Total Surface-Active Phospholipid/Sphingomyelin Ratio as an Index of Fetal Lung Maturity

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As an index of fetal lung maturity, the ratio of total surface-active phospholipid (phosphatidylethanolamine + phosphatidylylinositol + phosphatidylglycerol + lecithin) to sphingomyelin performs as well or better than any other indicator that we tested, showing 92% sensitivity and 98% specificity. The sensitivity and specificity, respectively, of the other methods were: (a) lecithin/sphingomyelin ratio, 92% and 95%; (b) alkaline phosphatase/glutamyltransferase ratio, 100% and 49%; (c) microviscosity, 75% and 97%; and (d) absorbance at 650 nm, 50% and 73%. The procedure requires 2 h or less and its simplicity allows it to be offered on a 24 h/day basis without necessitating special training.

Additional Keyphrases: fetal status • amniotic fluid • respiratory distress syndrome • cutoff values

The ratio of lecithin to sphingomyelin in amniotic fluid is a useful indicator of fetal lung maturity (1). Although its specificity for respiratory distress syndrome may be as great as 98% (2) its sensitivity may be as low as 54% (3). This prompted the development of "lung profiles," which included determination of the other phospholipids in amniotic fluid. The lung profile method of Gluck et al. (2) which includes determination of saturated lecithin, PG, and phosphatidylinositol has a sensitivity for RDS of 93%.1 However, this method involves two-dimensional thin-layer chromatography, which makes it inconvenient for routine use. Other methods for performing lung profiles (4–6) appear to offer improved sensitivity for RDS, but they all involve the interpretation of a number of specific phospholipid/sphingomyelin ratios, an interpretation that is not always clear when the ratios indicate conflicting estimates of fetal lung maturity. Other methods of estimating fetal maturity have been published: determination of amniotic fluid viscosity (7), alkaline phosphatase, γ-glutamyltransferase, and 5'-nucleotidase activities (8), and measurement of absorbance at 650 nm (9).

Here we describe a method of handling data from a lung profile that results in a single, easily interpreted number. The method is based on the hypothesis that all phospholipids that show surface activity contribute to fetal lung maturity. Therefore it is the ratio of total surface-active phospholipid to sphingomyelin that is important in assessing fetal lung maturity. With this approach, the lung profile showed 92% sensitivity and 98% specificity for RDS in this study. Microviscosity, alkaline phosphatase, and γ-glutamyltransferase determinations and absorbance measurements at 650 nm were also performed on some of the amniotic fluid samples, and their sensitivity and specificity for RDS were compared with that of the lung profile.

Materials and Methods

We used 102 consecutive amniotic fluids submitted to the laboratory. Samples were obtained by abdominal tap as required by clinical circumstances and were from normal pregnancies as well as those complicated by Rh incompatibility, diabetes (three class-A and two class-C patients), premature membrane rupture, toxemia, placenta previa, abruptio placentae, and a single case of fetal duodenal atresia. Samples were from single-birth patients except for three involving twins. One sample from a pregnancy complicated by abruptio placentae contained 10 mL of blood per liter. Samples were centrifuged for 5 min at 1000 × g and either were analyzed without delay or stored at −4 °C until the analyses could be performed.

Diagnosis of RDS was obtained by reviewing the chart or consulting with the attending physician, and was made only when no other explanation for the respiratory distress (e.g., respiratory tract infection) was present and chest roentgenographic findings and blood gas measurements indicated RDS.

Egg-yolk L-α-lecithin and phosphatidylglycerol, bovine brain sphingomyelin, phosphatidylethanolamine, and phosphatidylinositol were obtained from Sigma Chemical Co., St. Louis, MO 63178, and required no further purification. When dissolved in an equivalent mixture of chloroform and methanol they migrated as single, well-defined spots on the thin-layer chromatographic system described below. When mixtures were gravimetrically prepared, transmission densitometry yielded the expected ratios. All solvents were of ACS grade. Silica gel "high-performance" thin-layer chromatographic plates (cat. no. 58077) were obtained from Analtech Incorporated, Newark, DE 19711. After development, the plates were sprayed with a molybdenum blue spray (cat. no. 18213; Applied Science Laboratories, Inc., State College, PA 16801) with an aerosol sprayer (Air Brush; Badger, Franklin Park, IL 60131).

The extraction of the phospholipids was similar to that of Gluck and Kulovich (10), the major difference being a modification of the acetone step. In our procedure, direct treatment with cold acetone is used to remove the surface-active phospholipids from the dried chloroform extract, rather than precipitation of surface-active phospholipids with cold acetone after the dried extract has been re-dissolved in chloroform. This modification (a) shortens sample-preparation time, (b) removes impurities that do not take up the phospholipid spray but do interfere with densitometry of the chromatographic plate, and (c) improves the sensitivity for RDS by preventing false negatives caused by including non-surface-active phospholipids (11). Routine monitoring of the acetone supernate demonstrated the presence of lecithin in about 75% of all samples, and in virtually every sample from a pregnancy with a gestational age of 32 weeks or greater.

One volume of amniotic fluid was successively extracted with one volume of absolute methanol and two volumes of chloroform. After centrifugation, the chloroform layer was removed, filtered through Whatman No. 1 filter paper, and evaporated. One milliliter of cold acetone was added and the
samples were calculated from the fluorescence polarization ($P$) by using the equation $P = 2P/(0.46 - P)$ (12). Fluorescence polarization values were determined as described by Blumenfeld et al. (7) with a Turner filter fluorometer modified to hold polarizing filters in the lightpath before and after the sample cuvette. A broad-band, 365-nm filter (Corning no. 3-71) was used for excitation, and the fluorescence polarization of light emitted at wavelengths greater than 418 nm was determined with use of a filter with a sharp cutoff (Corning no. 7-60).

We measured absorbance of the samples at 650 nm at room temperature in a Gilford 300N spectrophotometer.

Results

With the thin-layer chromatographic system described, the following $R_f$ values were obtained: phosphatidylethanolamine, 0.59; phosphatidylglycerol, 0.47; phosphatidylinositol, 0.28; phosphatidylcholine (lecithin), 0.21; and sphingomyelin, 0.12 (Figure 1). All these are completely resolved except in the case of some patients' samples that contained large amounts of lecithin (L/S ratio >3:1), in which case phosphatidylinositol and lecithin were not completely resolved.

To determine the precision of the method, we analyzed two pools of amniotic fluid 10 times within one day. The mean TPL/S ratios were 3.8 (SD 0.3) and 1.6 (SD 0.2), yielding coefficients of variation of 6.4 and 7.0%, respectively. The mean L/S ratios were 3.3 (SD 0.2) and 1.5 (SD 0.1), yielding CVs of 4.4 and 5.2%. The amniotic fluid pools were analyzed five times over a six-day period, yielding mean TPL/S ratios of 3.7 (SD 0.3) and 1.5 (SD 0.2), with CVs of 8.5 and 15.0%, respectively. By comparing ratios obtained by extracting standard phospholipid mixtures with those obtained from an amniotic fluid pool supplemented with the standard solution, analytical recovery can be estimated. That for lecithin was about 17% higher than for the other phospholipids. This result is similar to that reported by Tsai and Marshall (19), who found about 10–15% higher recovery for lecithin.

Of the 102 samples used, 71 were collected within three days of birth, had appropriate clinical data to evaluate the respiratory outcome, and the data were used in the Tables. Immature lung was indicated in 21 specimens and labor was arrested (or not induced) and subsequent samples were taken. Eight patients were lost to followup. Data on a bloody sample and one other specimen (see Discussion) were not included in the Tables.

![Figure 1A](image1a.png)

**Figure 1A.** Representative densitometric tracings of human amniotic fluid phospholipid.

![Figure 1B](image1b.png)

**Figure 1B.**

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**FIGURE 1.** Representative densitometric tracings of human amniotic fluid phospholipid. A, L/S ratio = 2.5:1, TPL/S >3:1. Patient delivered a healthy child about 24 h after amniocentesis. B, L/S ratio = 2.7:1, TPL/S >3:1. Delivery eight days after amniocentesis.

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Figure 2 shows the relationships between the TPL/S ratio and gestational age for the 102 lung profiles performed, and lists the mean TPL/S ratio in each gestational age bracket, as well as the standard error for each mean. The TPL/S ratio is seen to increase with gestational age in a manner similar to the L/S ratio (9). Figure 2 also shows (in parentheses under the mean TPL/S ratio) the number of samples in each gestational age bracket for which the TPL/S ratio indicated mature lung.

Table 1 summarizes the correlation between the neonatal respiratory outcome and the estimates of fetal lung maturity when we used the cutoff values listed, which were chosen to yield maximum diagnostic efficiency (14). In the case of the alkaline phosphatase/glutamyltransferase ratio and the absorbance at 650 nm, the cutoff values were those previously reported (8, 9).

There was one case of RDS when the L/S ratio indicated mature lung (L/S = 3:1, PG present), i.e., a false negative for RDS. The mother was an insulin-dependent diabetic, and the infant was delivered by cervical section at 36 weeks because of fetal distress in utero as indicated by decreasing urinary estriol values. The infant experienced RDS and hypoglycemia, but survived. In three cases where the infants experienced no respiratory distress, the L/S ratio falsely predicted immature lung. PG was present in two of these samples, and in one case it was present in sufficient amount to increase the (L + PG)/S ratio to > 2.7.

The TPL/S ratio incorrectly predicted mature lung for the sample from the diabetic patient incorrectly predicted by the L/S ratio. However, this method correctly predicted the presence of mature lung for two of the three specimens incorrectly predicted by the L/S ratio, the difference in the ratios being caused by the presence of PG and PE in fluid from one patient and by PE in the other.

No cases of RDS occurred when the alkaline phosphatase/glutamyltransferase ratio was 2.0 or greater. However, the presence of mature lung was not reflected by results for 18 of the samples.

Measurement of amniotic fluid microviscosity incorrectly predicted mature lung for the sample from the diabetic patient missed by the L/S ratio. There also was one false positive.

Absorbance of amniotic fluid at 650 nm predicted mature lung in two cases where RDS developed, and failed to detect the presence of mature lung in nine cases.

Discussion

Any test performed on amniotic fluid, the results of which are going to be used in a clinical setting to predict fetal lung maturity, must show high sensitivity as well as specificity before it can be relied upon to help decide whether or not it is safe to allow the patient to deliver. Table 2 shows the sensitivity and specificity for RDS and the predictive values of a positive and negative result for the measures of fetal lung maturity that we used in this study.

Although the data show that the L/S ratio performs with high sensitivity and specificity, the presence of mature lung was missed in three samples. The lung status in two of these three samples was correctly predicted by the TPL/S ratio, because the TPL/S ratio takes into account all of the surfactant phospholipids present in the amniotic fluid. This view of lung surfactant seems to us to be conceptually more accurate than that presented by previous profiles, because no single phospholipid or combination of phospholipids yet tested completely matches the surfactant properties of naturally occurring lung surfactant. For example, Ikegami et al. (15) measured the ability of phospholipids to reduce the lung pressure-volume characteristics in rats. They used lecithin mixed separately with PG, phosphatidylcholine, and phosphatidylserine. Although the lecithin–PG mixture performed the best, it was not as effective as natural lung surfactant. Statland and Freer (16) measured the surface-tension-lowering ability of the lung surfactant in amniotic fluid with a du Nouy tensiometer and found no combination of phospholipids that could match naturally occurring lung surfactant. Such observations support our hypothesis on which this study is based.

Gluck et al. (11) say that PE isolated from human lung is not surface active, but this is the only such report yet in the literature. Nevertheless, PE has been included in the TPL/S ratio because: (a) it is acetone precipitable like other surface-active phospholipids; (b) when mono-layers of PE are investigated by using a Langmuir–Adams film balance their behavior is similar to lecithin (17); and (c) force vs area isotherms of Langmuir–Blodgett multilayers of phosphatidic acid and PE are very similar to those of phosphatic acid and other surface-active phospholipids (18). The decision to include PE in the ratio received support from one sample in particular. The TPL/S ratio without PE was 2.5 [L/S = 2.3; (L + PG)/S = 2.5]; with PE the TPL/S ratio was 3.2, and the infant experienced no respiratory distress.

The data reported in Tables 1 and 2 do not include a bloody specimen because blood interferes with determination of the L/S ratio (19). We also excluded one infant from a set of twins because sepsis could not be ruled out as the cause of the infant's respiratory distress.

The TPL/S ratio appears to be a sensitive and specific
measure for the possible occurrence of RDS in both normal and complicated pregnancies, performing better than the L/S ratio because it takes into account the presence of surface-active phospholipids not considered by the L/S ratio. Further studies on a larger population are needed to verify these findings. Measurement of amniotic fluid microviscosity performed as well as the TPL/S ratio, and offers the advantages of technical ease and short turnaround time if the necessary instrumentation is available. The alkaline phosphatase/glutamytransferase ratio, although showing good sensitivity, suffers from poor specificity. Measurement of absorbance at 650 nm performed poorly, making it unreliable in a clinical setting.

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References

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