Clinical Experience with the Helena Fetal-Tek Method of Lecithin/Sphingomyelin Determination

To the Editor:

Various techniques are currently used in assessing fetal lung maturity by analysis of amniotic fluid. These methods, recently reviewed by Freer and Statland (1), include quantification of lecithin (L), sphingomyelin (S), phosphatidylglycerol (PG), the L/S ratio, and other analytes in amniotic fluid. Thin-layer chromatographic determination of the L/S ratio by the Fetal-Tek (Helena Laboratories) procedure is the method used in our medical center. In it the major phospholipid classes found in amniotic fluid are resolved with use of a solvent system developed by Touchstone et al. (2). Not only the L/S ratio but also the presence or absence of PG and phosphatidylinositol (PI) can be qualitatively assessed with the Fetal-Tek procedure.

Whether or not precipitation with acetone is necessary after the initial lipid extraction with chloroform/methanol remains controversial. Some question the utility and reproducibility of this step (1); others simply omit it (3). We routinely perform the Fetal-Tek procedure according to the manufacturer’s instructions, with no modifications: precipitation with acetone is not performed or one of the surfactant phospholipids from amniotic fluid. Several obstetricians at our medical center questioned the validity of L/S results obtained by the Fetal-Tek method. Since the time this precipitation step was introduced by Gluck and Kulovich (4), extensive clinical studies of results by methods involving this step have established 2.0 as the cutoff value for the L/S ratio for prediction of fetal lung maturity (4, 5). We are aware of three clinical studies relevant to the validity of the Fetal-Tek method (6–8). Because these studies did not clearly establish a cutoff value that is highly predictive of mature lungs and the absence of respiratory distress syndrome (RDS), we were motivated to review, in retrospect, the prognostic value of our results obtained with the Fetal-Tek method.

We examined retrospectively all L/S and PG results reported during a 22-month period in our medical center, a total of 60 cases. After excluding cases in which the interval between amniotic fluid analysis and delivery exceeded 10 days or information on neonatal follow-up was unavailable, 40 records (maternal and neonatal) and results from 41 amniotic fluid analyses remained for review. In all but two of these remaining cases, PG was absent within five days of amniotic fluid analysis and RDS was verified in a total of eight cases (20%). The L/S ratio was less than 2.0 in seven of these eight cases (Figure 1). The eighth RDS case, with an L/S value of 3.9, involved the infant of a class C diabetic mother; PG was absent in amniotic fluid from this patient. Only a single infant showed an L/S ratio between 2.0 and 2.4, so we cannot be sure that 2.0 is necessarily the appropriate cutoff value by the Fetal-Tek method associated with mature lungs. However, with 2.0 as a working cutoff value for the L/S ratio associated with fetal lung maturity and the absence of RDS, the diagnostic sensitivity and specificity of the Fetal-Tek method were 97% and 87.5%, respectively.

PG was present in the amniotic fluid of 28 of 35 cases in which the chromatographic plate was further analyzed for the presence or absence of PG. No RDS was evident in infants in whom PG could be detected, but RDS was associated with six of the remaining seven infants in which PG was not detected. If the presence of PG was used as a positive indicator of fetal lung maturity and the absence of RDS was a true positive, the sensitivity and specificity of the Fetal-Tek method were 96.5% and 100%, respectively. A positive predictive value of 100% was obtained when we combined an L/S ratio exceeding 2.0 and the presence of PG. Similar results have been reported by others based on the L/S ratio alone (3). The predictive value of a negative result (i.e., L/S <2.0 or PG absent) was 88% for the L/S ratio and PG determinations, respectively. In addition, 33/35 (94.3%) of the cases we examined demonstrated concordance between L/S ratio and PG predictors of fetal lung maturity and the absence of RDS.

As a large referral center for the Air Force, our obstetrics service is biased toward fetal and maternal complications and consequent unavoidable early deliveries. Thus, the increased prevalence of infants at risk of developing RDS has enhanced the predictive value of both the evaluation of the L/S ratio and the PG tests.

Recently, the ability of the Fetal-Tek method to accurately assess the presence or absence of PG has been challenged and a prechromatography step suggested to remove "pseudo-PG" artifact comigrating with authentic PG (6). Tulley et al. (9) observed that 10 amniotic fluid samples, out of 12 in which PG was undetectable by a modified procedure involving silica-gel plates (Analtech, Newark, DE), were positive for PG when tested by the Fetal-Tek method. One infant in the discordant group developed "mild respiratory distress." In addition, Peterson et al. (9), of Helena Laboratories, reported that falsely positive PG results were observed for amniotic fluid samples from patients who were screened for fetal genetic defects at 12 weeks of gestation. With respect to clinical decisions as to delivery based on fetal lung maturity, this again makes one question the accuracy of PG determination by the Fetal-Tek method. Prechromatography successfully separates true PG from pseudo-PG, but this has not improved the predictive value of the results obtained (6). In amniotic fluid samples analyzed for PG and S, with and without prechromatography, six cases of RDS were associated with a PG signal lower than the cutoff value (0.5) for mature lungs. Nevertheless, the data of Tulley et al. (9) suggest that caution is warranted when the presence of PG is assessed by the Fetal-Tek method.

Guadagno and Sekimoto (7) found a

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Fig. 1. Comparison of L/S ratios with incidence of RDS
Solid bars, no. of RDS cases; cross-hatched bars, no. of cases with L/S values in range shown
close correlation between the L/S ratio as measured by use of the Fetal-Tek method and their reference procedure, and they saw no subsequent clear evidence of RDS in 24 cases that were followed in which L/S values by both methods were similar. Three cases in which the L/S ratio was > 2.5 were classified as "not persistent" RDS. Using the Fetal-Tek method for PG/S and L/S determination, Huang et al. (8) reported results of 62 amniotic fluid analyses in which a single case of RDS occurred when both the PG/S and L/S ratios were indicative of fetal lung maturity. In addition, in 6 of the 62 cases, the clinical outcome agreed with that predicted on the basis of values for either PG/S or L/S ratio. These results tend to validate the Fetal-Tek method in the assessment of fetal lung maturity, but additional studies are needed to establish the appropriate cutoff value for the L/S ratio, the PG/S ratio, or both.

Although our results demonstrate a high correlation between the presence of PG or an L/S ratio > 2.0 by the Fetal-Tek method and failure to develop RDS, data from a larger group of infants, especially those with L/S values near the cutoff value of 2.0, would be reassuring. A mature L/S ratio alone is a good predictor of RDS, and in our study was almost always concordant with the presence of PG. Therefore, it is possible that PG determination can be erroneous and the error masked by the predictive strength of the L/S ratio. Cases in which PG is detected but the L/S ratio indicates lung immaturity, albeit quite uncommon, should be studied to address this issue.

References

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Linearity of Results by Low-Concentration Glucose Strips Evaluated

To the Editor:

We carried out a study to determine the efficacy of using the BMD Stat TEK (I; Boehringer Mannheim Diagnostics, Biodynamics Div., Indianapolis, IN) with low-concentration glucose strips in a neonatal intensive-care unit because of the importance of measuring glucose in concentrations of < 3000 mg/L in the serum of newborns; the delays in reporting incurred in a centralized laboratory; and the difficulty of processing a specimen with low glucose concentration without values being falsely low because of erythrocytic glycolytic activity. We confined the study to low-birth-weight infants who were suspected of being hypoglycemic and who in many instances had three or more glucose determinations. We stopped the study when we had data for more than 100 such comparisons of the strips with results obtained with the Astra method (II; SmithKline Beckman Corp., Bres, CA), which involves the use of glucose oxidase (EC 1.1.3.4).

Multiple stepwise linear-regression analyses showed a linear relation between results by the two methods to 850 mg/L (Table 1). Method I gives results about 45 mg/L lower than method II and the slope of the regression increases at about 500 mg/L, in conformance with the manufacturer's recommendation. We believe that method I has a significant advantage only in those instances when a critically low glucose concentration is being monitored in newborns at the bedside, so to speak. The CV is between 25 and 30% at a glucose concentration of 400 mg/L, for either method.

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Table 1. Linear Regression Analyses of Glucose in Newborns, Determined by Two Methods

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<thead>
<tr>
<th>Glucose (SD), mg/L</th>
<th>Method I</th>
<th>Method II</th>
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<tbody>
<tr>
<td>Glucose (SD), mg/L</td>
<td></td>
<td></td>
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<tr>
<td>l</td>
<td>557 (216)</td>
<td>637 (364)</td>
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<tr>
<td>r</td>
<td>0.4080</td>
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<tr>
<td>502 (164)</td>
<td>540 (156)</td>
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<td>486 (147)</td>
<td>499 (126)</td>
<td>74</td>
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<tr>
<td>435 (130)</td>
<td>482 (122)</td>
<td>65</td>
</tr>
<tr>
<td>405 (118)</td>
<td>457 (118)</td>
<td>54</td>
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1. BMD Stat TEK; II, SmithKline Beckman Astra.