The role of phosphatidyl-glycerol in the determination of fetal lung maturity

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Summary

Phosphatidyl-glycerol (PG) and the lecithin/sphingomyelin (L/S) ratio were determined on amniotic fluid from 140 patients to evaluate the role of PG estimation as a functional fetal lung maturity test. PG was absent without associated respiratory distress syndrome (RDS) in 17 cases (12%) and present in 123; 3 of the infants born to these patients developed slight hyaline membrane disease. There were no neonatal deaths. When both PG and the L/S ratio were determined the accuracy of prediction of the RDS improved from 87% to 93%. PG determination should not be used as a substitute for the L/S ratio, but should be used in conjunction with it in high-risk situations.

S Afr Med J 1983; **63:** 45-47.

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Date received: 8 January 1982.

Since the introduction of routine amniocentesis with determination of the lecithin/sphingomyelin (L/S) ratio, the incidence of iatrogenic neonatal respiratory distress syndrome (RDS) has shown a marked decrease. Kulovich *et al.*[1] in a compilation of several reports involving more than 1 200 neonates showed that mature L/S ratios predicted absence of the RDS with about 98% accuracy. According to Harvey *et al.*[2] a low L/S ratio (less than 2,0) is only 54% accurate in predicting the development of the RDS. Infants born to patients with diabetes mellitus and Rh iso-immunization are at risk of developing the RDS, even if the L/S ratio is 2,0 or more. Kulovich *et al.*[1] therefore developed a 'lung profile', which includes the L/S ratio and the results of acetone precipitable lecithin, phosphatidyl-glycerol (PG) and phosphatidyl-inositol (PI) estimation. This lung profile improved the ability to diagnose unsuspected lung maturity in infants born to mothers with low L/S ratios to 93%.

Surfactant is a complex mixture of lipids (80 - 90% by weight), protein (10 - 20% by weight) and carbohydrates (1 - 2% by weight).[3] Eighty to ninety per cent of the lipid fraction is phospholipid, of which 70 - 80% is phosphatidylcholine (lecithin). Of the minor phospholipids, PG is the most abundant, accounting for up to 10% of the surfactant phospholipids in the adult. It is synthesized in the microsomal fraction of the type II alveolar cell. The remaining phospholipids are PI, sphingomyelin, phosphatidylethanolamine (PE) and others of minor importance.

The purpose of this study was to evaluate the role of PG determination as a functional fetal lung maturity test.

Patients and methods

Over a period of 13 months (July 1980 - August 1981) L/S ratio and PG determination and 'bubble tests' (foam stability tests) were performed on amniotic fluid from 182 unselected patients. For the patient to qualify for inclusion in this study, delivery of the infant had to occur within 72 hours of the amniocentesis. According to these criteria, 140 patients were found suitable for inclusion. A full record was kept for each patient (name, folder number, date of amniocentesis, diagnosis, gestational age, L/S ratio, bubble test result, presence or absence of PG, method of delivery and date of delivery). The sex, mass, Agpar score and respiratory status of each infant were recorded at birth. Neonates were evaluated by the paediatric staff, and particular attention was paid to the development of the RDS. The diagnosis of RDS was based on clinical features (tachypnoea of 60/min, rib retraction, sternal recession and grunting), the radiological appearance of the lung fields, and capillary blood gas values. The results of L/S ratio and PG determination and the bubble test were correlated with the patient's clinical diagnosis and the development of RDS in the neonate.

The L/S ratio was determined according to a modification of the technique described by Gluck *et al.*,[4] while the foam stability test was performed according to the method of Clements *et al.*[5] PG was determined by a modification of the technique used by Hallman and Teramo.[6]

Silica gel H plates, impregnated with 5% $(NH_4)_2SO_4$ and activated in an oven at 120°C for 15 minutes, were used for thin-layer chromatography. The phospholipids were isolated from the amniotic fluid by centrifugation after addition of methanol and chloroform. Acetone precipitation of the surface-active phospholipids was included in this technique.[3] The precipitate was dissolved in chloroform and applied to the activated silica gel plates, along with the standards of the different phospholipid fractions, namely L/S, PG, PI, and PE.

The plates were first run in one direction, in a solvent of chloroform-methanol-acetic acid-H_2O (130 ml-50 ml-16 ml-8 ml), and dried with warm air; subsequently they were run in another direction in a solvent of tetrahydrofuran-methylal-methanol-liquid ammoniac (100 ml-74 ml-16 ml-11 ml). They were then dried and left in a bath containing bromothymol blue detector (bromothymol blue 0,150 g, boracic acid 0,005 g, Na_2CO_3 50 g, made up to 1 000 ml with distilled water). Finally, they were blotted dry and dried in an oven.

The plates were interpreted with a Vitatron densitometer. The L/S ratio as well as the PG/PI ratio and the PG/S (PG/sphingomyelin) ratio were plotted in this way. For the purpose of this study PG was only reported as present or absent, along with the L/S ratio.

The majority of the tests were performed on the same day as the amniocentesis. If the amniotic fluid sample was collected too late it was kept in a refrigerator at −4°C and analysed the following day.

Results

The clinical diagnoses of the 140 patients are summarized in Table I. Diagnoses included diabetes mellitus (41 cases), hypertensive diseases (39 cases), intra-uterine growth retardation (13 cases), and Rh iso-immunization (9 cases); there were also 23 patients undergoing elective caesarean section. PG was absent without associated RDS in 17 cases (12,14% of the total) (Table II). Of these patients 3 had diabetes mellitus, 11 had hypertension, and 3 had other problems. PG was found to be present in 123 cases. Nine infants in this group developed the RDS (6,42%), although there were no neonatal deaths. Only 3 of these 9 infants had mild hyaline membrane disease (HMD); none required assisted ventilation. The other 6 had aspiration pneumonia (5 cases) and transient tachypnoea of the newborn (1 case), probably due to hypoglycaemia. These infants were born to mothers with diabetes (3 patients), hypertension (2), Rh sensitization (1) and other diseases (3) (Table II). Of the 123 patients in whom PG was found to be present, 3 had an L/S ratio below 2,0. PG was found to be absent in 3 of the 41 patients with diabetes. None of their infants developed RDS. Conversely, 3 of the infants born to the 38 diabetic mothers in whom PG was present developed RDS, although none had HMD. None of the diabetics had an L/S ratio of less than 2,0. The L/S ratio was 2,0 - 2,5 in 3 cases and 2,6 - 3,0 in 15; the rest had an L/S ratio of less than 3,0.

TABLE I. CLINICAL DIAGNOSES OF THE PATIENTS

		No. of patients
Diabetes mellitus*		**41**
Whites class A	**17**	
Whites class B	**5**	
Whites class C	**3**	
Not classified	**16**	
Hypertension and pre-eclampsia		**39**
Rh iso-immunization		**9**
Diverse		**51**
Elective caesarean section	**23**	
Postmaturity	**3**	
Intra-uterine growth retardation	**13**	
Other	**12**	
Total		**140**

*Class A = chemical diabetes; B = maturity-onset diabetes (age over 20 years), duration < 10 years, no vascular lesions; C = age 10-19 years at onset, and diabetes of 10-19 years' duration.

The accuracy of prediction of lung maturity is shown in Table III. As a single factor, the L/S ratio was 87% accurate, compared with the 86% of PG alone. This difference is statistically insignificant. In combination the accuracy of prediction was 93%.

TABLE II. RDS IN RELATION TO AMNIOTIC FLUID L/S RATIO AND PRESENCE OR ABSENCE OF PG IN 140 PATIENTS

	PG present with RDS	PG absent without RDS	L/S < 2,0 PG present without RDS
Diabetes	**3**	**3**	**0**
Hypertension	**2**	**11**	**2**
RH iso-immunization	**1**	**0**	**2**
Diverse	**3**	**3**	**4**
Total	**9*** **(6,42%)**	**17 (12,14%)**	**8 (5,51%)**

*See text for further discussion.

TABLE III. ACCURACY OF PREDICTION OF LUNG MATURITY WITH L/S RATIO, PRESENCE OF PG AND A COMBINATION OF BOTH

	Mature L/S (≥ 2)	PG present	Mature L/S and/or PG present
Diabetes	39	38	39
RH iso-immunization	7	9	9
Hypertension	35	28	37
Diverse	44	48	48
Total	125	123	133
HMD		3 cases (see text)	
Mature (no HMD)	122	120	130
Accuracy of prediction of maturity	97,60% (122 of 125)	97,56% (120 of 123)	97,74% (130 of 133)
Accuracy of prediction of total	87,14% (122 of 140)	85,71% (120 of 140)	92,85% (130 of 140)

TABLE IV. PREVALENCE OF RDS AND HMD IN RELATION TO AMNIOTIC FLUID L/S RATIO AND PRESENCE OR ABSENCE OF PG

	All cases					PG present					PG absent	
		RDS		HMD			RDS		HMD			No. with
L/S ratio	Total	No.	%	No.	%	Total	No.	%	No.	%	Total	RDS
≥ 2,0	125	6	4,8	2	1,6	120	6	5	2	1,7	17	0
< 2,0	15	3	20	1	6,7	3	3	100	1	33,3	0	0
Total	140	9	6,4	3	2,1	123	9	7,3	3	2,4	17	0

Discussion

For predicting the RDS in the newborn the presence of PG had a false-negative value of 12% and a false-positive value of 7,3% (Tables II and IV). Similar results were obtained when the L/S ratio was used alone (Table III). Combining these two improved the accuracy of prediction of the RDS from 87% to 93% (Table III). This compares favourably with the 93% accuracy of Gluck's lung profile.

When compared with the L/S ratio PG determination has the advantage that PG is not affected by contamination of amniotic fluid with blood, vaginal secretions or meconium.[1,7-9] Disadvantages of PG determination include the fact that it is technically more difficult, more time-consuming and more expensive than determination of the L/S ratio alone. The present study confirms previous findings that the mere presence of PG in amniotic fluid is a good indication of functional biochemical fetal lung maturity.[1,10,11] However, the absence of PG is not an indication that the RDS will develop. Seventeen patients (12%), of whom 11 had hypertensive disease, had a false-negative PG result. This was not found in previous studies, and needs further investigation.

In a previous study from our laboratory the foam test was compared with the L/S ratio as a determinant of lung maturity.[12] For the foam test a false-positive rate of 8,3% and a false-negative rate of 27,1% were found. This high false-negative rate made the foam test suitable for screening purposes, but the L/S ratio remained the more important diagnostic test. The present study confirmed these findings; it was possible to perform the foam test in 100 cases, and it was found to have a false-positive rate of 2% and a false-negative rate of 20%.

We conclude that PG determination is not a substitute for the L/S ratio but should be used in conjunction with it in high-risk situations. This will improve the accuracy of prediction of fetal lung maturity by 6%. When amniotic fluid is contaminated with blood or meconium determination of PG is still reliable, whereas the L/S ratio and foam test become unreliable.

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