Amniotic Fluid Fluorescence Polarization Value at Physiological Temperature: A Marked Improvement in Assessing Fetal Lung Maturity

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Determination of fetal lung maturity by measurement of the fluorescence polarization (P) value of the amniotic fluid at room temperature has become the method of choice in an increasing number of perinatal units because of its simplicity and relatively high predictive value. Nevertheless, its power to discriminate between cases with and without hyaline membrane disease (HMD) needs improvement. To this end, we assessed the discriminative power of the P value at the physiological temperature of 37 °C (P37) as compared with the power at 25 °C (P25). The study group consisted of 288 consecutive cases at risk for preterm delivery. Samples from all 288 cases were measured at 25 °C and samples from 112 of these were measured concurrently at 37 °C as well. HMD occurred in 27 infants of the total group, nine of whom belonged to the subgroup tested at both temperatures. When sensitivity was fixed at 100% the specificity of P25 was 97% as compared to 79% for P37 (p < 0.001). The percentage of cases with infants free of HMD who had borderline P values was also significantly smaller: 1% vs 21%, respectively (p < 0.001). Although data on more HMD cases are needed to establish the precise threshold of lung maturity for P37, we conclude that P37 is a considerably better discriminator for fetal lung maturity determination than P25.

Additional Keyphrases: fetal status · respiratory distress syndrome (hyaline membrane disease) · L/S ratio · cutoff value

Determination of fetal lung maturity by evaluating amniotic fluid microviscosity, as measured by fluorescence polarization (P), was first introduced by Shinitzky et al. (1). Since then, additional results confirming the advantage of this method over the widely accepted criterion of lecithin/sphingomyelin (L/S) ratio have been published (2–4).

Determination of fetal lung maturity by the bi-dimensional thin-layer chromatographic method has an excellent predictive value (5–7), but is time consuming, expensive, and requires sophisticated equipment and highly trained technicians. Therefore it is impractical for routine and urgent situations. On the other hand, microviscosity determination as expressed by the P value can be done easily within less than half an hour and requires no great technical proficiency (1, 8). This method more accurately predicts HMD than does the L/S ratio (3, 4). Nevertheless, in all reported series using the lowest P value ever observed for an HMD case as the cutoff point for defining lung maturity, an unsatisfactory specificity was noted: up to about 80% of infants for whom the P values exceeded the threshold for lung maturity did not develop HMD (4, 8). Any changes in the technique that diminish this rate of false positives would be of obvious benefit.

The present study investigates the effect on its predictive value of raising the temperature at which the microviscosity is determined.

Microviscosity of lipids is inversely related to temperature (9), and since 90% of the alveolar surfactant is lipids, temperature might be expected to affect the measured P values. In previous studies the P value for amniotic fluid was determined at room temperature (24–25 °C) (1–4, 8, 10, 11). Because at 37 °C the P value might more closely reflect the physiological state of the lung surfactant at body temperature than at 25 °C, we decided to compare the discriminative ability of the P value measured at both temperatures in relation to the clinical outcome in a prospective series of cases.

Materials and Methods

Determination of Microviscosity

In the study, microviscosity was determined by mixing two 1-mL samples of amniotic fluid (from the same amniocentesis) with 4 mL of 10⁻⁶ mol/L 1,6-diphenyl-1,3,5-hexatriene (DPH) dispersions. The mixture was then incubated for 20 min at 37 °C. The samples were measured for P value, one directly (at 37 °C), the other after cooling to 25 °C. P was determined in a FELMA microviscosimeter (Elscint Ltd.) with technical modification for regulation of temperature. For further details about the technique, see refs. 1 and 8.

Study Group

Samples of amniotic fluid, obtained by amniocentesis from 288 consecutive women of different gestational ages—at risk for preterm delivery, or before elective cesarian section—were tested at 25 °C. Of these, a recent subgroup of 112 was tested concurrently at 37 °C. The 288 cases comprised the following obstetric complications: (a) toxemia of pregnancy—72 cases, (b) placental insufficiency—20 cases, (c) third-trimester bleeding—11 cases, (d) premature contractions—70 cases, (e) diabetes mellitus, insulin dependent—18, and non-insulin dependent—44 cases, and (f) other states (most of them before elective induction or cesarian section)—37 cases.

The subgroup of 112 cases comprised the following obstetric complications: (a) toxemia of pregnancy—16 cases, (b) placental insufficiency—six cases, (c) third-trimester bleeding—five cases, (d) premature contractions—26 cases, (e) diabetes mellitus—26 cases, (f) premature rupture of membranes—19 cases, (g) others—16 cases.

All study participants conformed with the following criteria:

- Definitely known gestational age
- A time interval of no more than 72 h between last amniocentesis and delivery
- Samples free of blood or meconium
- Abdominal amniocentesis only

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Received Sept. 10, 1982; accepted Oct. 25, 1982.
Clinical Criteria for Lung Maturity

The decision to induce labor was made only if the P value at 25 °C (P_{25}) indicated lung maturity. The criterion for lung maturity was determined empirically as a P_{25} value = 0.316, based on the highest P value in which no case of HMD occurred. The attending medical staff was not informed of the P value at 37 °C.

Criteria for the diagnosis of HMD were: (a) grunting, (b) sternal and intercostal retractions, (c) a roentgenographic picture of reticulum and an air bronchogram, (d) tachypnea, (e) cyanosis in room air, (f) nasal flaring, and (g) negative culture for streptococcus group B.

Statistical Analysis

The significance of the difference between the mean P values of the HMD cases and those free of HMD (no HMD cases) at 37 °C (P_{37}) and at 25 °C (P_{25}) in the total group and in the subgroup measured at both temperatures was analyzed by use of two-sample t-test program "3D" (12). The independent contribution of P_{37}, P_{25}, and gestation week to the prediction of HMD in the subgroup of 112 was analyzed by logistic regression analysis by use of the "LR" program (12).

We compared the extent of the separation of the distributions of the P values of the HMD and the no-HMD cases measured at the two temperatures by computing for each individual HMD case, at each temperature, a standardized distance (S_dis). The S_dis was derived from the difference between each P value from the respective mean of the no-HMD group divided by the standard deviation of the no-HMD group for that temperature.

The means of S_dis, values at 25 °C and 37 °C were statistically compared in two ways: (a) In the subgroup of 112 examined at both temperatures: both by paired t-test and Wilcoxon's nonparametric test for matched pairs. (b) In the total group, i.e., analyzing the difference between the 112 tested at 37 °C from the 288 tested at 25 °C, both by the Mann–Whitney nonparametric test and the two-sample t-test.

The threshold value for lung maturity at 37 °C was defined similarly to 25 °C as the highest P_{37} value at which there was no case of HMD. The predictive capacity of the P values at both temperatures was evaluated by calculating the specificity with the sensitivity thus set at 100%. "Specificity" refers to the rate of negative test results (P equal to or below threshold value) in the no-HMD group. "Sensitivity" refers to the rate of positive test results—namely, P value above the threshold—in patients with HMD.

Results

Figure 1 presents the distribution of P_{25} values, by gestational week, in the total study group of 288 women. As shown, HMD developed in 27 of the infants. The ranges of P_{25} value between the HMD and the no-HMD cases overlapped considerably. Moreover, of the 206 no-HMD cases with P_{25} = 0.316, 45 (21%) had values in the range 0.310–0.316. A P_{25} value that is so close to the threshold of immaturity might put the physician in a predicament with respect to the patient's management.

Figure 2 presents the P_{37} values for the subgroup of 112 women tested at both temperatures, among whom nine HMD cases were diagnosed. HMD and no-HMD cases are better resolved. The highest P_{37} value at which no HMD case was observed was 0.286. Only one of the 103 no-HMD cases (1%) had a P_{37} value in the range adjacent to the respective threshold of lung maturity, namely 0.280–0.286; the rest of the cases had lower values. Thus, the rate of borderline values in the range defined as indicating lung maturity is also decreased by determination of the P value at 37 °C.

Table 1 summarizes the occurrence of HMD and no-HMD cases in the ranges, based on the cutoff point of 0.316 and 0.286 for the total group determined at 25 °C and the subgroup examined at 37 °C, respectively.
Table 1. Distribution of HMD and No-HMD Cases in the Ranges of P Values Defining Lung Immaturity at 25 and 37 °C

<table>
<thead>
<tr>
<th>Definition of lung maturity in cases with P value in the range</th>
<th>25 °C (total group)</th>
<th>37 °C (subgroup)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P value</td>
<td>HMD</td>
</tr>
<tr>
<td>Immature</td>
<td>&gt;0.316</td>
<td>27</td>
</tr>
<tr>
<td>Mature</td>
<td>≤0.316</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>206</td>
</tr>
</tbody>
</table>

Table 2 compares the sensitivity and specificity for both groups. When a $P_{25}$ value ≤0.316 was considered as indicating lung maturity, the sensitivity was by definition 100% (27/27), but 55 cases were misdiagnosed, yielding a specificity of only 79% (206/261). On the other hand when $P_{37}$ ≤0.286 was used to define lung maturity, the sensitivity was again by definition 100% (9/9), but only three no-HMD cases were misdiagnosed, yielding specificity of 97% (100/103)—significantly higher as compared with the measurements at 25 °C ($p < 0.001$).

Figure 3 illustrates the P values in the subgroup of 112 cases examined at 25 °C. It is evident that this subgroup was not selective and resembled the total group of the 288 cases in its $P_{25}$ values ($p > 0.30$).

Logistic regression analysis, evaluating simultaneously the predictive value of $P_{37}$, $P_{25}$, and gestation week in the subgroup of the 112 cases, revealed that $P_{37}$ was the best predictor and was sufficient to discriminate between HMD and the no-HMD cases with no additional effect of $P_{25}$ or gestational week on the discrimination ($p > 0.30$).

Further to demonstrate the better discrimination afforded at 37 °C, we compared the mean $S_{dis}$ (see Methods) of the HMD cases between 37 °C and 25 °C. The individual $P$ and $S_{dis}$ values of the nine HMD cases in the subgroup are presented in Table 3. The mean $S_{dis}$ was significantly higher at 37 °C than at 25 °C (paired $t < 0.0001$). The mean $S_{dis}$ at 25 °C of the HMD cases in the total study group was 1.62, which was near the mean $S_{dis}$ for the subgroup (1.78) and not significantly different from it ($p > 0.50$).

![Fluorescence polarization (P) values for the amniotic fluid at 25 °C, by gestation week, in the subgroup of 112 women tested at both temperatures](image)

Fig. 3. Fluorescence polarization (P) values for the amniotic fluid at 25 °C, by gestation week, in the subgroup of 112 women tested at both temperatures.

Table 2. Sensitivity and Specificity of P Values Measured at 25 and 37 °C

<table>
<thead>
<tr>
<th>25 °C (total group)</th>
<th>37 °C (subgroup)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td></td>
<td>100%</td>
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<td></td>
<td>(27/27)</td>
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<thead>
<tr>
<th>25 °C (total group)</th>
<th>37 °C (subgroup)</th>
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<tbody>
<tr>
<td></td>
<td>sensitivity</td>
</tr>
<tr>
<td></td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>(27/27)</td>
</tr>
</tbody>
</table>

Table 3. P and $S_{dis}$ Values for the Nine HMD Cases in the Subgroup of 112 Women Tested Prospectively at Both 25 and 37 °C

<table>
<thead>
<tr>
<th>$P_{25}$</th>
<th>$P_{37}$</th>
<th>$S_{dis25}$</th>
<th>$S_{dis37}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.346</td>
<td>0.301</td>
<td>2.13</td>
<td>2.47</td>
</tr>
<tr>
<td>0.344</td>
<td>0.294</td>
<td>2.03</td>
<td>2.17</td>
</tr>
<tr>
<td>0.344</td>
<td>0.304</td>
<td>2.03</td>
<td>2.58</td>
</tr>
<tr>
<td>0.331</td>
<td>0.291</td>
<td>1.40</td>
<td>2.05</td>
</tr>
<tr>
<td>0.317</td>
<td>0.286</td>
<td>0.71</td>
<td>1.84</td>
</tr>
<tr>
<td>0.341</td>
<td>0.312</td>
<td>1.88</td>
<td>2.92</td>
</tr>
<tr>
<td>0.335</td>
<td>0.298</td>
<td>1.59</td>
<td>2.34</td>
</tr>
<tr>
<td>0.336</td>
<td>0.304</td>
<td>1.62</td>
<td>2.58</td>
</tr>
<tr>
<td>0.358</td>
<td>0.320</td>
<td>2.67</td>
<td>3.25</td>
</tr>
</tbody>
</table>

Mean $S_{dis}$ 1.78 2.47

Significance $p < 0.0001$

$P_{25}$, $P_{37}$: the individual P values at the respective temperatures. $S_{dis25}$, $S_{dis37}$: the individual $S_{dis}$ values at the respective temperatures.

$S_{dis25} = P_{25} - 0.302$

$S_{dis37} = P_{37} - 0.021$

$0.302$ and $0.021$ are the mean and SD of $P_{25}$ in the $103$ no-HMD cases; $0.242$ and $0.024$ are the respective values for $P_{37}$.

As to the effect of obstetric complications on the discriminative power, a significantly lower specificity in toxemia, both mild and severe, was found both at 25 and 37 °C. Thus, in all categories except toxemia, the respective specificities at the two temperatures were 88.5% and 100%. In toxemia, both mild and severe, the respective specificities were significantly lower: 56.3% and 81.3% ($p < 0.005$). Consequently, at 37 °C all the false-positive cases (no-HMD cases with P value above the cutoff point for lung maturity) were observed in the toxemia group.

Discussion

Evaluation of lipid microviscosity by means of fluorescence polarization is a simple method of expressing the physical state of the lipid assembly in systems such as amniotic fluid. The procedure requires not more than 30...
min and no great skill in laboratory or technical work; therefore it can replace the traditional L/S ratio evaluation of fetal lung maturity. We have previously shown that the P25 value correlates well with the L/S ratio (1, 8) and affords a better predictive power as regards the antenatal diagnosis of HMD as compared with the latter technique (4). Nevertheless, measurement of the P value at room temperature, applied originally for the sake of convenience, is associated with an undesirably low specificity of 79% when sensitivity is fixed at 100%.

Our results indicate that, at 37 °C, when sensitivity is fixed at 100%, the specificity is considerably and significantly improved to 97%. These results resemble the specificity achieved by the lung profile suggested by Gluck et al. (6, 7).

At both temperatures, false positives were most common for the toxemia group. In contrast to all other obstetrical complications, this group remains the only one that failed to improve specificity up to 100% at 37 °C. Whether this is the result of some peculiar composition of the amniotic fluid phospholipids in pregnancies complicated by toxemia remains to be explored.

Although our conclusions are based on only nine HMD cases, the highly significant increase in the mean standardized distance of the HMD from the no-HMD cases at 37 °C as compared with 25 °C seems to establish unequivocally the better discrimination between the two groups. Nevertheless, more HMD cases will have to be studied before the lower limit of the P value for immature lungs at this temperature is conclusively known and the specificity estimated with satisfactory precision. In the meantime, readings at both temperatures should be continued.

References
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