Assessment of the Diagnostic Accuracy of the TDx-FLM II to Predict Fetal Lung Maturity

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Background: Because respiratory distress syndrome (RDS) affects 1% of live births, accurate and rapid assessment of markers of fetal lung maturity is critical to clinicians in deciding whether to deliver a preterm infant. Our objective was to determine the optimal diagnostic cutoff value for the TDx-FLM II assay (Abbott Laboratories) for predicting clinically significant RDS.

Methods: Amniotic fluid TDx-FLM II data were collected retrospectively over 4 years. Women were included in the study if they had delivered within 72 h of TDx-FLM II testing and both the mother and infant charts could be reviewed. Women who had been treated with steroids and delivered unaffected infants were excluded from the analysis. The diagnosis of RDS was defined as infants who either were treated with surfactant and/or were placed on a ventilator and/or required continuous positive airway pressure for >1 day.

Results: A total of 185 women met all entry criteria (15 RDS, 170 non-RDS). A cutoff value for a mature result of ≥45 mg/g gave a sensitivity of 100% (95% confidence interval, 82–100%) and a specificity of 90% (95% confidence interval, 78–89%).

Conclusions: The TDx-FLM II appears to predict clinically significant RDS when a cutoff of ≥45 mg/g is used for mature results. Further studies will be required to confirm these findings.

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Respiratory distress syndrome (RDS)5 is a major cause of death in the newborn. In 1998, RDS ranked fourth among leading causes of infant deaths, with 1328 deaths from RDS in the United States (1). RDS, also referred to as hyaline membrane disease, is associated with insufficient surfactant production by the neonatal lung. Laboratory assessment of fetal lung maturity (FLM) before delivery enables clinicians to either delay delivery and administer steroids to hasten fetal lung development or to elect to deliver a preterm infant whose lungs are mature.

The biochemical markers for assessment of FLM are the lecithin-to-sphingomyelin ratio (L/S) and phosphatidylglycerol (PG) in amniotic fluid, determined by thin-layer chromatography (2–7). Chromatography, although considered the gold standard, is technically difficult and time-consuming. Alternative, more rapid assays have emerged, including foam stability, lamellar body count, PG agglutination, and fluorescence polarization. The foam stability index test is fast, easy to perform, inexpensive, and sensitive when compared with L/S and PG, but it is less specific, and there are differences in operator interpretation of results (8, 9). Similarly, PG agglutination is also fast and easy to perform, but there is inconsistency in test interpretation (3, 6). The lamellar body count test takes advantage of the size similarities between platelets and the lamellar bodies in the amniotic fluid and allows for counting of lamellar bodies on automated cell counters. For this reason, this assay is widely available, requires a low sample volume, and is fast, easy, and...
inexpensive to perform. However, there are no standardized protocols, quality-control material, or well-established cutoffs for this assay (10–13). The TDx-FLM is an automated fluorescence polarization assay that measures mg surfactant/g of albumin in amniotic fluid and reports quantitative results (14–18).

Studies evaluating the first-generation TDx-FLM I assay indicated that the ≥70 mg/g cutoff for a mature result suggested by the manufacturer was conservative and could be lowered without compromising clinical sensitivity (14, 15, 19, 20). The TDx-FLM II is a second-generation fluorescence polarization assay in which the manufacturer’s cutoffs have been set as follows: mature ≥55 mg/g, intermediate 40–54 mg/g, and immature ≤39 mg/g. Preliminary studies examining the TDx-FLM II assay have indicated that the manufacturer’s cutoff for this assay may also be set conservatively (21–23). For this reason, we initiated a collaborative evaluation of the TDx-FLM II assay to determine a cutoff that would not miss prediction of clinically significant RDS. This is the largest study of its kind to date.

**Materials and Methods**

This study was a collaborative, retrospective cohort study in which a consecutive series of physician-ordered TDx-FLM II (Abbott Laboratories) samples, collected during a 4-year period, were examined. Data were collected from the charts of patients who had TDx-FLM II analysis performed at either Barnes-Jewish Hospital (St. Louis, MO) during 1998–2000 or Hennepin County Medical Center (Minneapolis, MN) during 1998. During this time, 1104 physician-ordered FLM tests were performed on 881 different patients. FLM testing was ordered on women presenting with signs and symptoms of preterm labor as well as asymptomatic women before scheduled cesarean sections.

Inclusion in the study required that delivery occurred within 72 h of amniocentesis and that charts for both mother and infant were reviewable (see below). Women who received steroids before delivery and delivered non-RDS infants were excluded from the study because it is impossible to determine whether the steroids impacted the respiratory status of the infant. Women who received steroids before delivery and delivered RDS infants were included in the study because it is clear that the steroids did not impact the presence of RDS. Indeed, steroids may have changed the severity of RDS, but data were analyzed only for the presence or absence of RDS.

Barnes-Jewish Hospital performs FLM testing for a total of 12 hospitals, so we were unable to obtain charts for many of the 881 patients. Charts were available on both the mother and infant for 281 (32%) patients. Nineteen of these patients delivered >72 h from the time of FLM sampling, giving a total of 262 patients. Of these, 77 were treated with steroids and delivered non-RDS infants. Therefore, 185 patients met the inclusion criteria. Institutional Review Board approval was obtained for these studies at both institutions.

Infants who were treated with surfactant and/or were placed on a ventilator and/or required continuous positive airway pressure (CPAP) for >24 h with no other congenital anomalies were given a diagnosis of clinically significant RDS. Indications for administering surfactant include prematurity, radiologic signs of hyaline membrane disease, and mechanical ventilation or CPAP.

Six sets of twins were included in the study (three RDS, three unaffected). In all six cases only one amniotic fluid sample was obtained. However, in all six cases both twins had the same clinical outcome. Therefore, the data were included in the study although each set of twins was counted only as a single data point.

Amniotic fluid was collected by transabdominal amniocentesis or free flowing vaginal pool if the membranes were ruptured. Both types of specimens are considered acceptable for analysis by the manufacturer (24). Samples with visible bilirubin, blood, or meconium were rejected. TDx-FLM II analysis was performed according to the manufacturer’s instructions. The upper limit of detection was 160 mg/g, and the lower limit of detection was 10 mg/g. The L/S and the phosphatidylglycerol-to-sphingomyelin ratio (PG/S) were determined by an outside reference laboratory (Quest Diagnostics, Saint Louis, MO) by thin-layer chromatography (4, 25, 26). Reference laboratory cutoffs for L/S were as follows: immature, <1.0; premature, 1.0–1.5; intermediate, 1.5–1.9; caution mature, 2.0–2.5; mature, >2.5. Reference laboratory cutoffs for the PG/S were as follows: immature, <0.3; mature, >0.3.

**Statistics**

TDx-FLM II cutoff values were established with the criterion that there be no false mature results. Sensitivity refers to the probability of an immature result in a patient with RDS. Specificity refers to the probability of a mature result in a patient without RDS. Confidence intervals (CIs) for sensitivity and specificity were computed as exact binomial 95% CIs, using Stata statistical software, release 7.0 (Stata Corp.).

**Results**

A total of 185 women met all the inclusion criteria, of whom 170 delivered unaffected infants and 15 delivered infants with RDS. The gestational ages at time of delivery ranged from 31 to 40 1/7 weeks (median, 37 weeks) for unaffected infants and 28 to 38 6/7 weeks (median, 33 4/7 weeks) for RDS infants. The frequency of all the TDx-FLM II results based on RDS diagnosis is shown in Fig. 1. The data demonstrate that none of the women who delivered infants with RDS had amniotic TDx-FLM II values ≥45 mg/g. Women who delivered unaffected infants and had not been treated with steroids had FLM II results ranging from 14.6 to >160 mg/g.

As shown in Table 1, the 15 women who delivered infants with RDS had TDx-FLM II values that ranged from...
from <10 to 40.7 mg/g. Eight of these women had L/S and/or PG/S values obtained concomitantly with the TDx-FLM II. The TDx-FLM II values correlated well with the L/S and PG/S data in five of the eight women. However, three of the women who delivered infants with RDS (patients 10, 11, and 14) had L/S in the caution mature or mature range, with TDx-FLM II values in the immature range. Patient 14 also had a mature PG/S (>0.3). In 56 cases, L/S and TDx-FLM II were measured concomitantly (Fig. 2). These data demonstrate fair correlation between the two tests ($r = 0.73$), although as shown in Table 1, three RDS patients tested falsely mature by L/S but correctly immature by the TDx-FLM II.

Listed in Table 2 are the sensitivity (95% CI), specificity (95% CI), predictive value of a mature result, and predictive value of an immature result for cutoff values ranging from the current cutoff of $\geq 55$ mg/g down to $\geq 40$ mg/g. As shown in the ROC curve (Fig. 3), lowering the mature cutoff value from $\geq 55$ mg/g to $\geq 45$ mg/g improved specificity, from 72% to 84%, without compromising the sensitivity of 100%. Sensitivity decreased to 93.3% when the cutoff was lowered to $\geq 40$ mg/g. In addition, this change in cutoff increased the predictive value of an immature result from 24% to 36% (Table 2).

**Discussion**

In the assessment of women who are at risk for preterm delivery, the laboratory plays an important role in predicting FLM. Following the development of the automated TDx-FLM method, we eliminated routine perfor-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gestational age, weeks</th>
<th>FLM II, mg/g</th>
<th>L/S ratio</th>
<th>PG/S ratio</th>
<th>Treatment, type (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34 3/7</td>
<td>&lt;10</td>
<td>ND</td>
<td>ND</td>
<td>Surfactant, ventilator (3)</td>
</tr>
<tr>
<td>2 (twins)</td>
<td>30 4/7</td>
<td>&lt;10</td>
<td>1.2</td>
<td>&lt;0.3</td>
<td>Surfactant, ventilator (3 and 6)</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>&lt;10</td>
<td>ND</td>
<td>&lt;0.3</td>
<td>Surfactant, ventilator (&gt;1)</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>&lt;10</td>
<td>1.7</td>
<td>&lt;0.3</td>
<td>Surfactant, CPAP (3)</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>&lt;10</td>
<td>0.8</td>
<td>&lt;0.3</td>
<td>Surfactant, ventilator (&gt;1)</td>
</tr>
<tr>
<td>6</td>
<td>34 2/7</td>
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<td>1.2</td>
<td>&lt;0.3</td>
<td>Ventilator (2)</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>12</td>
<td>ND</td>
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<td>CPAP (2)</td>
</tr>
<tr>
<td>8 (twins)</td>
<td>28</td>
<td>14.6</td>
<td>ND</td>
<td>ND</td>
<td>Surfactant, CPAP (5 and 6)</td>
</tr>
<tr>
<td>9</td>
<td>31</td>
<td>14.8</td>
<td>1.6</td>
<td>ND</td>
<td>CPAP (1)</td>
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<td>2.5</td>
<td>&lt;0.3</td>
<td>Surfactant, ventilator (5)</td>
</tr>
<tr>
<td>11 (twins)</td>
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<td>23.4</td>
<td>2.5</td>
<td>ND</td>
<td>Surfactant, ventilator (4 and 4)</td>
</tr>
<tr>
<td>12</td>
<td>36</td>
<td>23.4</td>
<td>ND</td>
<td>ND</td>
<td>Ventilator (3)</td>
</tr>
<tr>
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<td>30 4/7</td>
<td>27</td>
<td>ND</td>
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</tr>
<tr>
<td>14</td>
<td>33 4/7</td>
<td>29</td>
<td>4.6</td>
<td>&gt;0.3</td>
<td>Ventilator (&gt;1)</td>
</tr>
<tr>
<td>15</td>
<td>38 6/7</td>
<td>40.7</td>
<td>ND</td>
<td>ND</td>
<td>Surfactant, ventilator (&gt;7)</td>
</tr>
</tbody>
</table>

* ND, not determined.
Cutoff, mg/g | Sensitivity, % (CI) | Specificity, % (CI) | Predictive value, % | Immature result | Mature result |
---|---|---|---|---|---|
≥55 | 100 (82–100) | 72 (65–79) | 100 | 24 | 4 |
≥50 | 100 (82–100) | 78 (71–84) | 100 | 29 | 4 |
≥45 | 100 (82–100) | 84 (78–89) | 100 | 36 | 4 |
≥40 | 93 (68–100) | 89 (83–93) | 99 | 42 | 4 |

Table 2. Diagnostic efficiency of the TDx-FLM II in identifying clinically significant RDS.

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References
9. Clements JA, Platzer AG, Tierney DF, Hobel CJ, Creasy RK,


