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Amniotic Fluid Phospholipids. New Predictive Values of L/S and PG/S Ratios

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The lecithin/sphingomyelin (L/S) ratio after cold acetone precipitation is widely used to predict fetal lung maturity. The separation of saturated lecithin, the main component of surfactant, is the basis for using the precipitation procedure but there is still a controversy as to whether cold acetone precipitable lecithin can be equated with saturated lecithin. Following up a previous paper in which the effect of cold acetone precipitation on phospholipids of amniotic fluid was studied, the present work reports that non-precipitated L/S and phosphatidylglycerol/sphingomyelin (PG/S) ratios correlate well with the precipitated L/S ratio ($r = 0.93$, $r = 0.84$, $n = 92$). The predictive value of both non-precipitated L/S and PG/S ratios has been studied when a "positive" result predicts a precipitated L/S ratio ≥ 2.0, and proposes a L/S ratio ≥ 4.7 and a PG/S ratio ≥ 0.8 to predict fetal lung maturity, when cold acetone precipitation step is omitted.

Key words: Phospholipid, Amniotic fluid, Cold acetone precipitation, Fetal lung maturity.

Normal lung function requires the presence of a surfactant layer on the surface of the alveoli. Lung immaturity as far as its capacity to synthesize or secrete surfactant onto the alveolar surface is concerned seems to play an important role in the pathogenesis of hyaline membrane disease (HMD) in prematurely delivered infants.

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Various methods have been developed to provide the obstetrician with information about the state of lung maturity of the fetus before delivery (3, 12, 15). The basis of most methods for assessing fetal lung maturity is the detection of a sufficiently high concentration of surfactant phospholipid in amniotic fluid (5, 16).

The lecithin/sphingomyelin (L/S) ratio, after the isolation of disaturated phosphatidylcholine with precipitation in cold

acetone, proposed by GLUCK *et al.* (7) stand out, but there is still considerable controversy about the precipitation step.

While some authors (8, 13) report that leaving out the precipitation step may cause serious errors when predicting fetal lung maturity, several researchers (2, 11, 14) suggest that there are no significant differences between precipitated and non precipitated fractions. TORDAY *et al.* (20) concluded that the L/S ratio vas not modified by cold acetone precipitation and that precipitated lecithin could not be equated with saturated lecithin isolated by osmium tetroxide. Finally, HOBSON *et al.* (10) found significant differences between the L/S ratio numeric values obtained with and without the cold acetone precipitation step but they also reported that the clinical prediction of HMD was not significantly enhanced by using the precipitation step.

Other authors (1, 6, 9, 17) suggest that phosphatidylglicerol (PG) is also an important indicator of fetal lung maturity and in pregnancies complicated by maternal diabetes mellitus, the PG content in amniotic fluid may be more predictive of fetal lung maturity than the L/S ratio.

In a previous paper (4) a procedure for separating and measuring individual phospholipids is outlined: sphingomyelin (Sph), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). Here, we report the influence of cold acetone precipitation on the L/S ratio, and the fetal lung maturity predictive value of L/S and PG/S ratios without the cold acetone precipitation step.

Materials and Methods

TLC plates.– We used 10 x 10 cm silica gel F254 "High performance" thin-layer chromatography plates (HPTLC) (Merck, Darmstadt, Germany), especially designed for nanochromatography.

Equipment.– Hettich Rotanta K centrifuge; chromatography injector: Camag Nanomat 2770-1078-E with applicator 27750-278-E (Camag, Muttenz, Switzerland); chromatography tank: camag twin-trough chamber for HPTLC. Densitometer: Camag TLC scanner light source, equipped with an integrator Rockwell AIM 65 analogic digital interface (Rockwell, München, Germany).

Chromatographic solvent.– Chloroform/hexane/methanol/glacial acetic acid (5:3:1.6:1 vol/vol) (4).

Staining reagent.– A phosphomolibdic acid reagent previously reported (18) was used.

Amniotic fluid samples.– Ninety-two amniotic fluid samples were obtained by transabdominal amniocentesis or by transvaginal sampling after artificial amniorrexis, between 28 and 42 weeks of gestation. Amniocentesis was performed for medically indicated reasons when fetal lung status might influence management. A preliminary selection on clinical cases was not made because of the nature of the study, but the categories of amniotic fluid samples are shown in table I. All patients underwent ultrasonography before the procedure. Meconium and blood-stained fluids were discarded. If the sample could not be used on the day it was collected, it was stored at –20 °C until required. All the patients involved in this study were cared for by the Zaragoza University Clinical Hospital Perinatal Services.

Amniotic fluid samples were centrifuged (2500 xg 5 min) to remove cellular debris (19). The supernatant fluid was

Table I. *Categories of amniotic fluid specimens.*

Type	n
Uncomplicated pregnancy	54
Complicated pregnancy	
Diabetes	17
Preeclampsia or eclampsia	4
Intrauterine growth retardation	5
Hepatitis	3
Multiple pregnancy	6
Others	3
Total	92

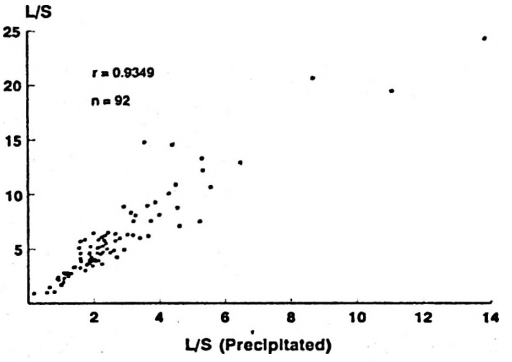

Fig. 1. *Correlation between the L/S ratio without cold acetone precipitation and the precipitated L/S ratio of Gluck.*

decanted and divided into two 2 mL aliquots. They were mixed with 2 mL methanol, vortexed for 10 s, then mixed with 5 mL chloroform and vortexed for 1 min, before centrifugation at 1500 xg for 5 min. The lower chloroform layers were carefully collected and evaporated to dryness at 60 °C under a stream of nitrogen. One of the dried lipidic extracts was redissolved in a minimal amount of chloroform. Excess cold acetone dropwise was added until no more white precipitate was formed and the sample was kept in a bath of crushed ice for 15 minutes before centrifugation at 1500 xg 5 min. Supernatant was discarded and the pellet evaporated to dryness under nitrogen. The two residues were reconstituted in 25 mL chloroform for spotting onto HPTLC plate.

Statistical methods.– All data were analyzed and are reported as means ± standard error of mean (media ± SEM). To determine if any relationship between the L/S or PG/S and L/S precipitated ratios existed the regression analysis was used.

Results and Discussion

Effects of cold acetone precipitation on the L/S ratio.– The L/S ratio with and without cold acetone precipitation changed (5.94 ± 0.43 vs 2.68 ± 0.21; n = 92).

The relationship between cold acetone precipitable lecithin, gestational age and lung maturity has been established (7). As sphingomyelin remained relatively constant in amniotic fluid the L/S ratio can be used to predict the respiratory distress syndrome.

Although a number of studies have contested the validity of the cold acetone precipitation procedure (2, 11, 14, 20), others (8, 13, 18) reported that the L/S ratio is changed by cold acetone precipitation and that the omission of this step would need a new value to predict lung maturity. In the same way, HOBSON *et al.* (10) found significant differences in the L/S ratio values because of precipitation step but they reported that this procedure did not increase the predictive value of the test to detect RDS.

In our study, a linear regression analysis has been made to compare the values of both L/S ratio with and without cold acetone precipitation (dependent and independent variable, respectively). Figure 1 shows the correlation between both variables (r = 0.93) and thus, a precipitated L/S ratio of 2.0 corresponds to a non-precipitated L/S ratio of approximately 4.5. The correlation between PG/S ratio without acetone precipitation vs precipitated

L/S ratio is shown in figure 2 (r = 0.84) where a precipitated L/S ratio of 2.0 is associated with a PG/S ratio without cold acetone precipitation of 1.2.

No amniotic fluid samples from cases with hyaline membrane disease, were available, so that it was impossible to use the predictive value of L/S and PG/S ratios to define specific "cut off" values for predicting fetal lung maturity. Thus, fetal lung maturity was defined by using the criteria proposed by GLUCK *et al.* (7), hence, a precipitated L/S ratio ≥ 2.0 reflects fetal lung maturity, although there is a transitional zone (with L/S ratios between 1.5 and 2.0) where lung maturity or immaturity cannot be diagnosed.

The values equivalent to the critical precipitated L/S ratio of 2.0 are shown in table II, where prediction of fetal lung maturity on the basis of the L/S ratio without cold acetone precipitation ≥ 4.7 results in four false positives out of 51 tests (7.8 %). These four false positive cases had a precipitated L/S ratio in the transitional zone of Gluck (1.55 to 1.9). There were also 7 false negatives out of 41 negative tests (17 %).

The prediction on the basis of PG/S ratio without cold acetone precipitation ≥ 0.8 results in eleven false positives out of 60 positive tests (18.3 %). Ten of these false positives were in the transitional zone of Gluck. There were also five false negatives out 32 negative tests (15.6 %).

When the precipitated L/S ratio ≥ 1.5 was used as the critical values, a L/S ratio without cold acetone precipitation 3.4 corresponds to no false positives and two false negatives (9.5 %). Table III shows on the basis of the PG/S ratio without precipitation ≥ 0.8, one false positive (1.6 %) but 14 false negatives (43.7 %).

From these results it follows that there is a transitional zone for the L/S ratio without cold acetone precipitation between the 3.4 and 4.7 values, which corresponds to the one proposed by Gluck for the precipitated L/S ratio (1.5 to 1.9). Nevertheless this transitional zone is not in the PG/S ratio.

To sum up, from our results it follows that the L/S ratios with and without cold acetone precipitation change and that the value of the precipitated Gluck L/S ratio is nearly twice as low as the one without the cold acetone precipitation step. The L/S and PG/S ratios without cold acetone

Table II. *Prediction of fetal lung maturity with L/S and PG/S ratios without cold acetone precipitation.* "Cut off" value L/S ratio of Gluck > 2.0 (n= 92).

Test	L/S ≥ 4.7	PG/S ≥ 0.8
True positives (TP)	47	49
False positives (FP)	4	11
True negatives (TN)	34	27
False negatives (FN)	7	5
Sensitivity	87.03	90.74
Specificity	89.47	71.5
PVP	92.16	81.67
PVN	82.93	84.37
Index of Youden	0.76	0.62

Sensitivity = TP/(TP+FN).
Specificity = TN/(TN+FN).
PVP = Predictive value of a positive test = TP/(TP+FP).
PVN = Predictive value of a negative test = TN/(TN+FN).
Index or Youden = relationship between sensitivity and specificity.

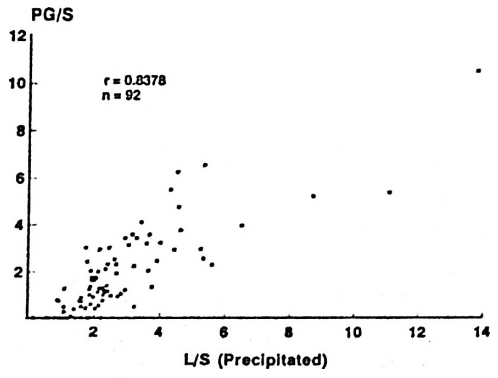

Fig. 2. *Correlation between the PG/S ratio without cold acetone precipitation and the precipitated L/S ratio of Gluck.*

Table III. *Prediction of fetal lung maturity with L/S and PG/S ratios without cold acetone precipitation.*
"Cut off" value L/S ratio of Gluck ≥ 1.5 (n = 92).
Abbreviations as table II.

Test	L/S ≥ 3.4	PG/S ≥ 0.8
True positives	71	59
False positives	0	1
True negatives	19	18
False negatives	2	14
Sensitivity	97.26	80.82
Specificity	100.00	94.73
PVP	100.00	98.33
PVN	90.48	56.25
Index of Youden	0.97	0.75

precipitation correlate well with the precipitated L/S ratio in the same samples (r = 0.93 and r = 0.84).

Finally, the study of the predictive value of these ratios when the prediction of fetal lung maturity (precipitated L/S ratio of Gluck ≥ 2.0) was compared by both L/S and PG/S ratios, showed a transitional zone between 3.4 and 4.7 for the L/S ratio without cold acetone precipitation. The PG/S ratio did not have this transitional zone and its "cut off" value for predicting fetal lung maturity was ≥ 0.8.

J. RUIZ-BUDRÍA, E. FABRE-GONZÁLEZ, M.T. HIGUERAS and J.L. SERRANO-OSTÁRIZ. *Fosfolípidos del líquido amniótico. Nuevo valor predictivo de los índices L/S y PG/S.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (1), 17-22, 1995.

La separación de la lecitina saturada es la base para el uso del procedimiento de precipitación con acetona fría. En relación con el efecto de la precipitación de los fosfolípidos del líquido amniótico con acetona fría, se muestra la correlación existente entre los índices lecitina/esfingomielina (L/S) y fosfatidilglicerol/esfingomielina (PG/S) no precipitados (r = 0.93, r = 0.84, n = 92). También se estudia el valor predictivo de ambos índices de fosfolípidos no precipitados cuando un resultado "positivo" predice un índice L/S precipitado ≥ 2.0, y se propone un índice L/S ≥ 4.7 y un índice PG/S ≥ 0.8, para predecir la madurez pulmonar fetal, cuando se omite el paso de precipitación con acetona fría.

Palabras clave: Fosfolípidos, Líquido amniótico, Precipitación con acetona fría, Madurez pulmonar fetal.

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