The Role of Amniotic Fluid Phospholipids in Determining Fetal Lung Maturity

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ABSTRACT

Assessment of the status of amniotic fluid phospholipids (qualitative and semi-quantitative) has become important in determining fetal lung maturity. In uncomplicated pregnancies, it has been documented that the ratio of lecithin to sphingomyelin (L/S ratio), with respect to pediatric gestational age, is a reliable predictor of maturity. In complicated pregnancies (e.g., toxemia, diabetes mellitus) the L/S ratio may result in erroneous clinical interpretation and premature intervention. The presence of phosphatidylglycerol (PG) in these cases has been acknowledged to be a significant factor indicative of fetal lung maturity. Thin-layer chromatography, which clearly elucidates the six phospholipids of amniotic fluid, is the method of choice.

Introduction

Within the past decade the vital importance of surface active phospholipids in the pathogenesis of neonatal respiratory distress syndrome (RDS) or hyaline membrane disease has been recognized. Several hours after birth, the neonate with true RDS usually develops symptoms of expiratory grunting, intercostal retractions, and cyanosis. Progressive atelectasis leads to further respiratory insufficiency, exhaustion, and possible death. The most common cause of RDS is a deficiency of surfactant secondary to pulmonary immaturity. Less frequent causes of RDS, other than neonatal immaturity, are aspiration or streptococcal bronchopneumonia. Respiratory distress syndrome, regardless of the etiology, remains the most common cause of neonatal morbidity and mortality.

The need to assess fetal pulmonary maturity is essential in pregnancies in which fetal well-being is jeopardized by maternal diabetes mellitus, toxemia, or erythroblastosis fetalis. In situations wherein labor may be induced or the infant delivered by caesarian section, the assessment of fetal pulmonary status is invaluable for the proper management of the delivery. The clinical laboratory must be able to provide the attending obstetrician and pediatrician with relevant and timely assessment of pulmonary surfactant, hence, status of fetal lung maturity.
**Pulmonary Surfactant**

When surfactant is present there is a significant reduction in the surface tension at the alveolar-air interface as the alveolus decreases in radius during expiration. According to Laplace's law, the pressure (P) needed to keep an alveolus from collapsing is equal to twice the surface tension (T) divided by the radius (R) of the alveolus (\(P = \frac{2T}{R}\)). Without surfactant, the alveolar surface tension remains constant as the alveolar radius decreases and the alveolus will collapse with generalized atelectasis resulting.

Pulmonary surfactant is a complex mixture consisting predominantly of lipids with lesser amounts of protein and carbohydrate. Surfactant is synthesized, stored, and secreted by large Type II alveolar lining cells. Within the cytoplasm of mature alveolar Type II cells are lamellar inclusions which act as storage vesicles for surfactant. The production of surfactant in the fetus is marked by the appearance of these cytoplasmic lamellar inclusions at the 34th to 36th week of gestation.

Phospholipids are the major lipid component of pulmonary surfactant. L-\(\alpha\)-dipalmitoyl phosphatidylcholine (lecithin) is the major, but by no means the only, phospholipid with surface-tension reducing properties. It is the bipolar chemical configuration of lecithin which imparts its surface active properties. Other phospholipids which may be present in smaller amounts include sphingomyelin (S), phosphatidylcholine (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG). Phosphatidylglycerol is an important "minor" phospholipid which has surface activity, has a stabilizing effect on lecithin, and which may represent the final biochemical pathway of pulmonary maturation.\(^{11,19}\)

Lecithin is synthesized in the lung through two major pathways.\(^8\) In the choline incorporation pathway, choline is phosphorylated and then conjugated with \(\alpha, \beta\)-diglyceride to form L-\(\alpha\)-dipalmitoyl lecithin. In the methylation reaction pathway, phosphatidylethanolamine is methylated to form palmitomyristoyl lecithin. These two major pathways can be summarized as follows:

**Choline incorporation:**

\[
\text{Cytidinediphosphate choline} + \alpha\text{-diglyceride} \rightarrow \text{dipalmitoyl lecithin}
\]

**Methylation:**

\[
\text{Phosphatidylethanolamine} + \text{CH}_3 \rightarrow \text{palmitomyristoyl lecithin}
\]

The choline incorporation pathway is the most important in the fetus and maturing neonate for the synthesis of total lecithin. This pathway begins significant production of lecithin at the 35th to 36th week of gestation and signifies pulmonary "maturity." If necessary, the methylation pathway matures at 22 weeks of gestation and can provide an alternate system for surfactant in the very premature neonate. However, frequent complications of prematurity such as hypoxia, hypothermia and/or acidosis inhibit the alternate methylation pathway. Thus, in a premature neonate with pulmonary insufficiency, the situation is further aggravated.\(^7\)

The amount of surfactant in amniotic fluid correlates with the concentration of surfactant in the alveoli. Early in pregnancy the concentration of lecithins in amniotic fluid is relatively low. After the 34th to 35th gestational week, a sharp increase in lecithin concentration occurs. This lecithin surge reflects the accelerated production by the choline incorporation pathway. The concentration of sphingomyelin is very stable and no significant changes are observed between the 32nd and 40th weeks of gestation (figure 1).\(^6\) Phosphatidylcholine appears prior to the 33rd week of gestation, increases slowly, and generally disappears at maturity. Phosphatidylglycerol appears during the 35th to 38th gestational week, in-
increases with gestation and correlates well with the increasing lecithin.15

Procedure for Determination of L/S Ratio

The procedure for L/S ratio determination by thin layer chromatography (TLC) requires initial organic solvent extraction of the phospholipids from the amniotic fluid. Using TLC, L, S, PI, PE, PS, and PG can be separated and their relative amounts estimated by densitometry.20,21 There are other methods suggested for measuring the surfactant properties of amniotic fluid by either biochemical or biophysical methods.17 The relative amounts of lecithin and sphingomyelin, expressed as the L/S ratio, determined by TLC has proven to be a clinically simple and reliable index of fetal pulmonary maturity in uncomplicated pregnancies.

Fundamental to the performance of amniotic fluid phospholipid analysis is the proper collection, storage, and preparation of the sample. Routinely, amniotic fluid is obtained by transabdominal amniocentesis. With premature rupture of amniotic membranes, free flowing vaginal (amniotic) fluid can be used.4 Phospholipid determination, if necessary, can be performed on neonatal tracheal or gastric aspirations to estimate pulmonary maturity.14 In these unusual clinical circumstances, it must be remembered that the amniotic fluid is mixed with other body fluids containing phospholipids and phospholipases which may interfere with the analysis. Analytical results from samples other than those obtained by transabdominal amniocentesis are less than ideal, and the results must be interpreted with caution.

Contamination of the amniotic fluid by blood or meconium can affect the measurement of the phospholipids. Plasma may contain significant concentrations of lecithin. The effect of blood on the L/S ratio may be variable with blood generally decreasing a high L/S ratio or increasing a low L/S ratio thus yielding spurious and, perhaps, misleading clinical direction. Meconium appears to have an inconsistent effect on the L/S ratio.5

Centrifugation of the specimen is not needed unless the amniotic fluid appears blood tinged or turbid. In this latter case, the specimen should be centrifuged at 150 to 300 g for two minutes to sediment the extraneous debris. The fluid should then be completely decanted and gently mixed manually before analysis.

In uncomplicated pregnancies, the L/S ratio increases in a consistent and predictable fashion with the gestational age and fetal pulmonary maturity. An L/S ratio of 2.0 or greater generally indicates pulmonary maturity and a minimal risk for developing RDS.6,16 However, since there are many methods for amniotic fluid analysis, each laboratory must establish a numerical L/S ratio threshold indicative of
pulmonary maturity. If the L/S ratio indicates immaturity (less than 2.0), the risk of RDS is greater. However, the L/S ratio alone is nonspecific for accurately predicting RDS. There is a high incidence of neonates with L/S ratios indicative of immaturity which have normal pulmonary function.1,17

Discussion

In pregnancies complicated by diabetes or toxemia, the L/S ratio may be accelerated irrespective of the actual pulmonary maturity (L/S ratio greater than 2.0), and RDS may thus occur in an infant with an apparently mature L/S ratio.3,17 The determination of other amniotic phospholipids such as PG and PI in complicated pregnancies may yield additional valuable information and permit a more accurate prediction of the actual status of pulmonary maturity. Accordingly, in such complicated pregnancies, an L/S ratio greater than 2.0 and the presence of PG indicates maturity. Likewise, the absence of PI may be indicative of pulmonary maturity (table I).2,17 In complicated pregnancies, the L/S ratio has proven not to be sufficient.24

The determination of the L/S ratio and other amniotic fluid phospholipids by thin layer chromatography affords the clinical laboratory a rapid, simple, and precise method of assessing fetal pulmonary maturity. There are numerous other biochemical and biophysical methods for analyzing the surfactant properties of amniotic fluid to predict fetal pulmonary maturity, but none has as extensive a clinical evaluation or as general an acceptance in the clinical laboratory.

The role PG plays as an additional predictor of fetal pulmonary maturity has been appreciated in complicated pregnancies. The predictive value of other amniotic phospholipids (PI, PE, PS) is presently under investigation and requires further clinical correlation.

<table>
<thead>
<tr>
<th>Fetal Lung Maturity</th>
<th>PG</th>
<th>PI</th>
<th>L/S Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td>±</td>
<td>±</td>
<td>1.5:1</td>
</tr>
<tr>
<td>Transitional</td>
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<td>+</td>
<td>1.5 to 1.9:1</td>
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<td>±</td>
<td>2.0+:1</td>
</tr>
<tr>
<td>Mature - uncomplicated</td>
<td>±</td>
<td>+</td>
<td>2.0+:1</td>
</tr>
</tbody>
</table>

PG = Phosphatidylglycerol
PI = Phosphatidylinositol
L/S Ratio = Ratio of lecithin to sphingomyelin

References


