J. Clin. Chem. Clin. Biochem.
Vol. 20, 1982, pp. 295–298

Comparison of the Values for Filter-Trapped Phospholipids with those for the Lecithin/Sphingomyelin Ratio of Amniotic Fluid
A Preliminary Report[1])

By *D. O. E. Gebhardt, W. Soederhuizen* and *J. Egberts*

Department of Obstetrics and Gynaecology, University Hospital, Leiden, The Netherlands

(Received June 29/December 10, 1981)

Summary: A comparison was made between the lecithin/sphingomyelin ratio of amniotic fluid and the amount of phospholipids which could be removed from the amniotic fluid by filtration. We found a good correlation between the two methods. The amount of filter-trapped phospholipids can be determined within one and a half hours compared to three and a half hours for the lecithin/sphingomyelin ratio. It has a higher reproducibility than the lecithin/sphingomyelin ratio and can be performed with the aid of a simple filtration apparatus.

Bestimmung der mit Filter abtrennbaren Phospholipide im Fruchtwasser und ihre Beziehung zum Lecithin/Sphingomyelin-Quotienten. Ein vorläufiger Bericht

Zusammenfassung: Die Reife der foetalen Lunge wird oft an der Größe des Lecithin/Sphingomyelin-Quotienten von Fruchtwasser beurteilt. Da dieser Quotient nicht problemlos zu bestimmen ist, haben wir eine neue quantitative Methode entwickelt, die gut mit dem Lecithin-Sphingomyelin-Quotienten korreliert. Die Methode besteht darin, daß man das Fruchtwasser durch ein Filter mit Poren von 0,45 μm Durchmesser abfiltriert. Man bestimmt vor und nach der Filtration den Phospholipid-Phosphor. Der Unterschied ist ein Maß für die Menge „Surfactant", die als „lamellar bodies" im Fruchtwasser anwesend ist. Die Bestimmung erfordert 1½ Stunde, entschieden weniger als die Bestimmung des Lecithin/Sphingomyelin-Quotienten.

Introduction

It is well known that the fetal lung produces phospholipids which are secreted into the amniotic fluid (1). The determination of the lecithin/sphingomyelin ratio of amniotic fluid is based on this fact and various studies have shown that respiratory distress will, in general, not occur if the ratio is greater than 2.0 (2, 3). There are, however, some objections to the lecithin/sphingomyelin test namely:

1) It is a semi-quantitative test which cannot easily be standardized (4–8);

2) It is unknown whether the lecithin and sphingomyelin are solely derived from the fetal lung since amniotic fluid also contains lipoproteins (9) which are probably of maternal origin (10).

Thus the specificity of the test, especially in the period when neonatal respiratory distress may occur, (before the 36th week of development), is still a matter of conjecture. There is need for a quantitative method of estimating those phospholipids of amniotic fluid which are produced by the fetal lung. Recently lamellar bodies, the major source of pulmonary surfactant phospholipids were detected by *Duck-Chong* (11, 12) in amniotic fluid. These insoluble structures with a diameter of about 2 μm were isolated by gradient ultracentrifugation and were then quantified by determining their phospholipid content. *Duck-Chong* suggested that the amount of lamellar body phospholipid of amniotic fluid might be an index of fetal lung maturity.

With this in mind we have devised a simpler method of removing lamellar bodies from amniotic fluid namely by filtration through a microporous filter of 0.45 μm pore size. We have determined the amount of phospholipid phosphorus before and after filtration and have correlated the difference to the lecithin/sphingomyelin ratio.

[1]) An abstract of this paper has appeared in the Wissensch. Zt. Humboldt Univ. Berlin Math. Nat. R. *29*, 579–580 (1980) "A new method of estimating fetal lung maturity by determination of amniotic fluid phospholipids" by *J. Egberts, W. Soederhuizen, D. O. E. Gebhardt & M. van der Ploeg.*

0340-076X/82/0020-0295$02.00

Material and Method

Amniotic fluid was obtained by amniocentesis at various stages of pregnancy. Immediately after collection, the samples were centrifuged for 10 minutes at 1500 *g* to remove cells. The supernatant was used for estimating:

a) the lecithin/sphingomyelin ratio as described by l.c. (13).

It consists of the following steps: 5 ml amniotic fluid are mixed with 5 ml methanol and 10 ml chloroform. The mixture is centrifuged (1500 *g* for 5 minutes) to separate the layers. 3 ml of the chloroform layer is evaporated almost to dryness. Then a few drops of cold acetone are added to precipitate dipalmitoyl lecithin and other acetone-insoluble phospholipids. The precipitate is dissolved in chloroform and the various phospholipids are separated by thin layer chromatography. The components are stained by spraying the plates with phosphomolybdic acid and the lecithin/sphingomyelin ratio is then determined densitometrically;

b) the total amount of phospholipid phosphorus in the amniotic fluid according to l.c. (14). For this purpose 2 ml of the chloroform extract (see above) were evaporated to dryness and the residue was digested with perchloric acid. Quantitation of the organic phosphorus was then done by colorimetry using the reagent of *Fiske & Subbarow*;

c) the amount of lamellar body phospholipid phosphorus: 3 ml of amniotic fluid was filtered through an AMICON model 12 stirred ultrafiltration cell containing a microporous filter of 0.45 µm pore size (Amicon, Lexington, Mass. U.S.A.). The cell was placed on a magnetic stirrer and was pressurized to 4.5 kg/cm^2 until the fluid had passed the filter. It was then washed under pressure with 3 ml saline. The filtration step took about 5 minutes and could also be performed by collecting the amniotic fluid in a syringe and passing it through an AMICON "Sterilet" disposable filter of 0.45 µm pore size. The phospholipid phosphorus content of the filtrate was then estimated in the same way as the total phospholipid phosphorus (14). The lamellar body phospholipid value was found by subtracting the phospholipid phosphorus content of the filtrate from that of the total phospholipid phosphorus of the amniotic fluid before filtration.

The ratio, lamellar body phospholipid/total phospholipid, which is independent of amniotic fluid volume, was also calculated and compared with the lecithin/sphingomyelin ratio. The time needed for the determination of the lecithin/sphingomyelin ratio was about 3½ hours whereas the total phospholipid and lamellar body phospholipid phosphorus determinations could be performed in 1½ hours.

For the statistical evaluation, which consisted of calculating the various correlation coefficients, only samples obtained in the "critical" period of gestation (30–36 weeks) were used.

Results

In figures 1, 2 and 3 the lamellar body phospholipid content, the total phospholipid phosphorus content and the lamellar body phospholipid/total phospholipid values of amniotic fluid samples are shown together with their lecithin/sphingomyelin ratios. The coefficient of variation of the lamellar body phospholipid phosphorus determination was 5% whereas the coefficient of variation of the lecithin/sphingomyelin determination was 10%. The values equivalent to the critical lecithin/sphingomyelin ratio of 2.0 were obtained graphically by determining the intersection between the perpendicular line at the lecithin/sphingomyelin ratio = 2.0 and the linear regression line. From there a line parallel to the x axis was drawn and the point where the line cut the

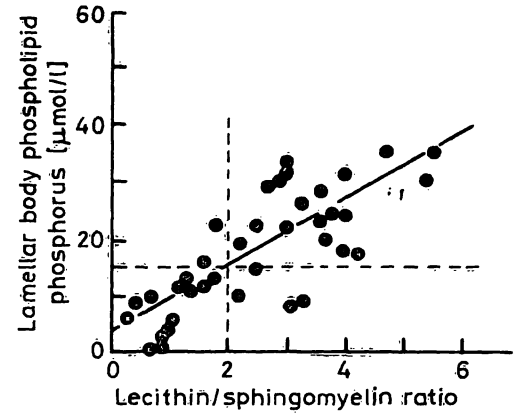

Fig. 1. Correlation between the amount of lamellar body phospholipid phosphorus of amniotic fluid and the lecithin/sphingomyelin ratio.
(y = 4.37 + 5.33 x; (n = 38) r = 0.76 P < 0.001).

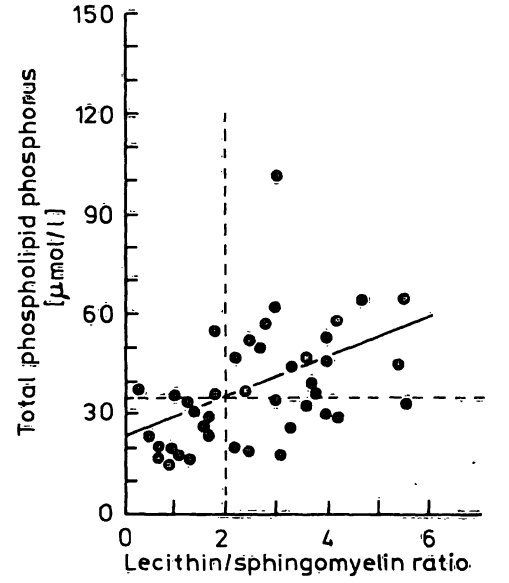

Fig. 2. Correlation between the total amount of phospholipid phosphorus of amniotic fluid and the lecithin/sphingomyelin ratio.
(y = 22.47 + 5.90 x (n = 41) r = 0.48 P < 0.002).

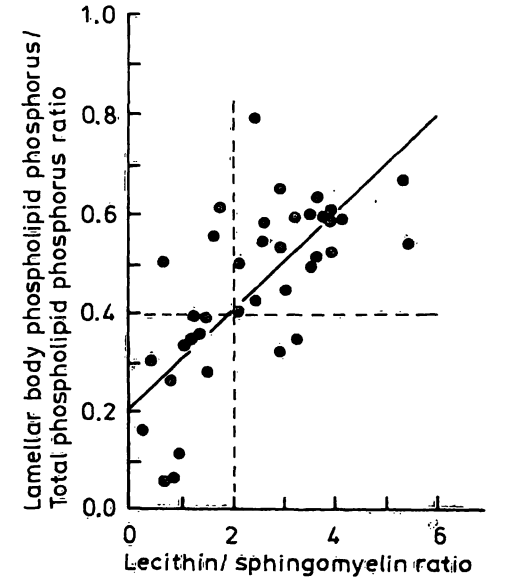

Fig. 3. Correlation between the lamellar body phospholipid phosphorus/total phospholipid phosphorus ratio and the lecithin/sphingomyelin ratio of amniotic fluid.
(y = 0.212 + 0.091 x (n = 38) r = 0.69 P < 0.001).

y axis was used as the equivalent value (for the lamellar body phospholipid phosphorus this was 15 μmol/l, for the total phospholipid phosphorus 35 μmol/l, and for the lamellar body phospholipid/total phospholipid ratio 0.4). Figures 4 and 5 show the distribution of the lamellar body phospholipid and the lamellar body phospholipid/total phospholipid values as a function of gestational age. It can be seen that the critical value was not reached before the 31st week of pregnancy. Finally in table 1 the number of cases is given in which there is agreement or disagreement between the lecithin/sphingomyelin ratio and the other parameters using the above mentioned threshold values.

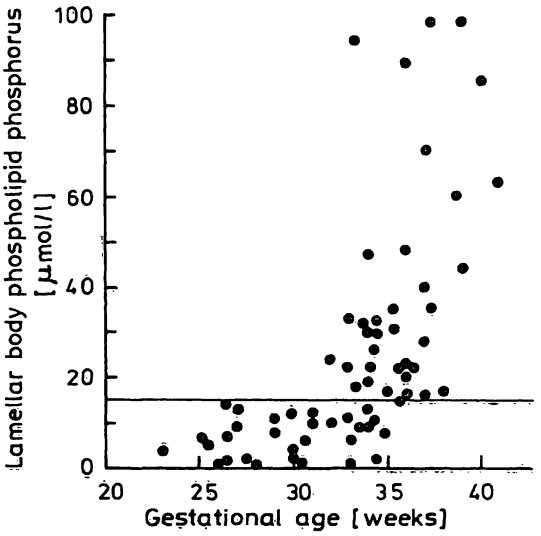

Fig. 4. The relationship between the lamellar body phospholipid phosphorus value of amniotic fluid and gestational age.

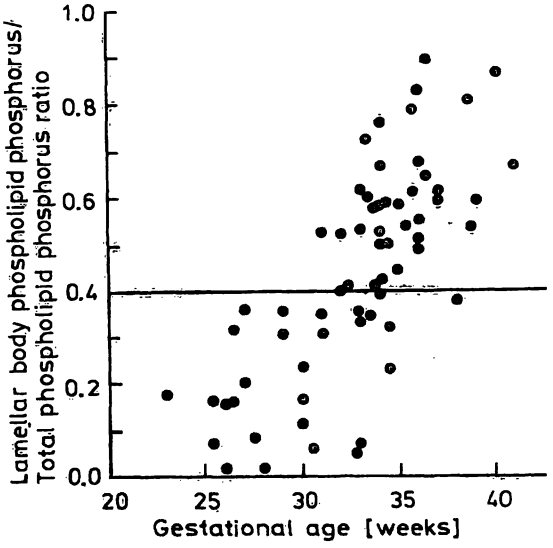

Fig. 5. The relationship between the lamellar body phospholipid/total phospholipid phosphorus ratio of amniotic fluid and gestational age.

Tab. 1. Comparison of lecithin/sphingomyelin (L/S) ratio and phospholipid values in amniotic fluids from 30–36 weeks of gestation.

	TPP (μmol/l)		LP (μmol/l)		LP/TPP	
	< 35	> 35	< 15	> 15	< 0.4	> 0.4
L/S ratio < 2	11	2	8	2	7	3
L/S ratio > 2	11	14	3	17	2	18

TPP = total phospholipid phosphorus
LP = "lamellar" or filter-trapped phospholipids

Discussion

The technical difficulties which exist in performing the lecithin/sphingomyelin test have restricted its use to those laboratories specialized in the analysis of amniotic fluid. In this study we have described a quantitative determination of the insoluble phospholipids, derived from the lamellar bodies of amniotic fluid, which correlates well with the lecithin/sphingomyelin ratio ($r = 0.76$; 95% confidence interval: 0.582–0.869). There is a complication, however, which holds also for the lecithin/sphingomyelin ratio, namely the (threshold) value depends on the speed at which the amniotic fluid is centrifuged prior to analysis (15, 16). Optimal conditions of centrifugation must still be determined before definite critical values can be introduced. The correlation coefficient, calculated from the relationship of total phospholipid phosphorus values versus lecithin/sphingomyelin ratios, ($r = 0.48$; 95% confidence interval: 0.203–0.686) is much lower than the one between the lamellar body phospholipid values and the lecithin/sphingomyelin ratio (see above). This can be explained by considering that the total phospholipid phosphorus value is a measure of both the lamellar and the non-lamellar body phospholipids of amniotic fluid, whereas the lamellar body phospholipids and also the lecithin/sphingomyelin ratio are indices of the fetal lung surfactant activity.

From figure 4 it follows that even before the 31st week of pregnancy some of the phospholipids of amniotic fluid can be removed by filtration. According to *Oulton* et al. (17) these phospholipids are derived from immature lamellar bodies.

Whenever a new technique is compared with an existing one, such as the lecithin/sphingomyelin ratio, the question arises which test will have the highest predictive value for the clinician. In table 1 the cases are given where there is lack of agreement between the methods. Since our amniotic fluid samples were obtained at least 48 hours before parturition, this question cannot yet be answered. Furthermore the lecithin/sphingomyelin ratio appears to be less reliable than originally assumed (18, 19) in diabetes and other fetomaternal diseases. It is to be hoped that in the near future a prospective study will take place in close cooperation with obstetricians and neonatologists to solve this problem.

References

1. Gluck, L., Kulovich, M. J., Borer, R. C. & Keidel, W. N. (1974) Am. J. Obstet. Gynecol. *120*, 142–155.
2. Gebhardt, D. O. E. & Beintema, A. (1975) Am. J. Obstet. Gynecol. *122*, 734–736.
3. Kulovich, M., Hallman, M. & Gluck, L. (1979) Am. J. Obstet. Gynecol. *135*, 57–63.
4. Gebhardt, D. O. E. & Dubbeldam, A. (1973) Lancet *I*, 726.
5. Wagstaff, T. I., Whyley, G. A. & Freedman, G. (1974) J. Obstet. Gynaecol. Br. Cwlth. *81*, 264–277.
6. Penney, L. L., Hagerman, D. D. & Sei, C. A. (1976) Clin. Chem. *22*, 681–682.
7. Fruchart, J. C., Ponthieu, A., Porchet, N., Dewailly, P., Sezille, G. & Puech, F. (1977) Clin. Chim. Acta *79*, 549–556.
8. Cherayil, G. D., Wilkinson, E. J. & Borkowf, H. (1977) Obstet. Gynecol. *50*, 682–688.
9. Gebhardt, D. O. E., Beintema, A. & Reman, F. C. (1979) Clin. Chim. Acta *94*, 93–100.
10. Sutcliffe, R. G. & Brock, D. J. H. (1973) J. Obstet. Gynaecol. Br. Cwlth. *80*, 721–727.
11. Duck-Chong, C. G. (1978) Life Science *22*, 2025–2030.
12. Duck-Chong, C. G., Gupta, J. M. & Storey, G. N. (1980) Clin. Chem. *26*, 766–769.
13. Verhoeven, A. G. J. & Merkus, H. M. W. (1974) Clin. Chim. Acta *53*, 229–232.
14. Bhagwanani, S. G., Fahmy, D. & Turnbull, A. C. (1972) Lancet *II*, 66–67.
15. Oulton, M. (1979) Am. J. Obstet. Gynecol. *135*, 337–343.
16. Duck-Chong, C. G., Brown, L. M. & Hensley, W. J. (1981) Clin. Chem. *27*, 1424–1426.
17. Oulton, M., Martin, T. R. & Faulkner, G. T. (1980) Pediatr. Res. *14*, 722–728.
18. Cruz, A. C. & Spellacy, W. N. (1976) Am. J. Obstet. Gynecol. *126*, 78–82.
19. Morrison, J. C. & Fish, S. A. (1977) Am. J. Obstet. Gynecol. *127*, 363–368.

D. O. E. Gebhardt, Ph. D.
Department of Obstetrics
University Hospital
Rijnsburgerweg 10
NL-2333 AA Leiden