Have Liley charts outlived their usefulness?

K. H. Nicolaides, M.D., C. H. Rodeck, M.D., R. S. Mibashan, M.D., and J. R. Kemp, M.D.

London, England

Fetal blood and amniotic fluid samples were obtained fetoscopically from 59 rhesus-isoimmunized pregnancies at 18 to 25 weeks' gestation. Fetal hemoglobin was measured and amniotic fluid optical density deviation at a wavelength of 450 nm determined. Two sets of normal reference values for optical density at 450 nm and fetal hemoglobin at 16 to 36 and 16 to 25 weeks were established from 475 amniotic fluid and 153 fetal blood samples obtained from pregnancies not complicated by fetal hemolysis.

As expected, there was a significant linear correlation between the degree of fetal anemia and the amniotic fluid optical density at 450 nm in rhesus-isoimmunized pregnancies. However, the values of optical density at 450 nm were widely scattered, thereby limiting their ability to predict accurately the severity of disease in these second-trimester pregnancies. In 25 of the patients, the value of optical density at 450 nm was determined at 6 to 16 days before fetoscopy. The severity of fetal anemia could not be predicted by the trend in optical density at 450 nm. These data suggest that the only reliable method to determine the severity of rhesus isoimmunization in the second trimester of pregnancy is the direct measurement of fetal hemoglobin. (AM J OBSTET GYNECOL 1986;155:90-4.)

Key words: Rhesus isoimmunization, Liley charts, fetoscopy

Although bilirubin staining of the amniotic fluid in cases of severe jaundice in the newborn infant was first described by Ballantyne as long ago as 1892, it was not until the 1950s that the association between the amount of blood pigment in the liquor amnii and the degree of erythrocyte destruction was recognized.1,2 Subsequently, Liley1,2 offered a systematic approach to amniotic fluid analysis in the management of rhesus-immunized pregnancies, an approach that has become the established method for assessing the severity of the disease and for timing obstetric interventions such as early delivery or intrauterine fetal blood transfusions.

In a series of 101 rhesus-immunized pregnancies, Liley1 performed amniocenteses at 27 to 41 weeks' gestation, measured spectrophotometrically the amniotic fluid bilirubin concentration by the deviation in optical density at a wavelength of 450 nm (ΔOD450), and plotted the results on semilogarithmic graph paper against the gestational age. He divided the values into three prognostic zones according to the outcome of pregnancy and the severity of the disease as assessed by the hemoglobin concentration at delivery. The upper zone included those severely affected fetuses who were at risk for intrauterine or neonatal death while the lower zone contained rhesus-negative or mildly affected rhesus-positive fetuses. In the middle zone, which was subsequently subdivided into upper and lower subzones,1,2 the prognosis was found to depend on the magnitude of the deviation as well as the trend in ΔOD450, that is, whether it was rising or falling after a second or possibly third determination. It was recommended that when the ΔOD450 values fell in the lower zone, no intervention was needed. However, if the values appeared in the upper zone, delivery should be undertaken or, if the fetus were too premature, an intrauterine blood transfusion given.1,2

In the 1960s, other investigators confirmed and elaborated on the findings of Liley, often devising their own systems for interpreting ΔOD450 measurements.4 However, the high perinatal mortality rate at the time for premature infants of 28 to 32 weeks' gestation and the high procedure-related mortality rate associated with intraperitoneal transfusion, especially for severely affected fetuses at less than 25 weeks' gestation, limited the need for the application of amniotic fluid analysis to the late second and early third trimesters of pregnancy.

During the last 5 years the development of safer techniques for fetal blood transfusions, either with the use of fetoscopy for direct intravascular access or by the intraperitoneal route with ultrasound guidance, and the improved prognosis for the very premature infant as a result of advances in neonatal intensive care, have encouraged the undertaking of obstetric interventions.

From the Harris Birthright Research Centre for Fetal Medicine, Department of Obstetrics and Gynaecology, and the Lions Mountbatten Blood Research Laboratories, Department of Haematology, King's College School of Medicine and Dentistry, and the Rhesus Isimmunisation Unit, Lewisham Hospital.

Received for publication November 12, 1985; revised March 7, 1986; accepted March 10, 1986.

Reprint requests: Dr. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, Department of Obstetrics and Gynaecology, King's College School of Medicine and Dentistry, London, England SE5 8RX.
much earlier in pregnancy. In some centers, the Liley graph has been extrapolated backward and intrauterine transfusions are begun as early as 20 weeks, on the basis of these \( \Delta OD_{450} \) values.\(^{1-6}\)

The aim of the present study was to correlate amniotic fluid \( \Delta OD_{450} \) values with the fetal hemoglobin in rhesus-isoimmunized pregnancies at 18 to 25 weeks to examine the predictive value of the \( \Delta OD_{450} \) as well as the validity of extrapolating the original Liley graph from the third trimester to the middle of the second trimester of pregnancy.

**Patients and methods**

The study included 59 rhesus-negative pregnant women at 18 to 25 weeks' gestation who were considered to be severely rhesus isoimmunized on the basis of their obstetric history. These women were referred from several centers in the United Kingdom and Europe for direct intravascular fetal blood transfusion by fetoscopy because of elevated maternal serum rhesus antibody levels or amniotic fluid \( \Delta OD_{450} \) measurements.

Linear-array real-time ultrasound scanning was performed to estimate gestational age, exclude external and internal anatomic defects, and detect fetal hydrops (skin edema and serous effusions). Fetoscopy and pure fetal blood sampling from an umbilical cord vessel was carried out with an Olympus Selfoscope 1.7 mm in diameter introduced transabdominally into the amniotic cavity.\(^{11}\) Pure fetal blood samples were obtained from all fetuses and, after assessment of the degree of fetal anemia, with a combined electronic hemocytometer and particle size analyzer (Coulter Channelizer C-100 with mean corpuscular volume–hematocrit attachment), an intravascular fetal blood transfusion was given. The data in this study were derived from pretransfusion fetal blood and amniotic fluid samples obtained at the first fetoscopy. Further fetal blood transfusions were given as needed (mean number, three; range, one to six). The survival rate achieved in this series was 85%.

Fetal blood samples (180 µl) were collected into 20 µl of isotonic edetic acid solution (0.5 mmol/L in 0.15 mol/L sodium chloride) and the hemoglobin concentrations in grams per deciliter were determined with the use of a Coulter S-Plus counter. Amniotic fluid samples (10 ml) were collected at the time of fetoscopy and transferred into darkened containers to protect the bilirubin from photodecomposition. Samples were centrifuged for 15 minutes at 500 g to remove vernix caseosa, amniocytes, and erythrocytes and analyzed within 24 hours of collection. A spectrophotometric scan of the amniotic fluid was made with a double-beam, continuous-recording spectrophotometer (Pye Unicam SP 800). Optical density readings were plotted automatically on semilogarithmic graph paper, with wavelength as the linear abscissa (horizontal) and optical density deviation as the logarithmic ordinate (vertical). The bilirubin absorption band was measured manually as the vertical distance between the absorbance at 450 nm and the line connecting the curve between 550 and 365 nm.\(^3\) In 25 patients, another amniotic fluid sample, obtained by amnioncentesis between 6 and 16 days before the fetoscopy, was also analyzed and the \( \Delta OD_{450} \) determined.

Normal reference ranges for amniotic fluid \( \Delta OD_{450} \) and fetal hemoglobin between 16 and 36 and between 16 and 25 weeks were established from 475 amniotic fluid and 153 fetal blood samples, respectively, obtained from pregnancies not complicated by fetal hemolysis. The mean and standard deviation for successive weeks of pregnancy and the correlation coefficient between each variable and fetal maturity in weeks were calculated. The probability of the correlation coefficient being significant was determined by Student's \( t \) test.

**Results**

The distribution of fetal hemoglobin and amniotic fluid \( \Delta OD_{450} \) measurements from pregnancies not complicated by fetal hemolysis is shown in Fig. 1 and 2. Within the gestational range of 16 to 25 weeks, there was no significant change in either fetal hemoglobin (mean = 12.14, SD = 1.22) or liquor \( \Delta OD_{450} \) \((n = 246, \text{mean} = 0.103, \text{SD} = 0.045)\). For the log-transformed values of liquor \( \Delta OD_{450} \) between 26 and 36 weeks' gestation, however, there was a highly significant negative linear correlation \((n = 229, \text{correlation coefficient} r = -0.738, \text{intercept} = 2.776, \text{slope} = -0.127, p < 0.0001)\).

The amniotic fluid \( \Delta OD_{450} \) values from the 59 rhesus-isoimmunized pregnancies are plotted against gestation (on the semilogarithmic graph) in Fig. 3 and divided.
Fig. 2. Reference range (mean + 2SD) and distribution of 497 values of amniotic fluid ΔOD_{550} obtained from pregnancies not complicated by fetal hemolysis.

Table I. Accuracy of amniotic fluid ΔOD_{550} measurements in predicting severity of rhesus isoimmunization, as defined by fetal hemoglobin, in 59 pregnancies at 18 to 25 weeks’ gestation

<table>
<thead>
<tr>
<th>Cutoff value of ΔOD_{550}</th>
<th>&lt;6 gm/dl</th>
<th>n</th>
<th>%</th>
<th>6-9.7 gm/dl</th>
<th>n</th>
<th>%</th>
<th>&gt;9.7 gm/dl</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.1</td>
<td>31*</td>
<td>100</td>
<td>15</td>
<td>100</td>
<td>13</td>
<td>100†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0.2</td>
<td>28</td>
<td>90</td>
<td>15</td>
<td>87</td>
<td>7</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0.3</td>
<td>22</td>
<td>71</td>
<td>10</td>
<td>67</td>
<td>2</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0.4</td>
<td>13</td>
<td>42</td>
<td>6</td>
<td>40</td>
<td>2</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0.5</td>
<td>7</td>
<td>23±</td>
<td>3</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liley zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>27</td>
<td>8</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B</td>
<td>16</td>
<td>52</td>
<td>7</td>
<td>47</td>
<td>4</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10%</td>
<td>32</td>
<td>4</td>
<td>27</td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There is no ΔOD_{550} value (upper half) or extrapolated Liley zone (lower half) that clearly separates severely affected fetuses (hemoglobin <6 gm/dl) from nonanemic ones (hemoglobin >9.7 gm/dl). If the cutoff level is set at 0.1 nm in order to achieve detection of all 31 severely anemic fetuses(*), the false positive rate would be 100%(†). At ΔOD_{550} >0.5 nm the false positive rate is 0%(‡) but the detection rate is simultaneously reduced to 23%(§). Similarly, if obstetric intervention(s) is/are restricted to those pregnancies with amniotic fluid ΔOD_{550} values in extrapolated Liley zone 3 only 10(11) of the 31 severely anemic fetuses would be identified (32%).

into three prognostic zones by extrapolating the Liley lines backward to meet the vertical axis at 18 weeks’ gestation; zone 2 is further subdivided into upper and lower subzones. The ΔOD_{550} values are correlated with the severity of the disease as defined by the fetal hemoglobin and the presence or absence of fetal hydrops at the time of sampling. Thus, if the hemoglobin fell within the normal range (<9.7 gm/dl) the disease was considered to be mild and the fetus therefore not in need of blood transfusion. If the hemoglobin was <6 gm/dl the disease was thought to be severe and the fetus at high risk of intrauterine death. Ultrasonographic evidence of hydrops was found in 14 of the 31 fetuses in this group. Moderate disease was defined by a fetal hemoglobin of 6 to 9.7 gm/dl. Although not at risk of imminent death, these fetuses would probably require an intrauterine blood transfusion within a few weeks.

The accuracy of predicting the severity of fetal anemia, and thus the need for obstetric intervention, by the Liley method and by specified cutoff levels of amniotic fluid ΔOD_{550} are shown in Table I. There is no ΔOD_{550} level that clearly separates severely from mildly affected pregnancies. If the cutoff level is set low enough to include 90% of the severely affected pregnancies, for example, at ΔOD_{550} >0.2 nm, the upper level of the normal ΔOD_{550} range (Fig. 2), it would also include 54% of the mildly affected ones. Alternatively, if the cutoff level is set high enough to exclude all mildly affected pregnancies (ΔOD_{550} >0.5), then the false negative rate would be 77%.

There is an overall negative linear correlation (n = 59, r = -0.310, p < 0.05) between fetal hemoglobin and ΔOD_{550}. Separate analyses of fetal hemoglobin and ΔOD_{550} in hydropic and nonhydropic fetuses reveal a
highly significant positive correlation for the former (n = 14, r = 0.750, p < 0.01) and a negative one (n = 45, r = -0.487, p < 0.001) for the latter group of fetuses (Fig. 4). Thus the pregnancies with the most severely anemic hydropic fetuses had low values of liquor $\Delta OD_{450}$. Ultrasonography, by identifying the hydropic fetuses, increases the sensitivity of elevated $\Delta OD_{450}$ measurements in detecting severely anemic fetuses from 32% to 71% (14 hydropic fetuses irrespective of $\Delta OD_{450}$ values plus eight nonhydropic ones with $\Delta OD_{450}$ values in Liley zone 3) without increasing the false positive rate.

The trends in $\Delta OD_{450}$ for the 25 patients who had had amniocentesis 6 to 16 days before fetoscopy are illustrated in Fig. 3. In the 19 cases with measurements in the extrapolated Liley zones 1 and 2, the severity of fetal anemia could not have been reliably predicted by the trend in $\Delta OD_{450}$ values. Thus in four of the 11 (36%) severely affected pregnancies the trend in optical density deviation was downward. Moreover, of the 14 cases of $\Delta OD_{450}$ values that remained the same or increased, seven had severe disease, four moderately severe disease, and three mild disease.

Comment

In the present study the fetal hemoglobin, measured in samples obtained fetoscopically, was used as the basis for evaluating the severity of rhesus isoimmunization. The fetal hemoglobin rather than the outcome of pregnancy was chosen because: (1) fetal hemolysis and consequent anemia constitute the underlying pathophysiology of the disease; (2) in the natural history of the
disease, and three mild disease.

Comment

In the present study the fetal hemoglobin, measured in samples obtained fetoscopically, was used as the basis for evaluating the severity of rhesus isoimmunization. The fetal hemoglobin rather than the outcome of pregnancy was chosen because: (1) fetal hemolysis and consequent anemia constitute the underlying pathophysiology of the disease; (2) in the natural history of the
disease, and three mild disease.

Comment

In the present study the fetal hemoglobin, measured in samples obtained fetoscopically, was used as the basis for evaluating the severity of rhesus isoimmunization. The fetal hemoglobin rather than the outcome of pregnancy was chosen because: (1) fetal hemolysis and consequent anemia constitute the underlying pathophysiology of the disease; (2) in the natural history of the
disease, and three mild disease.
values in pregnancies not complicated by fetal hemolysis decreases with gestation (Fig. 2) and lies in the upper subdivision of Liley's zone 2. For the second trimester, however, this limit is horizontal and coincides with the action line for fetal transfusions advocated by other investigators (for instance, 0.2 nm by Gordon et al.15). Had we adopted the criteria of these investigators, seven of the 13 (54%) mildly affected fetuses might have received unnecessary and potentially hazardous transfusions, while three (10%) of the severely anemic fetuses would not have been transfused (Table I). The positive correlation between fetal hemoglobin and ΔOD₉₀₀ in the pregnancies complicated by fetal hydrops (Fig. 4) could be explained by the dilutional effect of associated polyhydramnios. This problem has been clearly demonstrated by Queenan,15 who advocated the determination of the amniotic fluid volume, when polyhydramnios is suspected, to allow appropriate correction of the measured ΔOD₉₀₀. Similarly, Whitfield16 found that, although the estimated amniotic fluid volume is within the normal range for gestation in most rhesus-immunized pregnancies, rapidly developing hydrops, is usually associated with severely or fatally affected fetuses. Ultrasonography allows the diagnosis of fetal hydrops, thereby identifying a group of severely anemic fetuses in urgent need of blood transfusion. In the absence of hydrops, however, neither ΔOD₉₀₀ (Table I) nor ultrasonographic measurements of intrauterine volume, placental thickness, umbilical vein diameter, fetal abdominal circumference, or intraperitoneal volume can reliably distinguish mild from severe fetal disease (unpublished results).

In the management of rhesus-immunized pregnancies, it has traditionally been recommended that the first amniocentesis be performed 10 weeks before the time of the earliest previous fetal or neonatal death, fetal transfusion, or birth of a severely affected baby.16 In the absence of such a history, amniocentesis is undertaken when a "critical level" of maternal rhesus antibody is reached. A value of 4 IU/ml has recently been recommended.17 In the third trimester, amniocentesis and interpretation of ΔOD₉₀₀ values by the Liley method have proved to be reliable. Fetal blood sampling may be associated with a marginally greater risk of fetal mortality and maternal morbidity than amniocentesis.17 However, our present findings suggest that fetal hemoglobin determination on samples obtained with this technique is the only accurate method to assess the severity of fetal anemia in the second trimester of pregnancy.

REFERENCES