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# Prenatal prediction of respiratory distress syndrome

## Measurement of surface properties and Lecithin/Sphingomyelin ratio in human amniotic fluid

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The postnatal changes of the lung in the neonatal respiratory distress syndrome (RDS) resemble in their pathological appearance [52] to a large extent those seen in adult shock lung [6, 23, 27, 38].
In addition to neurological [22] and morphological [54] immaturity, RDS is primarily caused by a lack of surface active substances [43].
The various constituents of surfactant, such as protein, mucopolysaccharides, electrolytes and phospholipids form a complex biological system [53] the sum of which ensures alveolar stability [30].
Phospholipids are considered to be most effective fraction [14, 15, 16, 18]. They enter the amniotic fluid and thus are available for diagnostic procedures which form the basis for all conventional assays for pulmonary maturity [summarized in 2, 40, 50].
Among these methods the determination of the L/S ratio according to GLUCK [17] is the most widely used [3, 4, 9, 10, 11, 12, 13, 19, 21, 25, 26, 31, 34, 46, 48]. With few exceptions [39] it is undisputed in its predictive power. Therefore, the determination of the lecithin/sphinomyelin ratio (L/S ratio) is the method with which others have to be compared [5, 7, 29, 33, 37, 41, 42, 45, 49, 51].

### Curriculum vitae

PETER KRIEGLSTEINER, *MD, was born in 1943 in Eger/CSR. High school at Regensburg, study of medicine at the University of Munich. State Examination in 1969, MD graduation in 1970. Since 1970 at the department of Gynecology and Obstetrics of the "Technische Universität", Munich. In 1975 he was certified as a specialist in gynecology and obstetrics. Since 1975 in laboratory research together with the staff of Prof.* G. BLÜMEL, *"Institut für Experimentelle Chirurgie der Technischen Universität", Munich.*

The function of the total fetal surfactant system can be judged by determining the surface tension of amniotic fluid with the WILHELMY balance [28, 32, 35, 36, 44].
This study will describe the possibilities of the prenatal prediction of RDS by biomechanical surface activity measurements in comparison to the L/S ratio.

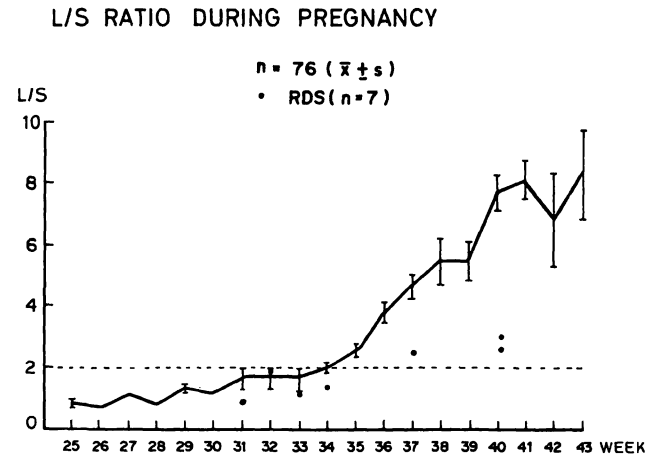


Fig. 1. L/S ratio: normal values and RDS cases.

## 1 Material and methods

### 1.1 Material

One hundred and eighteen amniotic fluid samples were obtained from 102 pregnant women between the 25th and 43rd week of gestation. In 43 cases the samples were obtained during labor through amniotomy under amnioscopic control; all other samples were obtained by transabdominal amniocentesis. We got in each case about 30 ml of amniotic fluid. Samples contaminated with blood or meconium were discarded [5].

Normal pregnancies yielded 76 samples in which the history did not suggest acceleration or retardation of pulmonary maturity [20, 21]. From these samples normal values were established. In 37 cases the sample was obtained within the 72 hours required by GLUCK [2] for a valid RDS prediction. In 7 cases the infants developed idiopathic respiratory distress syndrome [24]. Diagnosis was made radiographically and was documented clinically by the SILVERMAN retraction score. In all samples the following examinations were carried out:
out:

(a) biomechanical measurement of surface tension (ST) with a WILHELMY balance,
(b) determination of L/S ratio.

### 1.2 Biomechanical measurements

The amniotic fluid was centrifugated for 10 min at 3000 rpm (1250 g) to remove visable contaminants. Then the undiluted samples were filled into the teflon trough (internal dimensions 132 X 59 X 14 mm) of the WILHELMY balance (manufacturer BIEGLER, model ATF 0.1).

After the immersion of the platinum wire float half an hour was waited in order to facilitate the formation of a surfactant monolayer. By means of a transducer the float measures the surface tension. During the measurement, a moveable barrier compresses the area of the monolayer from an original value of 100% (ST at 100% = $\gamma$ max) to 20% (ST at 20% = $\gamma$ min). Subsequently, the barrier is reversed toward the original position. The barrier and the float-transducer system were connected to an x-y recorder. Cycles of 4 minutes were repeated until two successive hystersis curves coincided. The stabilization lasted about one hour. The following standard conditions were maintained: 760 mm Hg barometric pressure; 50% relative humidity; 22 °C temperature. The surface tension area diagrams (SAD) were evaluated according to the following standard criteria [28, 32, 44]:

1. $\gamma$ max (at the point of 100% monolayer area) [dyne · cm$^{-1}$]
2. $\gamma$ min (monolayer compressed to 20% of its original area) [dyne · cm$^{-1}$]
3. index of stability (8) $S = \frac{2(\gamma \text{ max} - \gamma \text{ min})}{\gamma \text{ max} + \gamma \text{ min}}$
4. hysteresis area (A). Determined planimetrically (ARISTO Planimeter) [cm$^2$].

### 1.3 Determination of L/S ratio

The L/S ratio was determined simultaneously according to the method of GLUCK [17, 20] as modified by BORER [13, 20]. Following a FOLCH lipid extraction and thin layer chromatographic separation of the lecithin and sphingomyelin fractions we stained with ammonium molybdate and measured the spots planimetrically.

## 2 Results

### 2.1 Normal values [28]

#### 2.1.1 Biomechanical measurements

The samples were divided into three groups:
Group I – 25th to 30th week of gestation
Group II – 31th to 35th week of gestation
Group III – 36th to 43rd week of gestation

Tab. I. Normal values of the ST criteria expressed in the groups ($\bar{x} \pm 1$ S.D.).

| | n | $\gamma$ max (dyn. $cm^{-1}$) | $\gamma$ min (dyn. $cm^{-1}$) | S | F $cm^2$ |
|---|---|---|---|---|---|
| group I | 8 | 55,4 ± 2,5 | 34,5 ± 3,5 | 0,46 ± 0,15 | 10,1 ± 2,2 |
| group II | 14 | 52,0 ± 1,5 | 25,9 ± 3,5 | 0,65 ± 0,12 | 17,5 ± 1,3 |
| group III | 54 | 47,1 ± 3,0 | 17,6 ± 4,0 | 0,93 ± 0,19 | 26,5 ± 5,3 |

In Group I survival chances are considerably diminished because of general and especially CNS immaturity. In Group III one may expect infants with pulmonary maturity. Stimulation of surfactant synthesis would appear most desirable in Group II. The results of the four single criteria can be seen in Tab. I.

### 2.1.2 L/S ratio

The steep rise of the L/S ratio from the 36th gestational week indicates the increased activity of the lecithin biosynthesis.

## 2.2 Prediction of RDS

### 2.2.1 Patient outcome

For the prediction of RDS, we used only those samples which had been obtained within 72 hours of birth ([29]. Among these 37 cases, there were 7 patients with RDS (Tab. II).
Up to the 34th gestational week it is thought that the L/S ratio of 2:1 indicates sufficient surfactant production, but it does not allow unequivocal interpretation. Afterwards all RDS cases have an L/S ratio far below the value found in the corresponding week during normal pregnancies (Fig. 2). The SAD of the cases with RDS show high initial values ($\gamma$ max) and little decrease of surface tension during compression (elevated $\gamma$ min). This is reflected in a decrease of the stability index. Noticable is the considerable decrease of the hysteresis area (Fig. 3). A comparison of the mean values of cases without

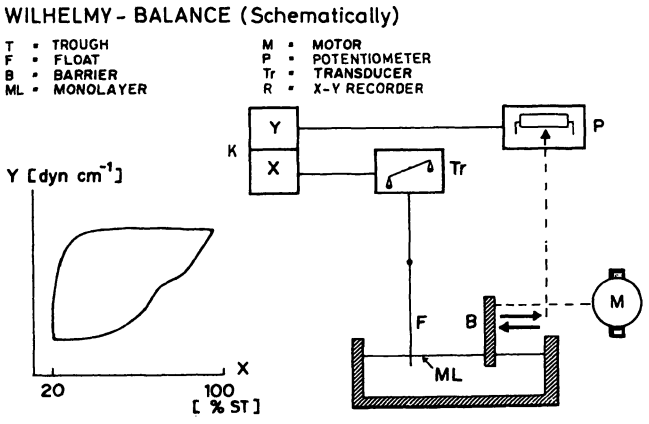


Fig. 2. WILHELMY balance.

Tab. II. Patients with RDS: Outcome.

| Nr. | Pat. | wk. gest. | birth weight | length (cm) | biparietal Ø (cm) | APGAR 1'/5'/10' | delivery | L/S ratio | $\gamma$ max (dyn. $cm^{-1}$) | $\gamma$ min (dyn. $cm^{-1}$) | S | A ($cm^2$) | RDS (class) | outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M. C. | 31 | 1400 | 43 | 8.0 | 5/ 7/ 7 | Spont | 1.0 | 59.5 | 41.0 | 0.36 | 7 | I | died on day 5 |
| 2 | S. A. | 32 | 1570 | 41 | 8.1 | 6/ 9/10 | breech | 1.9 | 55.0 | 32.0 | 0.52 | 10 | I–II | Normal |
| 3 | S. S. | 33 | 1780 | 43 | 8.2 | 6/ 9/10 | Spont | 1.3 | 55.0 | 32.0 | 0.52 | 11 | I–II | Normal |
| 4 | A. Z. | 34 | 1950 | 45 | 8.0 | 9/10/10 | Spont | 1.5 | 56.0 | 33.5 | 0.50 | 13 | I–II | Normal |
| 5 | E. S. | 37 | 2320 | 46 | 8.7 | 7/ 9/10 | Spont | 2.5 | 55.0 | 27.5 | 0.67 | 16 | I–II | Normal |
| 6 | C. F. | 40 | 2650 | 48 | 9.3 | 7/ 9/10 | Spont | 3.1 | 53.5 | 33.0 | 0.42 | 17 | II | Normal |
| 7 | S. G. | 40 | 2400 | 48 | 9.2 | 8/10/10 | Section | 2.6 | 51.5 | 28.5 | 0.58 | 20 | I–II | Normal |

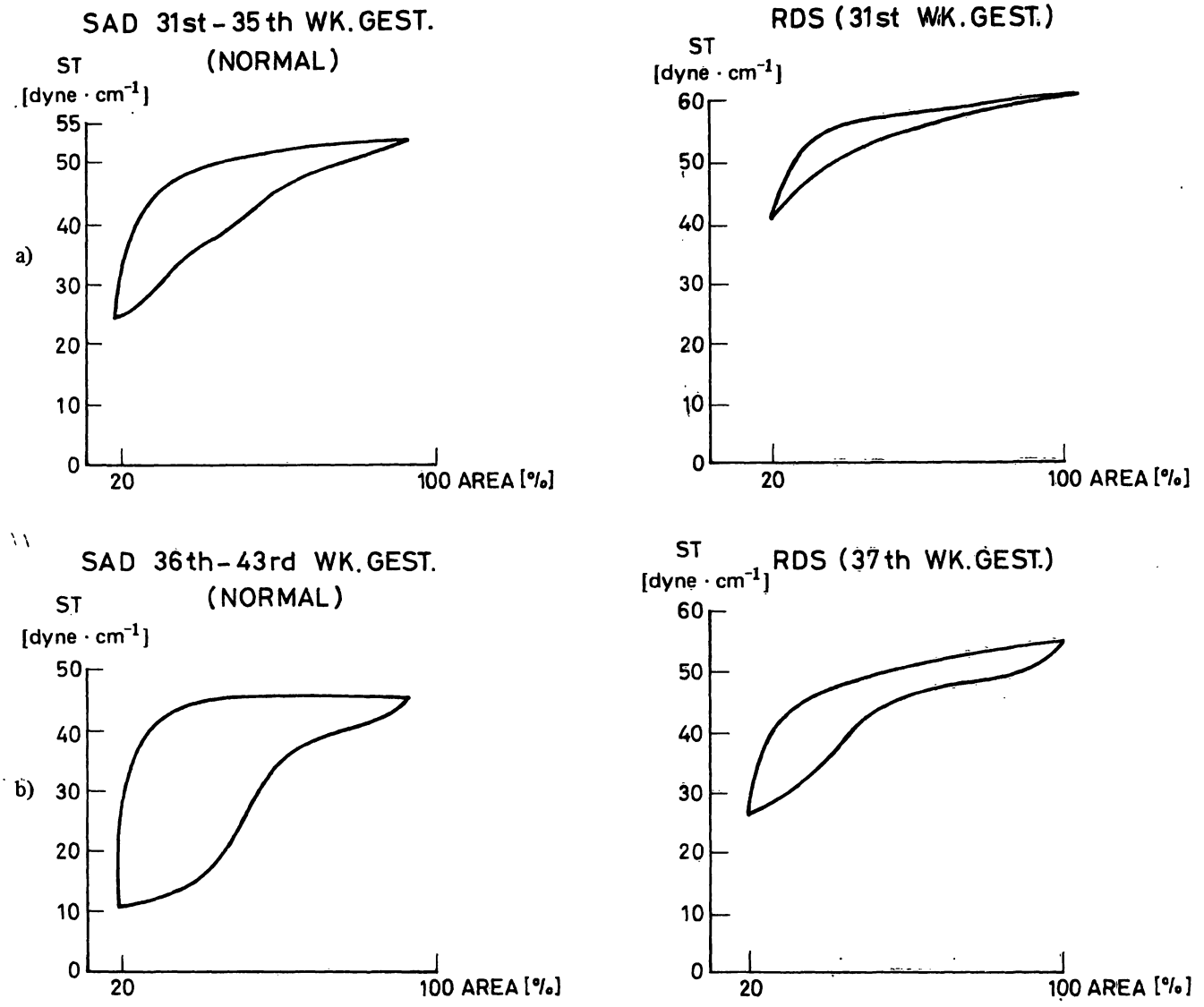


Fig. 3. Surface tension/area diagram,
a). Group II – normal cases and RDS at 31 weeks gestational age,
b). Group III – normal cases and RDS at 37 weeks gestational age.

RDS (n = 30) and those with RDS (n = 7) show statistically significant differences (WILCOXON test) for the L/S ratio as well as for each of the four criteria (Tab. III).

### 2.2.2 Prognostic determination

In order to give the prognostic relevance our results were tested as suggested by LORENZ [33]:

(1). Probability of prediction (%) =

$$\frac{\text{predicted RDS cases}}{\text{actual RDS cases}}$$

Tab. III. L/S ratio and values of ST criteria of amniotic fluid samples, which have been taken within the limit of 72 hours.

| | no RDS n = 30 | RDS n = 7 |
|---|---|---|
| LS | 5,90 ± 2,56 | 2,00 ± 0,77 * |
| $\gamma$ max [dyne · cm$^{-1}$] | 47,75 ± 2,54 | 55,00 ± 2,44 * |
| $\gamma$ min [dyne · cm$^{-1}$] | 19,47 ± 4,62 | 32,50 ± 4,38 * |
| S | 0,86 ± 0,19 | 0,51 ± 0,10 * |
| A [cm$^2$] | 25,00 ± 5,37 | 13,42 ± 4,50 * |

* $p < 0.01$

(2). Probability of occurrence (%) =

$$\frac{\text{number of RDS with values below threshold}}{\text{number of values below threshold}}$$

(3). Probability of exclusion (%) =

$$\frac{\text{number of cases of RDS with values above threshold}}{\text{all values above threshold}}$$

The denominator is 100% in each case.

Tables IV–VII indicate the different values for the probabilities of prediction, occurrence and exclusion with various values for the limits for the single SAD criteria as determined by us.

The L/S ratio was examined in a similar fashion. The values for the threshold were chosen as described by LORENZ [33] (Tab. VIII).

Tab. IV. The different values for the probabilities of prediction, occurence, and exclusion. Criterion: $\gamma$ max.

| | thresholds (dyn. $cm^{-1}$) | | |
|---|---|---|---|
| | $\geqslant 56$ | $\geqslant 53$ | $\geqslant 50$ |
| Probability of prediction | 29% | 86% | 100% |
| Probability of occurence | 100% | 86% | 54% |
| Probability of exclusion | 86% | 97% | 100% |

Tab. V. The different values for the probabilities of prediction, occurence, and exclusion. Criterion: $\gamma$ min.

| | thresholds (dyn. $cm^{-1}$) | | |
|---|---|---|---|
| | $\geqslant 30$ | $\geqslant 25$ | $\geqslant 20$ |
| Probability of prediction | 71% | 100% | 100% |
| Probability of occurence | 83% | 64% | 35% |
| Probability of exclusion | 94% | 100% | 100% |

Tab. VI. The different values for the probabilities of prediction, occurence, and exclusion. Criterion: Index of stability (S)

| | thresholds | | |
|---|---|---|---|
| | $\leqslant 0,5$ | $\leqslant 0,6$ | $\leqslant 0,7$ |
| Probability of prediction | 43% | 72% | 100% |
| Probability of occurence | 100% | 75% | 47% |
| Probability of exclusion | 86% | 97% | 100% |

Tab. VII. The different values for the probabilities of prediction, occurence, and exclusia. Criterion: Area (A)

| | thresholds ($cm^2$) | | |
|---|---|---|---|
| | $\leqslant 15$ | $\leqslant 17,5$ | $\leqslant 20$ |
| Probability of prediction | 57% | 86% | 100% |
| Probability of occurence | 100% | 86% | 47% |
| Probability of exclusion | 91% | 97% | 96% |

Tab. VIII. Prognostic validity of the L/S ratio. Criterion: L/S Ratio

| | thresholds | | |
|---|---|---|---|
| | $\leqslant 2,0$ | $\leqslant 2,5$ | $\leqslant 3,0$ |
| Probability of prediction | 57% | 71% | 86% |
| Probability of occurence | 67% | 56% | 46% |
| Probability of exclusion | 91% | 93% | 95% |

## 3 Discussion

In judging the prognostic validity of a method, it is assumed that laboratory errors are kept at a minimum by standardization and exact performance of the assays. For the determination of the L/S ratio this is easily achieved by use of the GLUCK set. Likewise we attempted with our measurement in the WILHELMY balance to minimize methodological errors: Samples contaminated with blood and meconium were discarded [5,47] and float, trough, and barrier were cleaned with non-detergents. Each assay was preceeded by a run with NaCl to control the purity of the trough and environmental

conditions were kept standard (22°C, 760 mmHg, 50% humidity).

With the L/S ratio we achieved probabilities for prediction and exclusion similar to those of previous investigators (summary in [33]). The probability of occurrence resulting from our planimetrically estimations of thin-layer chromatography are better than usual [12, 37], the results of assays of densitometry, however, are somewhat more precise.

It has to be taken into account that the values for the threshold are not uniform: With increasing L/S ratio the probability of prediction increases, the probability of occurrence decreases and the probability of exclusion rises slightly.

The normal values of our biomechanical measurements conform well with those of previous investigators [32, 35, 36]. In establishing normal values one must exclude the influence of factors which accelerate biosynthesis (cardiac or renal hypertension, placental insufficiency, Group D, E, F diabetes mellitus, heroin addiction of the mother, etc.) as well as those which retard the synthesis (Group A, B, C diabetes mellitus, hydrops fetalis, glomerulonephritis without hypertension, etc.) [20, 21]. The validity of RDS prediction depends on the maintenance of the 72 hour limit as suggested by GLUCK.

The threshold values for the biomechanical determinations had to be assumed by ourselves. The only other information about definitely pathological values is found in the work of MÜLLER–TYL [36].

RÜTTGERS [42] compared the predictive power of the static biophysical surface tension measurements with biochemical standard methods and found similar results.

The difficulty in the evaluation of a surface tension area diagram is that the hysteresis loop can be described mathematically only in a very complex fashion. The four criteria used by us ($\gamma$ max, $\gamma$ min, S. A) probably are the only way which can be done in clinical routine [1, 31, 36]. However, it is necessary to compare $\gamma$ max, $\gamma$ min, S. and A separately with the standard method of the L/S ratio: Correlation analysis demonstrated good correlation between the biomechanical criteria method and the L/S ratio [28]. If the prognostic power of these four criteria is regarded in reference to the probabilities of prediction, occurrence and exclusion of RDS it is found at least equal to that of the conventional L/S ratio.

**Summary**

118 samples of amniotic fluid were taken from 102 patients in the 25th to the 43th week of pregnancy by amniotomy or by transabdominal amniocentesis. 76 samples were obtained from patients with normal pregnancies without evidence for either induction or retardation of lung maturity; they served to establish normal values. In 37 cases samples were taken within 72 hours prior to delivery, the decisive period for the prediction of a respiratory distress syndrome. In seven cases a respiratory distress syndrome occured which was diagnosed by an independent pediatrician.

The following tests were performed for all samples:

1. **Surface tension** was measured biomechanically using the Wilhelmy balance and a surface tension area diagram (**Fig. 1**) was made:

A platinum plate is immersed into the liquid placed in the trough; the surface tension is measured via a transducer. The surface film of the liquid to be studied is compressed cyclically from an initial area of 100% to 20% by a moveable barrier and is expanded afterward. Platinum plate and barrier are connected to an X-Y recorder. The surface tension area diagrams are interpreted on the basis of the following criteria:

a) $\gamma$ max (100% surface film area)

b) $\gamma$ min (compression of surface film area to 20%)

c) stability index $S = \frac{2\,(\gamma\text{max} - \gamma\text{ min})}{\gamma\text{ max} + \gamma\text{ min}}$

d) hysteresis

2. The **determination of the L/S ratio** (simultaneous comparison with the standard method): The method of GLUCK as modified by BORER (planimetric interpreatation of the spots) was employed. Both methods produced normal values. For the biomechanical measurements a classification into three groups was made (25th–30th, 31st–35th, 36th–43rd week of pregnancy) (Tab. I); the L/S ratio showed the usual steep increase from the 36th week of pregnancy onwards.

In cases with RDS (Tab. II) surface tension-area-diagrams show high values for $\gamma$ max and a slight decrease in surface tension on compression (high $\gamma$ min values); the stability

index is low, the hysteresis is reduced. (Fig. 3). Up to the 34 th week of pregnancy the 2:1 L/S ratio assumed as the threshold for a sufficient surfactant concentration does not permit a clear interpretation; in subsequent weeks all cases fail to coincide with the normal pattern of distribution (Fig. 2). Both the L/S ratio and the criteria of the surface tension area diagrams show significant differences in cases with and without RDS.
The prognostic validity of both methods was tested for the probability of predicting occurrence of RDS, probability of RDS occurence and probability of non-occurrence of RDS. **The prognostic value seems equal to the L/S Ratio.**

**Keywords:** Amniotic fluid, L/S ratio, RDS, WILHELMY-balance

## Zusammenfassung

**Antepartale Prognose des Atemnotsyndroms.**
**Biomechanische Messung oberflächenaktiver Substanzen und Lecithin/Sphingomyelin-Ratio-Bestimmung im Fruchtwasser.**
102 Schwangeren wurden in der 25.–43. Schwangerschaftswoche durch Amniotomie oder transabdominale Amniozentese 118 Fruchtwasserproben entnommen. 76 verwertete Proben entstammen normal verlaufenden Schwangerschaften, bei denen anamnestisch weder Induktion noch Retardierung der Lungenreife zu erwarten war, und dienten zur Erstellung von Normalwerten. In 37 Fällen erfolgte die Entnahme innerhalb der für eine aktuelle RDS Voraussage maßgeblichen 72-h Grenze. Siebenmal kam es postpartal zum Auftreten eines RDS, dessen klinische Diagnose durch einen unabhängigen Pädiater erfolgte.
Bei allen Proben wurden folgende Untersuchungen durchgeführt:

1. **Biomechanische Messung der Oberflächenspannung** (OF) in der Wilhelmy-Waage und Erstellung eines Oberflächenspannungs-Flächen-Diagramms (OFD) (Fig. 1):

Ein Float taucht in die Flüssigkeit im Trog ein und mißt über einen Transducer die OF. Die Fläche des Monolayers der Prüfsubstanz wird durch eine mobile Barriere zyklisch von 100% Ausgangsfläche auf 20% komprimiert und anschließend expandiert. Float und Barriere stehen mit einem Koordinatenschreiber in Verbindung. Die entstehenden OFD werden nach folgenden Kriterien ausgewertet:

a) $\gamma$ max (100% Monolayerfläche)

b) $\gamma$ min (Monolayer auf 20% komprimiert)

c) Stabilitätsindex $S = \frac{2\,(\gamma\,\max - \gamma\,\min)}{\gamma\,\max + \gamma\,\min}$

d) Hysteresefläche F

2. **L/S Ratio Bestimmung** (simultaner Vergleich mit der Standardmethode): Es kam die Methode nach GLUCK in der Modifikation nach BORER (planimetrische Auswertung der Spots) zur Anwendung.
Mit beiden Methoden wurden Normalwerte erstellt. Bei den biomechanischen Messungen erfolgte eine zusammenfassende Einteilung in 3 Gruppen (25.–30., 31.–35., 36.–43. SS) (Tab. 1), die L/S Ratio zeigte den bekannten steilen Anstieg ab der 36. SSW (Fig. 2).
Die OFD zeigen bei Fällen mit RDS (Tab. II) hohe Ausgangswerte ($\gamma$ max) und geringe Oberflächenspannungsminderung bei Kompression ($\gamma$ min erhöht), der Stabilitätsindex ist klein, die Fläche vermindert (Fig. 3). Bis zur 34. SSW läßt die für eine ausreichende Surfactantrate angenommene L/S 2:1-Schwelle keine eindeutige Interpretation zu, danach liegen alle Fälle außerhalb der Normalverteilung (Fig. 2). Sowohl für die L/S Ratio, wie für die OFD-Kriterien ergeben sich signifikante Unterschiede bei Fällen mit und ohne RDS.
Die prognostische Aussagekraft wurde auf Erkennungswahrscheinlichkeit, Eintretenswahrscheinlichkeit und Ausschlußwahrscheinlichkeit eines RDS untersucht. Hierbei **erweisen sich die einzelnen OFD Kriterien der herkömmlichen L/S Ratio als zumindest ebenbürtig.**

**Schlüsselwörter:** Fruchtwasser, L/S Ratio, RDS, WILHELMY-Waage.

## Résumé

**Pronostic de l'asphyxie néonatale (RDS)**
**Mesure biomécanique de substances actives en surface et détermination de la proportion lécithine: sphingomyéline dans le liquide amniotique.**
118 échantillons de liquide amniotique ont été prélevés sur 102 parturientes entre la 25ème et la 43ème semaine de grossesse par amniotomie ou par amniocentèse transabdominale. 76 de ces spécimens provenaient de grossesses normales sans prévision d'induction ou de retardement de maturité pulmonaire et ont servi à établir les valeurs-normes. Dans 37 cas, les prélèvements ont eu lieu moins de 72 h. avant l.accouchement, c.à.d. durant la période décisive pour le pronostic d'asphyxie. Dans sept cas, on observa une asphyxie postpartale dont le diagnostic clinique fut établi par un pédiâtre indépendant.

Tous les spécimens prélevés ont subi les tests suivants:

1. **Mesure biomécanique de la tension en surface à l'aide du bilan** WILHELMY et établissement d'un diagramme des zones de tension en surface (Fig. 1):
Une plaque de platine est immersée dans le liquide placé dans le récipient; la tension en surface est mesurée à l'aide d'un transducer. Le film de surface du liquide examiné est comprimé cycliquement, à partir d'une zone initiale de 100%, à 20% par une barrière movible et répandu ensuite. La plaque de platine et la barrière sont reliées à un enregistreur X-Y. Les diagrammes des zones de tension en surface sont évalués en vertu des critères suivants.
a) $\gamma$ max (zone de film de surface de 100%)
b) $\gamma$ min (compression jusqu'à 20% de la zone du film de surface)

c) index de stabilité $S = \frac{2\,(\gamma\,\max - \gamma\,\min)}{\gamma\,\max + \gamma\,\min}$

d) hystérésis

2. Détermination de la proportion L/S (comparaison simultanée avec la méthode standard): On a eu recours à la méthode GLUCK modifiée par BORER (interprétation planimétrique des taches). Les deux méthodes ont donné des valeurs normales. Pour les mesures biomécaniques, on a procédé à un classement en trois groupes (25–30ème, 31–35ème, 36–43ème semaine de grossesse) (Tab. I), la proportion L/S montra la hausse rapide usuelle à partir de la 36ème semaine de grossesse (Fig. 2).
Dans les cas avec RDS (TAB'II), les diagrammes de zones de tension en surface ont révélé des valeurs élevées ($\gamma$ max) et une légère baisse de la tension en surface consécutive à la compression (valeurs élevées $\gamma$ min); l'index de stabilité est bas, l'hystérésis réduite (Fig. 3). Jusqu'à la 34ème semaine de grossesse la proportion L/S 2:1, considérée comme le seuil d'une concentration surfactante suffisante, n'autorise aucune interprétation précise; dans les semaines suivantes tous les cas se situent en dehors des normes de répartition (Fig. 2). Tant pour la proportion L/S que pour les critères des diagrammes des zones de tension en surface, on observe des différences significatives dans les cas avec et sans RDS.
On a vérifié la valeur de pronostic des deux méthodes sur les points suivants: probabilité de détection, d'apparition et de nonapparition d'un RDS. **Il semble que la valeur de pronostic sur ces critères soit au moins égale à la proportion L/S.**

Mots-clés: Bilan de WILHELMY, liquide amniotique, proportion L/S, (asphyxie néonatale).

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