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## Relationship between amniotic fluid optical density and L/S ratio

Jacobus H. P. Slothouber<sup>1</sup>, Peter K. Flu<sup>1</sup>, and Henk C. S. Wallenburg<sup>2</sup>

<sup>1</sup>Department of Obstetrics and Gynecology of the Ikazia Ziekenhuis, Rotterdam, and <sup>2</sup>Department of Obstetrics and Gynecology, Erasmus University Medical School, Rotterdam, The Netherlands

### 1 Introduction

Determination of the lecithin-sphingomyelin (L/S) ratio as described by GLUCK et al. [8] is generally considered an accurate test to predict fetal lung maturation. A serious disadvantage of determination of the L/S ratio is that it requires rather complicated laboratory techniques which are not always available in small hospital laboratories.

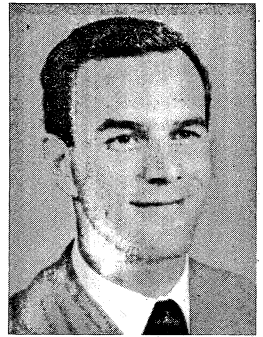
In 1976 SBARRA et al. [16] reported a simple test based on the determination of amniotic fluid optical density at 400 nm (OD 400) which demonstrated good agreement with the L/S ratio. A later study suggested an even better correlation between the L/S ratio and amniotic fluid OD 650 [17]. The clinical reliability of the determination of the amniotic fluid OD 650 to predict fetal lung maturation was assessed in various studies [1, 3, 4, 18] with varying and even conflicting results. This stimulated us to further evaluate the relationship between amniotic fluid optical density at 650 nm and L/S ratio.

### 2 Material and methods

Ninety samples of amniotic fluid were obtained by transabdominal amniocentesis between 26 and 39 weeks' gestation from 90 pregnant women who attended the antenatal clinic or

### Curriculum vitae

JACOBUS HENDRIK PIETER SLOTHOUBER was born on December 9, 1956 in The Hague, The Netherlands. He studied medicine at the Erasmus University of Rotterdam from which he was graduated in 1982. Having completed a fellowship at the Ikazia Hospital in Rotterdam, he has been a resident in Obstetrics and Gynecology at the Erasmus University Hospital since 1985.



were admitted to the Department of Obstetrics and Gynecology of the Ikazia Hospital, Rotterdam. Patients in group A (n = 49) underwent amniocentesis for determination of fetal lung maturation because of threatened premature labor. They were eventually delivered of live and adequate-for-gestational age infants with a birth weight above the 10th percentile of the normal intrauterine growth curve corrected for parity and fetal sex [10]. All women in group B (n = 25) underwent amniocentesis because of fetal growth retardation, confirmed by repeat ultra sonography. All women were delivered of live infants with birth weights below the 10th percentile of the intrauterine growth curve. Group C (n = 16) consisted of rhesus negative pregnant women with circulating

rhesus antibodies. The primary reason for amniocentesis in this group was spectrophotometric determination of the bilirubin concentration. Most of these women underwent several amniocenteses, but only the last one was used in this study. The three groups did not contain patients with hypertension or diabetes mellitus. All women were delivered at least one week after amniocentesis, between 34 and 41 weeks of gestation.

Sonographic placental localization was performed by means of an M. C. U. Technique before amniocentesis. The amniotic fluid samples were centrifuged at 2000 g for 10 minutes within one hour, and optical density at 650 nm was determined in an aliquot of the supernatant using a Zeiss spectrophotometer and 1 cm light-pass cuvettes [17], with saline as a blank. A value of 0.15 or more was considered to indicate fetal lung maturation [17]. In an aliquot of the same supernatant the L/S ratio was determined as described by GLUCK et al.[8] with modifications [19]. All values obtained were corrected to a standard ratio of 2.0, which allows the use of a value of 2.0 or larger as predictive of fetal lung maturation [6]. Samples contaminated with blood or meconium were excluded.

The statistical association between the values of the L/S ratio and the OD 650 was evaluated by Spearman's non-parametric test. A p value of less than 0.05 was considered to represent statistical significance.

### 3 Results

The relationship between the OD 650 and the L/S ratio in group A is shown in figure 1. The statistical correlation is significant ( $p < 0.001$ ). However, 7 of the 24 optical density readings of 0.15 or larger are associated with L/S ratios of less than 2.0. This means that 29% of the optical density readings can be considered false positive against the "gold standard" of an L/S ratio of 2.0 or higher indicating fetal lung maturity. Similarly, 7 of 25 OD 650 readings can be considered false negative (28%). Also in the samples from group B and C (figures 2 and

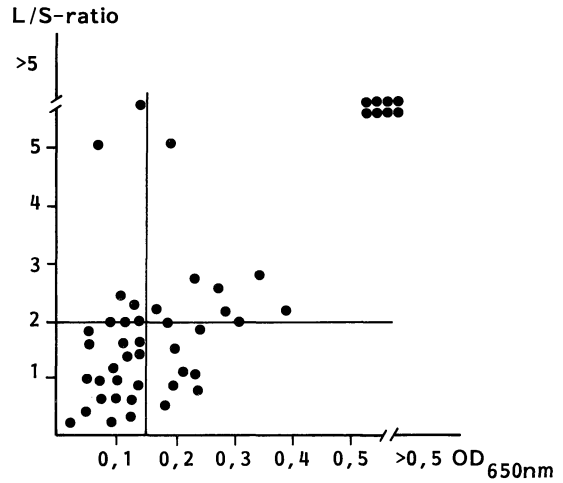


Figure 1. Correlation between L/S ratio and OD 650 in group A (n = 49);  $p < 0.001$ ;  $R_s = 0,59$ .)

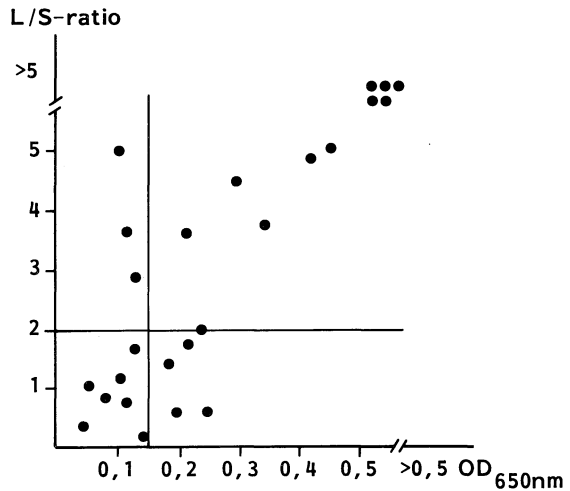
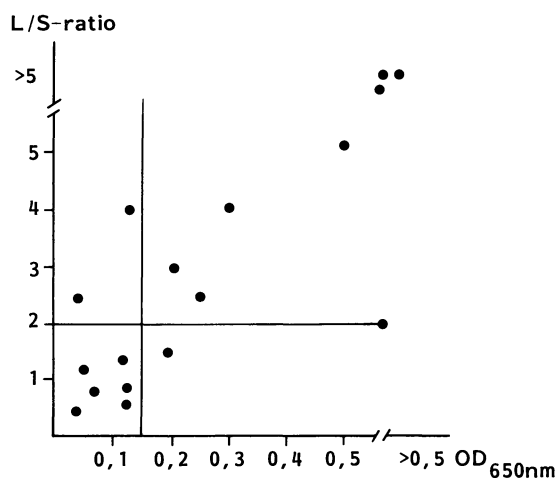


Figure 2. Correlation between L/S ratio and OD 650 in group B (n = 25;  $p < 0.001$ ;  $R_s = 0,76$ .)

3) a significant positive correlation between the two variables is found ( $p < 0.001$  and  $p < 0.05$ ). The percentages of false positive and false negative values of the optical density readings in group B are similar to those in group A (26 and 30%), respectively). When all samples are taken together, 25.5% of the OD 650 readings appear to be false positive, and 28% are false negative.



**Figure 3.** Correlation between L/S ratio and OD 650 in group C (n = 16; p < 0.05;  $R_s = 0,66$ .)

#### 4 Discussion

Determination of the L/S ratio in amniotic fluid is generally considered the most reliable test of fetal lung maturation. Although the method of L/S determination has been simplified after its initial introduction by GLUCK and associates [8], the method is still complicated and cannot easily be done on a 24-hour basis in routine obstetric practice. For that reason more simple tests were developed, including optical density readings at 400 and 650 nm. Most likely the changes in optical density of amniotic fluid during the course of pregnancy reflect turbidity changes dependent on the total phospholipid concentration [2, 14].

The results of the comparative study between values of the OD 650 and the L/S ratio reported here are at variance with the earlier findings of SBARRA et al. [16, 17]. There appears to be

a high percentage of false positive and false negative optical density readings when the L/S ratio is used as the standard. The predictive value of a positive optical density test, i.e. a reading of 0.15 or above, varies between 71 and 73% in our three groups of patients. The predictive value of a negative test is approximately 70% in all three groups. Other investigators [4, 12, 15] have reported the same or slightly better results. Because all women were delivered after 34 weeks and the neonatal respiratory distress syndrome did not occur, we could not examine the clinical relationship between OD 650 and neonatal lung maturity.

It has been shown that OD 650 determinations are markedly influenced by the speed of centrifugation [13] and by the presence of blood or meconium in the amniotic fluid samples. In our samples no blood or meconium was present. The morphologic and biochemical properties of the so-called lamellar bodies in amniotic fluid, which contain dipalmityl-lecithine and other phospholipids are known to change in the course of pregnancy [5, 9, 14]. Centrifugation will remove most of the phospholipid containing lamellar bodies from the supernatant and also a varying amount of phospholipids will disappear [17]. This may, at least in part, be responsible, for the frequent discrepancy between the L/S ratio and the OD 650, and may be prevented by using a differential centrifugation procedure [11].

We conclude from our study that a correlation exists between the OD 650 and the L/S ratio in amniotic fluid, but that the relatively high number of false positive and false negative optical density readings precludes a reliable clinical application of this simple test developed to assess fetal lung maturity.

#### Summary

Determination of the lecithin-sphingomyelin (L/S) ratio to predict fetal lung maturation requires rather complicated laboratory techniques which are not always available on a 24-hour basis in all hospitals. For that reason more simple tests have been developed. One of these tests, the determination of amniotic fluid optical density

at 650 nm (OD 650), was initially reported to correlate well with the L/S ratio, but later studies gave varying and conflicting results. To assess the possible usefulness of measurement of amniotic fluid OD 650 we determined the correlation between L/S ratio and OD 650 in 90 amniotic fluid samples from 90 pregnant women. All

samples were obtained by transabdominal amniocentesis between 26 and 39 weeks' gestation. Indications for amniocentesis were threatened premature labor ( $n = 49$ ), fetal growth retardation ( $n = 25$ ), and rhesus sensitization ( $n = 16$ ). The OD 650 and the L/S ratio were determined in the same amniotic fluid sample using standard techniques. A statistically significant positive correlation was found between the OD 650 and the L/

S ratio. Considering an OD 650 reading of 0.15 or greater and an L/S ratio of 2.0 or higher as indicative of fetal lung maturation, 25.5% of the OD 650 readings appeared to be false positive, and 28% were false negative as compared with the L/S ratio. It is concluded that the low predictive values of positive and negative optical density readings preclude the clinical application of this simple test.

**Keywords:** Amniocentesis, amniotic fluid, L/S ratio, lung maturity, optical density.

## Zusammenfassung

### Beziehung zwischen der optischen Dichte von Fruchtwasser und der L/S-Ratio

Die Bestimmung des Lezithin-Sphingomyelin (L/S) Verhältnisses zur Fragestellung der fetalen Lungenreife erfordert relativ komplizierte Laboruntersuchungen, die nicht jedes Krankenhaus innerhalb von 24 Stunden zur Verfügung stellen kann. Aus diesem Grunde wurden einfachere Methoden entwickelt. Einer dieser Tests, die Bestimmung der optischen Fruchtwasserdichte bei 650 nm (OD 650), sollte nach anfänglichen Berichten gut mit der L/S-Ratio korrelieren, aber spätere Untersuchungen brachten keine übereinstimmenden Ergebnisse. Um eine Aussage über den möglichen Nutzen der Messung der optischen Dichte von Fruchtwasser zu treffen, verglichen wir die Korrelation zwischen L/S-Ratio und OD 650 in 90 Fruchtwasserproben von 90 schwangeren Frauen.

Alle Proben wurden durch die transabdominale Amniozentese zwischen der 26. und 39. Gestationswoche gewonnen. Indikationen für die Amniozentese waren vorzeitige Wehen ( $n = 49$ ), fetale Hypotrophie ( $n = 25$ ), und Rhesus-Sensibilisierung ( $n = 16$ ). Die OD 650 und die L/S-Ratio wurden in derselben Probe mit Standardmethoden bestimmt. Eine statistisch signifikante positive Korrelation wurde zwischen diesen beiden Parametern gefunden. Wenn man eine OD 650 Ableseung von 0,15 oder größer und eine L/S-Ratio von 2,0 oder höher als Hinweis für eine fetale Lungenreife wertet, sind, verglichen mit der L/S-Ratio, 25,5% der OD 650 Ableseungen falsch positiv, und 28% falsch negativ. Daraus ergibt sich, daß der geringe Aussagewert einer positiven oder negativen OD Ableseung eine klinische Anwendung dieses einfachen Tests ausschließt.

**Schlüsselwörter:** Amniozentese, Fruchtwasser, L/S-Ratio, Lungenreife, optische Dichte.

## Résumé

### Relation entre la densité optique du liquide amniotique et le rapport L/S

La détermination du rapport L/S nécessite des techniques de laboratoire plutôt compliquées, techniques qui ne sont pas toujours réalisables 24 heures sur 24 dans tous les hôpitaux. C'est pour cette raison qu'on a mis au point des tests plus simples. Un de ces tests est la détermination de la densité optique du liquide amniotique à 650 nm (OD 650); cette détermination au début a été estimée comme bien corrélée avec le rapport L/S, mais des études ultérieures ont apporté des résultats variables et contradictoires. Nous avons déterminé la corrélation entre le rapport L/S et l'OD 650 du liquide amniotique sur 90 liquides amniotiques provenant de 90 femmes enceintes pour évaluer l'utilité potentielle de l'OD 650 du liquide amniotique. Tous les liquides ont

été obtenus par amniocentèse transabdominale entre 26 et 39 semaines de gestation. Les indications de l'amniocentèse ont été une menace d'accouchement prématuré ( $n = 49$ ), un retard de croissance intra-utérin ( $n = 25$ ) et une immunisation rhesus ( $n = 16$ ). On a déterminé l'OD 650 et le rapport L/S par des techniques standards sur les mêmes échantillons. On a trouvé une corrélation positive significative sur le plan statistique entre l'OD 650 et le rapport L/S. Si l'on considère qu'une OD 650 supérieure ou égal à 0,15 et qu'un rapport L/S supérieur ou égal à 2 indiquent une maturation pulmonaire fœtale suffisante, 25,5% des OD 650 sont des faux positifs et 28% sont des faux positifs en comparaison avec les rapports L/S. On en conclut que la faible valeur prédictive des densités optiques exclue l'utilisation clinique de ce test simple.

**Mots-clés:** Amniocentèse, densité optique, liquide amniotique, maturité pulmonaire, rapport L/S.

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Dr. Peter K. Flu  
Department of Obstetrics and Gynecology  
Ikazia Ziekenhuis  
Montessoriweg 1  
3083 AN Rotterdam, The Netherlands