SAMPLING OF AMNIOTIC FLUID FOR L/S RATIO DETERMINATION AFTER RUPTURE OF MEMBRANES

W. GRUBER, A. EULLER and E. GOSS

1nd Department of Obstetrics and Gynecology, University of Vienna Medical School, Vienna, Austria

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After rupture of membranes (RM), amniotic fluid (AF) leaking from the external os can be collected by means of a sterile bladder catheter inserted into the vagina and connected to a plastic bag for determination of the L/S ratio. This study was designed to ascertain whether contamination of AF with cervical or vaginal mucus alters the L/S ratio determined by Gluck's method. 50 gravidas in term or preterm labor had their amniotic sac punctured by a long gauge-20 needle under amnioscopic sight. Two samples of AF were collected from the needle. After RM, a sterile plastic bladder catheter was placed in the vagina and connected to a plastic bag ordinarily used for urine collection. Two additional samples of AF were taken from the plastic bag. All 4 samples were marked with code numbers and submitted for L/S ratio determination. There were no significant differences in the L/S ratio between samples obtained by the two different techniques. It is concluded that sampling of AF by means of a vaginal catheter and a plastic bag does not alter the L/S ratio, and is therefore practicable for assessing fetal lung maturity after RM.

lung maturity; premature labor; respiratory distress syndrome

INTRODUCTION

Rupture of membranes (RM) prior to term may present a serious therapeutic problem. Treatment with tocolytics is often unsuccessful once membranes have ruptured (Gruber and Baumgarten, 1975). Mere observation of the patient bears both the hazards of amnionitis and of the delivery of a premature newborn. Active induction of labor may again result in the delivery of a premature infant, possibly jeopardized by the development of a respiratory distress syndrome (RDS). Information on the fetal lung maturity would be particularly helpful when choosing the appropriate management in these cases, especially since cortisone administration to the mother is known to be capable of accelerating the maturation of the fetal lungs (Liggins and Howie, 1972). Amniocentesis to obtain amniotic fluid (AF) for L/S ratio determination may be technically difficult after RM. It appears therefore reasonable to attempt collection of AF, leaking from the cervix, in the vagina. AF col-
lected in the vagina may be contaminated with cervical or vaginal mucus, which possibly affects the interpretation of the L/S ratio (Gluck et al., 1974).

This study was designed: (1) to examine the practicability of sampling of AF in the vagina; (2) to evaluate the reproducibility of the procedure followed to determine the L/S ratio; (3) to assess the reliability of the L/S ratio analyzed in specimens collected from the vagina; and (4) to compare the L/S ratio with the incidence of RDS in the newborns included in this investigation.

MATERIAL AND METHODS

69 Gravidas in spontaneous active labor, between 30 and 43 wk gestation, underwent amniotomy at a cervical dilatation of 2–5 cm for purposes like acceleration of labor, internal monitoring of labor and obtaining AF. Amniotomy was performed by puncturing of the lower pole of the membranes with a long gauge-20 needle through an amnioscope. Two samples of AF were collected from the needle. After removal of the amnioscope, a sterile plastic bladder catheter was placed in the vagina and connected to a plastic bag ordinarily used for urine collection. The catheter was left in place for 30 min. Two additional samples of AF were taken from the plastic bag. All 4 specimens were centrifuged at 3000 rpm for 10 min. The supernatant fluids were marked with code numbers and stored at −20°C until analysis. Samples were included in the study only if all 4 portions reached 10 ml in volume and appeared clear after centrifugation. The L/S ratio was determined by a modification of the procedure described by Gluck (Gluck et al., 1971) and Borer (Borer et al., 1971).

Commercial silica-gel thin-layer plates (DC Fertigplatten, Kieselgel 60, Art. Nr. 5721, Merck, Darmstadt, G.F.R.) (250 μm thick) were utilized. Samples were developed in chloroform:methanol:water, 95:34:4, up to 12 cm, dried and sprayed with a solution of ammonium molybdate to visualize compounds. The sizes and the intensities of the spots, representing the two phospholipids, were visually compared and expressed as L/S ratio. The variation coefficient of this method of L/S ratio determination amounts to 17%.

According to the code, the 4 L/S ratio values of each patient were identified. The 2 samples obtained by transcervical amniocentesis were named Ia and Ib, the specimens collected in the vagina IIa and IIb. Means of groups I and II were computed and compared. Correlations between groups Ia and Ib, IIa and IIb, Ia and IIa, and x Iab and x IIab were statistically examined. Student’s t-test for matched pairs and Spearman’s rank correlation were used to study differences between L/S ratios of the groups.

RESULTS

Out of 69 gravidas entering the study, 50 (72%) sets of samples of AF could be evaluated. In 19 instances one or both techniques of collection
Fig. 1. Correlation between L/S ratio values, determined in duplicate, of samples of amniotic fluid obtained by transcervical amniocentesis (groups Ia and Ib). Graphed are the regression lines and the 95% confidence limits. Values of fetuses that developed RDS are encircled.

Fig. 2. Correlation between L/S ratio values, determined in duplicate, of samples of amniotic fluid collected in the vagina (Groups Ila and IIb). Regression lines and 95% confidence limits are graphed. Values of fetuses that developed RDS are encircled.
yielded less than 10 ml/sample or one or more specimens appeared meconium- or blood-stained after centrifugation, and were therefore excluded.

L/S ratio values of the 2 groups of samples gained by transcervical amniocentesis (groups Ia and Ib) correlated significantly with each other (t-test: \( P < 0.01, r = 0.56; \) Spearman’s test: \( r = 0.54 \)) (Fig. 1). The same applies for the L/S ratios of specimens taken from the plastic bag (groups IIa and IIb) (t-test: \( P < 0.01, r = 0.84; \) Spearman’s test: \( r = 0.82 \)) (Fig. 2). The mean L/S ratio of all 100 samples obtained by transcervical amniocentesis (groups Ia and Ib) was 3.28 ± 1.43; the mean of the 100 specimens collected in the vagina (groups IIa and IIb) was also 3.28 ± 1.40. The t-test revealed no significant difference between groups I and II.

The correlations between groups of samples obtained by the two different techniques were significant \( (P < 0.01) \). The \( r \)-value of the correlation between groups Ia and IIa was 0.66 (Fig. 3), and that of the correlation of the means \( \bar{x} \) Ia and \( \bar{x} \) IIa amounted to 0.69 (Fig. 4).

All 50 gravidas included in the study gave birth to live single fetuses, between 10 min and 13 h (mean 3 h 57 min) after amniotomy. No neonatal death occurred. Three newborns, however, developed a mild transient RDS (Table I). Two of them had an L/S ratio of >2 in all 4 samples. One was

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![Fig. 3. Correlation between L/S ratio values of samples of amniotic fluid obtained by transcervical amniocentesis (group Ia) and by collection in the vagina (group IIa). Regression lines and 95% confidence limits are graphed. Values of fetuses that developed RDS are encircled.](image-url)
Fig. 4. Correlation between means of duplicate determinations of the L/S ratio in samples of amniotic fluid, obtained by transcervical amniocentesis ($\bar{X}_{Iab}$) and by collection in the vagina ($\bar{X}_{IIab}$). Regression lines and 95% confidence limits are graphed. Values of fetuses that developed RDS are encircled.

Born at 30 wk gestation 5 h 10 min after RM and weighed 1750 g. The other one was delivered at 37 wk gestation 9 h after RM, weighing 2700 g. Both had normal Apgar scores and no evidence of intrapartum asphyxia. The third newborn developing a mild RDS was one of the 8 fetuses with a borderline L/S ratio (all 4 values between 1.5 and 2.5): this was a 3100 g/47 cm female, 39 wk gestation, born 1 h after RM with Apgar 8/9 and requiring oxygen treatment for cyanosis.

The only newborn with an immature L/S ratio (between 0.75 and 1.25) failed to develop RDS. Possibly the 3 h elapsing between amniotomy and birth were sufficient to accelerate lung maturation (Seidl et al., 1977). None of the patients included in this study had received cortisone treatment.

TABLE I
L/S RATIO AND FETAL OUTCOME

<table>
<thead>
<tr>
<th>L/S ratio</th>
<th>n</th>
<th>No RDS</th>
<th>RDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>41</td>
<td>39</td>
<td>2</td>
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</table>
DISCUSSION

In the majority of cases (72%), the described technique of collecting AF in the vagina yielded AF sufficient in amount and quality for L/S ratio determination in duplicate. This percentage may be different when sampling of AF is attempted after spontaneous premature RM. When amniotomy was performed during delivery with the fetal head already engaged and the cervix dilated more than 4 cm, sufficient volumes of AF could frequently no longer be obtained. This limitation may not apply for cases of spontaneous RM prior to the onset of labor.

Duplicate estimations of the L/S ratio correlated significantly with each other. Correlation coefficients between 0.56 and 0.84 (Figs. 1 and 2), however, demonstrate a relatively poor reproducibility of the L/S ratio values determined with the described procedure. Correlation coefficients closer to 1 would be desirable. The identity of the means of samples obtained by the two different techniques suggests that collection of AF in the vagina has no specific influence on the L/S ratio. Coefficients of correlation between specimens sampled by the two different methods were 0.66 and 0.69 respectively, which is within the range of correlation coefficients of duplicate estimations (0.56–0.84).

Mature lungs had been correctly predicted in 39 of 41 cases (95%). All 4 L/S ratios of those fetuses were greater than 2. Two newborns of this group, however, developed mild RDS. A rate of 95% of correct negations of RDS is similar to those reported by other investigators utilizing visual determination techniques (Nakamura et al., 1972; Boehm et al., 1973; Dunn and Bhatnagar, 1973; Lemons and Jaffee, 1973; Lorenz et al., 1975). Gluck, however, reports a 100% prediction rate, using reflectance densitometry (Gluck and Kulovich, 1973), possibly stressing the superiority of densitometric methods over visual ones.

Although collection of AF in the vagina after RM by means of a plastic bladder catheter may not always yield amounts sufficient for L/S ratio determination, the attempt appears worthwhile. The technique imposes no additional risk upon mother and fetus, and, despite possible contamination of the AF, the L/S ratio determined in such samples seems to be as reliable as that of specimens obtained by amniocentesis. Information on fetal lung maturity gained with the help of this method may be a valuable parameter for the management of pregnancies complicated by premature RM.

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REFERENCES


