (n = 8)	
Race			
White	118 (58.4)	3 (37.5)	0.23
Black	36 (17.8)	1 (12.5)	
Hispanic	26 (12.9)	3 (37.5)	
Asian/South Asian	7 (3.5)	0 (0)	
Other/Not documented	15 (7.4)	1 (12.5)	
Tobacco use			
Non-Smoker	173 (85.6)	8 (100)	0.60
Smoker	29 (14.4)	0 (0)	
Diabetes status			
No diabetes	135 (66.8)	8 (100)	0.23
Diet-controlled diabetes	20 (9.9)	0 (0)	
Insulin-requiring diabetes	47 (23.3)	0 (0)	
Hypertension			
No hypertension	176 (87.1)	8 (100)	0.60
Chronic hypertension	26 (12.9)	0 (0)	
Preeclampsia			
No preeclampsia	188 (93.1)	8 (100)	0.57
Preeclampsia	14 (6.9)	0 (0)	
Neonatal Sex			
Male	102 (50.5)	3 (37.5)	0.72
Female	100 (49.5)	5 (62.5)	
Antenatal Steroids			
Steroid Course Given	33 (16.3)	7 (87.5)	< **0.0001**
No steroids	169 (83.7)	1 (12.5)	
L/S Ratio*	3.6 (± 1.1)	1.8 (± 0.7)	< **0.0001**
Gestational Age*	36.5 (± 1.2)	32.6 (± 3.6)	< **0.0001**
Maternal Age*	31.0 (± 6.0)	30 (± 9.9)	0.78

RDS = respiratory distress syndrome, PG = phosphatidylglycerol band, L/S = lecithin/sphingomyelin
Values expressed as *n (%)*, except where *. * = values expressed as *avg (standard dev.)*
P-values calculated using Chi square or Fisher's exact test

Odds ratios (ORs) were calculated for the variables of interest in this study – gestational age and lecithin/sphingomyelin ratio – as well as other commonly cited predictors of neonatal RDS, and are shown in Table 2. Both gestational age and L/S ratio were significant predictors of RDS, with ORs of 0.40 (95% CI 0.21 - 0.75) and 0.08 (95% CI 0.02 - 0.42), respectively. When adjusted for gestational age, the L/S value remained significant with an OR of 0.16 (95% CI 0.03 - 0.86) and $P = 0.03$. As predicted, the OR for administration of antenatal steroids appeared significant (OR 21.5; 95% CI 2.43 - 190.3), $P = 0.005$; however, once adjusted for gestational age, steroids did not increase the risk of neonatal RDS (OR 6.83; 95% CI 0.64 - 72.5), $P = 0.11$. The presence of the phosphatidylglycerol (PG) band also predicted a decreased risk of neonatal RDS (OR 0.05; 95% CI 0.01 - 0.42) with $P = 0.006$, yet once adjusted for gestational age the PG band became insignificant in our population (OR 0.11; 95% CI 0.01 - 1.14), $P = 0.06$. Finally, ORs for African-American race, male sex of the neonate, and complication of PPROM were found not to predict neonatal RDS. While the data was inestimable for calculating true ORs for maternal tobacco use, preeclampsia, and insulin-requiring diabetes, the Chi-square and Fisher's exact tests reported above indicated that these variables were also not associated with RDS in our study sample, with p-values greater than 0.05.

Table 2: Odds Ratio for Logistic Regression Modeling of Neonatal Respiratory Distress Syndrome

	Odds Ratio (95% Confidence Interval)	*P*	Adjusted Odds Ratio£ (95% Confidence Interval)	*P*
Gestational Age (wk) †	0.40 (0.21 – 0.75)	* 0.005	----	
L/S Ratio †	0.08 (0.02 – 0.42)	* 0.003	0.16 (0.03 – 0.86)	* 0.03
PG Band	0.05 (0.01 – 0.42)	* 0.006	0.11 (0.01 – 1.14)	0.06
Antenatal Steroids	21.5 (2.43 – 190.3)	* 0.005	6.83 (0.64 – 72.5)	0.11
African American race¥	0.60 (0.07 – 5.37)	0.65	0.87 (0.08 – 9.45)	0.91
Male Infant	0.62 (0.14 – 2.85)	0.54	1.87 (0.25 – 13.8)	0.54
PPROM	3.93 (0.31 – 49.1)	0.29	0.45 (0.01 – 16.9)	0.67
Smoking	--- Inestimable ---			
Preeclampsia	--- Inestimable ---			
DM requiring insulin	--- Inestimable ---			

L/S = lecithin/sphingomyelin, PG = phosphatidylglycerol, GA = gestational age, DM = diabetes mellitus, PPROM = preterm premature rupture of membranes, * = statistical significance ($p < 0.05$), £ = adjusted for gestational age, ¥ = compared to all other races, †=change in risk for each 1 week increment in gestational age or 1 unit increase in L/S ratio.

The data relating gestational age and lecithin/sphingomyelin ratio – the two significant variables in our study – were utilized to derive a predictive equation showing the probability of neonatal respiratory distress syndrome. Equation 2 shows Equation 1 rearranged to solve for the probability of RDS:

(Eq. 1) $$\log_e\left(\frac{prob(RDS)}{1-prob(RDS)}\right) = 36.1943 - 2.0712(L/Sratio) - 0.9611(GA(wks))$$

(Eq. 2) $$prob(RDS) = \frac{e^{36.1943-2.0712(L/Sratio)-0.9611(GA(wks))}}{1+e^{36.1943-2.0712(L/Sratio)-0.9611(GA(wks))}}$$

The results are displayed in Table 3. At each week of gestation from 26 weeks, the L/S cutoffs are shown with a corresponding risk of neonatal RDS. Bolded values represent a probability of neonatal RDS approximating 0.100, or 10%.

Table 3: Predicted Probability of Neonatal Respiratory Distress Syndrome by Gestational Age and Lecithin/Sphingomyelin Ratio

	Lecithin/Sphingomyelin Ratio													
Gestational Age (wks)	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6
26	1.00	1.00	1.00	1.00	.999	.999	.999	.998	.997	.996	.993	.990	.985	.977
27	1.00	1.00	.999	.999	.999	.998	.997	.995	.992	.988	.983	.974	.961	.942
28	.999	.999	.998	.997	.996	.994	.991	.987	.980	.970	.956	.934	.904	.861
29	.998	.997	.996	.993	.990	.985	.977	.966	.950	.926	.892	.845	.783	.704
30	.995	.992	.989	.983	.974	.962	.943	.916	.878	.827	.759	.676	.579	.476
31	.987	.980	.971	.956	.935	.905	.863	.807	.734	.646	.547	.443	.345	.258
32	.967	.950	.927	.893	.847	.785	.700	.615	.513	.411	.316	.233	.168	**.117**
33	.917	.880	.829	.762	.679	.583	.480	.379	.288	.211	.150	**.104**	.071	.048
34	.809	.737	.650	.551	.447	.349	.261	.189	.134	**.093**	.063	.043	.029	.019
35	.619	.518	.415	.319	.236	.170	.119	**.082**	.056	.038	.025	.017	.011	.007
36	.383	.291	.213	.152	**.106**	.073	.049	.033	.022	.015	.010	.006	.004	.003
37	.192	.136	**.094**	.064	.043	.029	.019	.013	.009	.006	.004	.002	.002	.001
38	**.083**	.057	.038	.026	.017	.011	.008	.005	.003	.002	.001	.001	.001	<.001
39	.034	.022	.015	.010	.007	.004	.003	.002	.001	.001	.001	<.001	<.001	<.001
40	.013	.009	.006	.004	.003	.002	.001	.001	<.001	<.001	<.001	<.001	<.001	<.001

Values are expressed as odds ratios.
Bolded values represent risk cutoff of approximately 0.100, or 10%

Discussion

The timing of a delivery remains a difficult and highly debated topic within the field of obstetrics. Clinical providers must weigh risks and benefits to both mother and baby when faced with the possibility of a preterm delivery. Given what is known about fetal pulmonary development, underdeveloped lungs and respiratory complications including neonatal RDS are some of the most significant risks of prematurity. Data showing that the incidence of respiratory distress syndrome declines significantly as mothers approach full term encourages obstetricians to prolong gestation, avoid early elective deliveries, and tocolyze in cases of preterm labor long enough to administer a course of antenatal steroids. However in some cases – when there is danger to the mother or child – delivery is the safest option. Clinical judgment remains the critical factor in assessing these situations case by case; amniotic fluid analysis for fetal lung maturity can aid these decisions when used properly.

Since its development in the early 1970's, the lecithin/sphingomyelin ratio has been used to help predict fetal lung maturity. Institutions generally use a threshold for maturity of 2.0 or 2.5; this threshold has not changed appreciably over the years. What complicates the use of such a cutoff is the fact that the incidence of RDS decreases with increasing gestational age, which our data supports. When the incidence of a disease changes over a short time course, e.g. toward the end of gestation, a screening test with one defined cutoff value is bound to have significant false-positives and false-negatives and variable predictive power (*57)*. Fetal lung maturity studies including the L/S ratio generally perform well for ruling out RDS (predicting absence of disease), while falling short in terms of ruling in RDS (predicting presence of disease). Still studies have shown

that neonatal morbidity can occur despite “mature” lung indices (*37, 38)*, reminding us of the need for improvement in the interpretation of these tests.

Gestational age-specific probabilities of RDS have recently been determined for the TDx-FLM II surfactant-to-albumin ratio assay (*39-41)*. Our analysis offers a similar gestational age-specific risk of RDS for the lecithin/sphingomyelin ratio, the first-line test in many institutions. By modeling the odds of RDS using multivariate logistic regression based on our data, a probability of RDS around 10% was found at an L/S value of 3.6 at 32 weeks, 2.8 at 34 weeks, 1.8 at 36 weeks, and 1.4 at term (37 weeks or greater). This shows that in a frequently critical window for decision making regarding preterm delivery, 32 to 37 weeks of gestation, the L/S value conferring the same probability of RDS ranges from 3.6 down to 1.4; this significant variation supports the need for an algorithm like this one, as opposed to a single cutoff of 2.0.

Above 38 weeks, an L/S ratio as low as 1.0 would still confer less than 10% probability of RDS, although such a result would be uncommon. This reflects the finding that neonatal respiratory distress is uncommon at such a late gestational age, and therefore risk is low regardless of the L/S value. At the other end of the spectrum in cases of very early gestation, a probability of RDS as low as 10% is not yielded by our algorithm. Even at 31 weeks, an L/S ratio of 3.6 yields a probability of RDS approximating 25% with our model. The lowest gestational age at which we have data, 26 weeks, yields a probability of RDS of 97-99% irrespective of the L/S ratio; again this reflects the trend that neonatal respiratory distress is a disease of underdevelopment with higher rates of incidence at low gestational age. Based on our table, assessing fetal lung

maturity with the L/S ratio before 28-29 weeks may have limited utility, as the likelihood of acquiring a reassuring test result would be low.

The main strength of our study was also somewhat of a limitation. Requiring that only mother-neonate pairs delivering within 72 hours of amniocentesis for fetal lung maturity ensured that the L/S ratio and the neonatal outcome were temporally related, and that information could be gleaned from the assay as a predictor for fetal pulmonary status. However, a test-to-delivery interval greater than 72 hours also turned out to be our most common cause for exclusion, ruling out 158 mothers from inclusion in the study and limiting our total number of cases. The most significant weakness in the study was the low number of neonates with respiratory distress syndrome in our study population. With only 8 cases in our sample, the confidence intervals surrounding our predictions for probability of RDS in Table 3 are wide. Thus more data may be needed before these values are used to guide clinical practice and decision making regarding timing of delivery. The precision of the regression model would be improved with additional data to increase the number of cases of RDS.

Nevertheless, our results show that both the lecithin/sphingomyelin ratio and gestational age were significant predictors of neonatal respiratory distress in our population, and that the L/S ratio remained a significant predictor when adjusted for gestational age. Furthermore the trend according to the logistic regression analysis displayed in Table 3 is clear. The L/S cutoff for a probability of RDS around 10% ranges from 1.4 to 3.6 between 32 and 37 weeks of gestation. The probability of RDS is not accurately represented by a single threshold of 2.0, but instead can be more accurately determined by a moving threshold based on the gestational age.

Aside from gestational age and the L/S ratio, several maternal-fetal characteristics may influence the risk of a given fetus developing RDS; these include maternal race (*58),* antenatal tobacco use (*59),* preeclampsia (*60),* and diabetes mellitus (*61),* among others. These variations in the host environment may affect fetal cortisol levels, impacting the progression of fetal lung maturation (*12).* In this study, we included data on maternal age, maternal race, tobacco use, presence or absence of preeclampsia, chronic hypertension, PPROM, diabetes, and neonatal sex as possible characteristics that may affect the prediction of RDS. Despite the evidence that suggests that these factors do modify the risk of neonatal respiratory distress, this did not prove to be the case in our study population. This may be partially due to the small number of cases of RDS, which make it difficult to establish definitive trends and variations. However it is also likely that the effects of these characteristics factor less significantly into the overall prediction of lung maturity when compared to gestational age and fetal lung maturity assays.

As discussed briefly in the results, the administration of antenatal steroids was the only other significant predictor of RDS in our study; however once adjusted for gestational age, these results were no longer statistically significant. This is likely due to the fact that the average gestational age in our sample was 36 weeks, whereas evidence for antenatal corticosteroid therapy has been demonstrated in pregnancies less than 34 weeks of gestation (*2, 56).* Steroid administration in our sample was more of an indicator of early gestation, and not an independent predictor of neonatal respiratory distress. Additional data with a broader range of gestational ages would allow further analysis regarding the role of antenatal steroids in the prediction of neonatal RDS using our prediction equation.

In conclusion, this analysis offers a gestational age- and test- specific algorithm for determining the probability of neonatal RDS throughout pregnancy. While additional data is needed to increase the precision of the regression model, these initial results show a clear trend in the gestational age-dependent L/S cutoffs when predicting neonatal respiratory distress. We hope that our data will contribute to the growing body of evidence that prediction of fetal lung maturity is not a dichotomous endeavor, but rather the evaluation of a continuum of risk dependent on gestational age, biochemical assays including the L/S ratio, and clinical factors. As has always been the case, risks and benefits must be weighed. For conditions placing the mother or fetus at risk, such as severe preeclampsia or chorioamnionitis, immediate delivery may be indicated regardless of gestational age or lecithin/sphingomyelin ratio. However in cases where urgency is less of an issue, an accurate assessment of fetal pulmonary status and probability of RDS will likely aid clinical decision making and improve neonatal outcomes.

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