ABSTRACT

Objective: To assess the accuracy, sensitivity, specificity, and the positive and negative predictive values of amniotic fluid fluorescence polarization for neonatal respiratory distress syndrome in high risk pregnancies. Methods: A prospective descriptive study of 54 patients with high risk pregnancies. Fetal lung maturity was assessed using amniotic fluid fluorescence polarization obtained by amniocentesis up to 72 hours before delivery. Respiratory distress syndrome, stratified by gestational age at birth, was the primary outcome analyzed. Amniotic fluid fluorescence polarization values equal to or over 50 mg/g (indicating fetal lung maturity) were considered as negative results. Results: The mean gestational age at birth was 35 weeks (SD 2.0). Respiratory distress syndrome was seen in 14 newborns (24%). Amniotic fluid fluorescence polarization had high sensitivity (86%) and specificity (81%), with 14% false-negative and 19% false-positive results. The positive predictive value was 60% and the negative predictive value was 94%. The area under the ROC curve indicated the 50 mg/g albumin/surfactant ratio as the best cutoff point (85% sensitivity and 81% specificity). Conclusion: A negative value in amniotic fluid fluorescence polarization (results equal to or over 50 mg/g) confirms lung maturity which translates into a very low risk of a newborn developing respiratory distress syndrome in high risk pregnancies.

Keywords: Fetal organ maturity; Amniocentesis; Respiratory distress syndrome; Fluorescence polarization; High risk pregnancy

INTRODUCTION

Respiratory distress syndrome (RDS) is still the main cause of mortality in premature newborns (NB). Establishing fetal lung maturity is, therefore, a constant challenge for obstetricians.
Maternal conditions usually associated with premature birth are premature rupture of membranes and premature labor, which are considered as causes of “spontaneous” or unavoidable prematurity\(^{(1)}\). There are, however, situations in which preterm delivery is elective or programmed for maternal and neonatal benefits, such as when there are complications resulting from hypertension, diabetes, Rh isoimmunization, placental insufficiency or restricted fetal growth\(^{(2)}\). In this context, knowledge of fetal lung maturity is extremely important, allowing the obstetrician to choose the best moment for delivery.

The aim of most fetal maturity tests is to assess the risk of RDS. The most effective methods use amniotic fluid (AF) samples usually obtained by amniocentesis for specific analyses of fetal lung surfactant components, given that RDS develops when the surfactant is altered or absent in the fetal lung.

The most frequently used direct methods to study fetal lung maturity include analysis of the lecithin/sphingomyelin ratio (L/S ratio), phosphatidyl-glicerol (PG) dosage, the percentage of desaturated phosphatidylcholine (DDPC) and uni-or bidimensional phosphatidylinositol (PI) chromatography\(^{(3)}\). Chromatographic methods, however, cannot be readily adapted to many hospitals, laboratories or clinical contexts. Lipid extraction and interpretation in silica-gel thin plates requires time, expert personnel and sophisticated procedures. The cost for services to provide full time exams and delays in delivering results also complicate the application of such methods to most cases that require prompt and crucial clinical decisions.

Fluorescence polarization (TDx-FLM) is a test that assesses fetal lung maturity based on the surfactant and albumin AF concentration. It is based on the observation that AF protein concentration remains constant in the last months of pregnancy, followed by a progressive rise in surfactant levels\(^{(4)}\). In this procedure a fluorescent reagent is added to a sample of AF and is distributed between albumin and the surfactant. Total fluorescence polarization in each sample reflects the distribution of the stain between the protein and the surfactant and is a form to establish the surfactant/albumin ratio in the liquid. By creating a window, polarization specifies an orientation for light, which then vibrates in a single plane. The albumin polarization level is high, for the molecule is large and moves little. The surfactant polarization level is low because the molecule is small and moves rapidly. Therefore, an increased concentration of surfactant in the AF leads to increased depolarization and lower polarization.

A polarimeter (TDx-FLM analyzer), easily found in many laboratories and commonly used to monitor therapeutic levels of different drugs, is needed for this test. The method is easy to perform and provides results in approximately 40 minutes, although the price is relatively high. The TDx-FLM assay was first studied by Shinitzky et al.\(^{(5)}\), who considered the total surfactant composition regardless of lipid concentration in the AF. Steinfeld et al.\(^{(6)}\) compared the L/S ratio and PG levels in the AF with the TDx-FLM assay. They found an excellent correlation between and L/S. The negative predictive value was 100%, considering a cutoff point of 50mg/g or higher. Herbert et al.\(^{(4)}\) suggested the TDx-FLM assay as a first screening test to assess fetal maturity, given its reliability, ease and rapidity. They considered a cutoff point of 30mg/g to indicative maturity without reducing the test sensitivity. Many papers were published as experience accumulated on the efficacy of the TDx-FLM assay in a variety of conditions, particularly in diabetes mellitus during pregnancy.

**OBJECTIVE**

This study aims to assess fetal pulmonary maturity using the AF TDx-FLM assay in relation to the rate of RDS in high risk pregnancies.

**METHODS**

The study population included 54 patients admitted into hospital for delivery, during twenty consecutive months. Pregnant and/or parturient patients with gestational age between 29 and 40 weeks and an interval between AF collection and delivery of less than 72 hours. Exclusion criteria were blood or meconium contaminated AF samples, Rh factor isoimmunized pregnant women or patients that had corticotherapy after AF sample collection. Other exclusion criteria were NB with congenital malformations that involved the central nervous, respiratory and/or cardiac systems, and NB with severe asphyxia and/or neonatal sepsis.

All candidate patients were consulted and authorized AF study, signing the free and informed consent form previously approved by the Research Ethics Committee of the Universidade Federal de Sao Paulo (UNIFESP). AF was obtained by ultrasound guided amniocentesis. AF was obtained after hysterotomy and prior to membrane rupture in patients undergoing cesarean section. For women in labor with unruptured membranes, progressing to normal delivery with at least 6cm cervical dilation, a vaginal speculum was placed for needle aspiration of AF under direct view. Five to ten milliliters of AF were obtained in all cases using a plastic 20ml syringe and a 30x8mm needle. Samples were obtained and analyzed within not more...
than 2 hours from collection, without using frozen liquid. The AF TDx-FLM assay was done with a TDx – Abbott Laboratories fluorescent analyzer, using the TDx-FLM assay technique. This method finds the relative quantity of phospholipid surfactants in the AF by measuring fluorescence distributed between the surfactant (low polarization) and albumin (high polarization). Results were expressed in surfactant milligrams per gram of albumin.

The procedure required filtering 1 to 3 ml of AF not contaminated with blood, meconium or bilirubin. The technique consisted of placing AF in a syringe coupled to a device containing a fiberglass filter. A 0.5 ml filtered specimen, was pipetted into a disposable plastic cubette and placed in the analyzer carousel together with the fluorescent reagent PC16, control cartridges, and the TDx buffer solution. After incubation for 7 minutes at 34°C, the device reads the intensity of fluorescence and polarization of 0.3 ml of AF and 0.6 ml of the buffer solution. The TDx adds 0.025 ml of PC16, 0.15 ml of the sample and 0.275 ml of the buffer to reach a final concentration of PC16 at 1.2 mmol/L, in 1.35ml of solution. The final fluorescence reading was made after a 15-minute incubation. Rather than reporting the polarization result immediately, the system is calibrated using six phospholipid/albumin solutions from 0 to 160 mg/g that produce a non-linear equivalence curve. The TDx-FLM assay uses calibrators and controls. The calibrators comprise six 4.5 ml flasks containing an aqueous solution of surfactant and albumin, with precisely known ratio values expressed in milligram of surfactant per gram of albumin. Flasks are identified as: A, B, C, D, E and F, respectively containing 0.0, 10.0, 20.0, 40.0, 80.0 and 160.0 mg/g. The device was calibrated previously with these solutions. Controls were placed in six flasks and divided two by two with the following values: low, medium and high quantity of a solution containing known amounts of surfactant/albumin, also expressed in milligrams per gram and classified as follows: low quantity flask – 12.00 to 18.00; medium quantity flask – 45.00 to 55.00; high quantity flask – 85.00 to 115.00. These controls were placed together with the AF in the carousel and served to obtain readings to verify calibration. If the system was not calibrated, controls stopped the reading and gave an error message. The full procedure, from sample preparation to reading the final results, took about 40 minutes.

Positive (immature) final polarization results were values equal to or lower than 49 mg of surfactant per gram of albumin. The result indicating maturity was defined as final polarization values over 50 mg of surfactant per gram of albumin.

Gestational age was defined based on a reliable menstrual period history, associated with obstetric ultrasound particularly for examinations made until 12 weeks from the last menstrual period for confirmation. In all cases the Capurro et al. (7) postnatal method was used; this last method was used to define gestational age if there was any disagreement between the first two methods.

Severe neonatal asphyxia was defined by the simultaneous occurrence of an Apgar score (8) below 3 in first and fifth minutes of postnatal life. RDS was defined by the need to give oxygen following birth for at least 24 h. Oxygen therapy was given by continuous positive airway pressure (CPAP) with mechanical ventilation following orotracheal intubation or using humidified and warmed oxygen at an inspired fraction (FiO2) of at least 30%. The typical clinical presentation of RDS included early respiratory failure, usually during the first minutes after birth, increased respiratory frequency (over 60 breaths per minute), and intercostal and xiphoid retraction. Other findings were: well-established dyspnea after four to six hours of life, nasal flaring, chin movement at each inspiration, in some cases expiratory grunts due to respiratory effort, inspiration generally faster than expiration and varied degrees of cyanosis.

All NB with RDS underwent chest X-rays. A reticular-granular aspect and the presence of air bronchograms established the diagnosis. Treatment of RDS aimed to keep PaO2 between 50 and 70 mmHg and PaCO2 between 35 and 45 mmHg.

The TDx-FLM assay results were analyzed according to accuracy, sensitivity, specificity, and positive and negative predictive values for RDS. Results were also analyzed in three gestational age groups (NB up to 34 weeks; NB between 34 weeks and one day less than 36 weeks and six days; and NB with 37 weeks or more). Finally, different cutoff points were identified with the method to establish the value of greater accuracy (highest sensitivity and specificity simultaneously). The sensitivity of a method is defined as the percentage of individuals with the disease (NB with RDS) with a positive test for the condition (“immature” result). Specificity is the percentage of individuals without the disease (NB without RDS) that have a negative test for the condition (“mature” results). The positive predictive value assesses the probability of disease (RDS) in cases where the test is positive (“immature” result). The negative predictive value assesses the probability of absence of RDS in cases where the test is negative (“mature” result). We used the ROC (receiver operator characteristic) curve to find the best cutoff point, since the results are given in a continuous interval scale. This curve is used to describe the accuracy of a test for a range of cutoff points and can be used...
as a nomogram to identify a specificity that matches a given sensitivity; it may be used to decide the best cutoff point (simultaneous highest sensitivity and specificity).

The quantitative variables were the average, standard deviation, and maximum and minimum values and the qualitative variables were absolute (n) and relative (%) frequencies. Agreement between RDS and positive tests was assessed by accuracy, sensitivity, specificity and positive and negative predictive values.

RESULTS
The prospective study to assess fetal lung maturity using fluorescence polarization initially included 56 patients, with two exclusions due to AF contamination with blood. Nine pregnant patients had multiple gestations. AF samples were obtained of both fetuses in only two of these cases. In six others, AF was obtained from only one fetus. In the remaining case of multiple pregnancy, there were three fetuses, and AF was obtained from only two, as the third fetus did not survive. The final number of AF samples for TDx-FLM assay analysis was 57, matching the number of NB.

Maternal characteristics – The average maternal age was 29 ± 6 years, ranging from 19 to 41 years; most were Caucasian (92%). As to parity, 52% were primigravida, 33% were gravida 2 and 15% were gravida 3 or more. Cesarean section was performed in 93% of cases. Indications for cesarean sections are shown on Table 1. Conduction anesthesia was given to 98% of patients as follows: 89% received rachianesthesia (48 cases), and 9% epidural blockade (5 patients).

General anesthesia was used in one case (2%).

Perinatal characteristics – Fifty-seven NB with a mean gestational age of 35 weeks and 6 days, standard deviation of 2 weeks (range 29 weeks – 39 weeks and 3 days) were assessed. Twelve fetuses (21%) were delivered at a gestational age of 34 weeks or less; 23 (40%) had a gestational age between 34 weeks and one day and 36 weeks and six days; the remaining 22 NB (39%) were delivered at gestational age of 37 weeks or over. There were more female NB (58%) compared to males (42%). The mean birth weight was 2633 ± 778 g, ranging from 730 g to 4020 g. The mean duration of hospital stay was 10 days ± 20 days, ranging from 2 to 147 days. The mean 1 minute Apgar score was 8 ± 0.7, ranging from 6 to 10. The mean 5 minute Apgar score was 9 ± 0.6, ranging from 8 to 10. NB morbidity included 11 NB (19%) with hypoglycemia and 7 NB with jaundice (12%). Respiratory morbidity, of special interest in this study, included RDS in 14 NB (24%).

Results of gestational age on the TDx-FLM assay – The AF maturity tests showed that the TDx-FLM assay mean was 59.58 ± 30.24 mg/g, ranging from 7.80 to 160.00 mg/g. Twenty NB (35%) had a positive test indicating lung immaturity (TDx-FLM < 50 mg/g). Table 2 shows the agreement between AF test results and RDS. Analysis revealed that the TDx-FLM assay is very sensitive and specific to predict RDS. By and large, the agreement was good, with 19% false-positive and 14% false-negative results. Figure 1 shows TDx-FLM assay sensitivity and specificity values.

Table 1. Indications of cesarean sections in 50 pregnancies submitted to assessment of fetal lung maturity

<table>
<thead>
<tr>
<th>INDICATION</th>
<th>N (%)</th>
</tr>
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<tbody>
<tr>
<td>Premature rupture of membranes</td>
<td>4 (8.00)</td>
</tr>
<tr>
<td>Previous cesarean section</td>
<td>12 (24.00)</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>9 (18.00)</td>
</tr>
<tr>
<td>Hypertensive syndrome</td>
<td>9 (18.00)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (4.00)</td>
</tr>
<tr>
<td>Fetal distress</td>
<td>6 (12.00)</td>
</tr>
<tr>
<td>Functional dystocia</td>
<td>3 (6.00)</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>1 (2.00)</td>
</tr>
<tr>
<td>Elective</td>
<td>4 (8.00)</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100.00)</td>
</tr>
</tbody>
</table>

Table 2. Accuracy (A), Sensibility (S), Specificity (Sp) and Positive (PPV) and Negative (NPV) Predictive Values of Fluorescence Polarization of amniotic fluid as to presence or absence of neonatal respiratory distress syndrome (RDS)

<table>
<thead>
<tr>
<th>RDS</th>
<th>TDX-FLM</th>
<th></th>
<th></th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>Positive</td>
<td>12 (85.71%)</td>
<td>8 (18.60%)</td>
<td>20 (35.09%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2 (14.29%)</td>
<td>35 (81.40%)</td>
<td>37 (64.91%)</td>
</tr>
<tr>
<td>Absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>14 (100.00%)</td>
<td>43 (100.00%)</td>
<td>57 (100.00%)</td>
</tr>
</tbody>
</table>

A = (12 + 35)/57 = 82%
S = 12/14 = 86%
Sp = 35/43 = 81%
PPV = 12/20 = 60%
NPV = 35/37 = 94%

Figure 1. Sensibility (S) and Specificity (Sp) of Fluorescence Polarization (TDX-FLM) in the amniotic fluid as to occurrence of neonatal respiratory distress syndrome (RDS)

The agreement between AF tests and RDS in NB up to 34 weeks gestational age (table 3) was low; in that, the TDx-FLM assay was very sensitive but not very specific to predict RDS. There were many false-positive results (43%), but no false-negative results (0%).
Fluorescence polarization of amniotic fluid to assess fetal lung maturity in high risk pregnancies

Table 6 shows the proportion of true and false-positive results for different cutoff points proposed for the AF fluorescence polarization test. Figure 2 shows the ROC curve for AF fluorescence polarization, showing graphically the data presented on Table 6.
DISCUSSION
Assessing fetal lung maturity by AF tests is not a relevant matter for most pregnancies. In high risk pregnancies, however, this procedure becomes an important strategy to achieve improved perinatal results. The efficiency of a test in predicting maturity depends essentially on the expected neonatal RDS risk for the gestational age and the associated maternal risk. The clinician must decide the moment when the risk of lung immaturity is low enough to assure a therapeutic delivery, even if preterm. Lung maturity assessment tests are also relevant in other clinical contexts. Lung maturity should be established before cesarean sections or elective birth induction in patients with imprecise clinical estimates. Investigation of maturity also has a role in premature labor, as the use of uterolytic agents may successfully delay birth by up to 72 hours, allowing pharmacological acceleration of fetal lung maturity with corticosteroids. The use of uterolytic agents, however, may bring undesirable side effects for the mother. If a quick laboratory test indicates maturity, it may provide the option of allowing progression of delivery with no use of uterolytic agents.

On the other hand, lung maturity investigation may be discussed when mothers may benefit from anticipated delivery. Assessment of fetal well-being by ultrasound and the improved capability of treating premature NB in neonatal ICUs and nurseries – increasing survival and progressively improving the limits of theoretical viability – underline this issue and encourage the assisting physician to take more active measures even when not directly assessing fetal lung maturity. However, it is important to emphasize that prematurity is not only extremely costly but also increases the risks of mortality, morbidity and sequelae that may compromise the quality of life of patients and families.

The chromatographic methods to assess fetal lung maturity introduced during the 1970s(9-12) are the accepted clinical tests and the gold standard. The methodology, however, requires specialized equipment, a trained team, adequate facilities and a test time ranging from 4 to 24 hours. The cost for full-time availability of this test is prohibitive in most laboratories and hospitals, meaning that clinicians frequently find themselves without this information. There have been regular attempts at finding an efficient, fast, low cost and reproducible test that correlates well with chromatographic methods, particularly the L/S ratio. The choice of the TDx-FLM assay as a test in our study is based on the fact that it is fast, easy, readily accessible in most laboratories and hospitals (it does not use specific equipment), and provides similar or even superior results compared to chromatographic methods(15).

The results showed that fluorescence polarization had a high negative predictive value and variable false-positive rates (between 20 and 30%), similar to values seen in literature.(14-16) Sensitivity is proportional to negative predictive value, demonstrating the capability of this test to predict the absence of RDS. The accuracy (the proportion of all positive and negative correct results) of the TDx-FLM assay was 82%. The positive predictive value was relatively low (60%) revealing the recognized difficulty for any fetal lung maturity test to identify NB that will truly develop RDS following a positive (immature) result. The analysis of results in the three gestational age groups did not allow final conclusions due to the small number of our samples in each age group. However, the test performed well in gestational ages under 34 weeks, which includes NB most susceptible to present complications due to prematurity, particularly respiratory morbidity. The results of the TDx-FLM assay in this study were similar to those found in literature, specifically with trials using the same maturity cutoff point (50 mg/g). Herbert et al. (4) found 100% sensitivity and 39% false-positive results. Hagen et al. (13) demonstrated 89% sensitivity and 26% false-positive results. In our study we found 86% sensitivity and 19% false-positive results, with an 82% accuracy, which we considered extremely satisfactory.

The analysis of different cutoff points for sensitivity and specificity in the ROC curve suggested 50 mg/g as the point of greatest accuracy for fluorescence polarization, which agrees with the manufacturer’s recommendation and with most studies we reviewed. Our results allow us to conclude that the AF TDx-FLM assay has good accuracy, high sensitivity and high negative predictive values, which may be useful to assess fetal lung maturity in high risk pregnancies.

CONCLUSION
The AF TDx-FLM assay is an efficient method to assess fetal lung maturity in high risk pregnancies, identifying normal NB who will not develop RDS with greater precision than finding premature NB more likely to develop this condition. The optimal cutoff point for fluorescence polarization in our study was 50 mg/g, which agrees with the manufacturer’s recommendation.

REFERENCES
Fluorescence polarization of amniotic fluid to assess fetal lung maturity in high risk pregnancies


