Reactivity of Amniotic Fluid Alpha-Fetoprotein with Concanavalin A in Relation to Gestational Age: Clinical Implications

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We used concanavalin A crossed-line affinity immunoelectrophoresis to determine the percentage of concanavalin A nonreactive \( \alpha \)-fetoprotein in amniotic fluid samples from pregnancies with normal and abnormal fetuses. In 167 samples from pregnancies with a normal outcome and normal values for total \( \alpha \)-fetoprotein concentration in amniotic fluid the percentage decreased from a median value of 27.4% in the 13th week to 8.5% in the 21st week of gestation, and a statistically significant \((p < 0.001)\) average decrease of 1.7% per week was found from the 14th to the 19th week. A similar average decrease (2.2%) was found in 22 pregnancies from which two or more samples were obtained. The clinical significance of this decrease is discussed. Of 108 samples from patients with above-normal values for total \( \alpha \)-fetoprotein and a normal outcome, seven had a total \( \alpha \)-fetoprotein above recommended cut-off values, and only one of these had a low percentage of concanavalin A nonreactive \( \alpha \)-fetoprotein. In contrast, for all 27 samples from pregnancies with a severe fetal malformation this percentage was low, even in one case where the total \( \alpha \)-fetoprotein concentration was below the recommended cut-off value.

Additional Keyphrases: fetal status • neural tube defect • cut-off values • crossed-line affinity immunoelectrophoresis

In the second multicenter report (1) by the United Kingdom Collaborative Study on Alpha-Fetoprotein in Relation to Neural Tube Defects it was concluded that quantitation of the amniotic fluid concentration of \( \alpha \)-fetoprotein (AFP) can be considered as almost diagnostic for the presence or absence of a fetus with neural tube defect. Pathological AFP values are defined as those equal to or exceeding a given cut-off value, usually expressed as a multiple of the median for the relevant gestational week.

However, a small proportion of unaffected pregnancies will show values exceeding the cut-off value and conversely a few neural tube defect pregnancies will have "normal" values. These false-positive and false-negative values are the main problem in all neural tube defect-screening programs.

Recently, several reports have shown that the percentage of AFP that does not react with concanavalin A (con A) is significantly lower in amniotic fluids surrounding a fetus with a neural tube defect than in amniotic fluids from a unaffected pregnancy (2-6). Measurement of the percentage of con A nonreactive amniotic fluid AFP might therefore decrease the problem of false negatives and false positives. Furthermore, if this test was used in conjunction with measurement of total AFP in amniotic fluid, the cut-off values for suspecting an neural tube defect might be set lower, so that fewer neural tube defects would be missed, without increasing the risk of terminating unaffected pregnancies.

However, the con A nonreactive fraction of AFP has been reported (4, 5) to decrease with increasing gestational age, and this might reduce the clinical value of the test. We therefore wished to investigate more extensively the gestational age dependency of the percentage of con A nonreactive AFP, and to define the clinical implication and interpretation of the test.

Materials and Methods

All amniotic fluid samples obtained by amniocentesis in early pregnancy for any purpose were examined for degree and type of blood contamination. Samples with any blood contamination were excluded from this study.

"Rocket" immunoelectrophoresis was used to quantify the AFP (7, 8). The samples were stored at \(-20^\circ C\); repeat assay after storage showed no change in AFP concentrations or con A reactivity. The 95% reference interval for total AFP in amniotic fluid has been described earlier (9).

Amniotic fluids studied were grouped as follows: (a) 167 samples from pregnancies with a normal AFP concentration (i.e., within the 95% reference interval) and a normal outcome of pregnancy. (b) 45 samples from 22 pregnancies with a normal outcome, where two or more samples were obtained at different gestational ages. (c) 108 samples from pregnancies where AFP was above the 95% reference interval, but with a normal outcome of pregnancy. (d) 27 samples from pregnancies with severe fetal malformations.

Con A crossed-line affinity immunoelectrophoresis was performed as described elsewhere (4), but with one slight modification: both the first- and second-dimension gels were molded between two glass plates (10). This modification gave a day-to-day coefficient of variation of 5% or less.

Electrophoresis in the first dimension took place in a gel containing free con A, 300 \( \mu g \) per cm\(^3\) (Pharmacia Fine Chemicals). We put 10 \( \mu L \) into the wells, and they were electrophoresed at 5 V/cm until a bromphenol blue-stained albumin marker had migrated 3.8 cm (a little longer than 1 h). Four first-dimension gel slabs were then transferred to the lower end of a 20 \( \times \) 10 cm glass plate with an upper gel containing specific rabbit anti-AFP antibody (3.75 \( \mu L \) per cm\(^3\); DAKO Immunoglobulins Ltd.). Between the con A gel and the antibody gel was molded an AFP-containing gel 3 mm wide.

The second-dimension electrophoresis was run at 2.5 V/cm for at least 16 h. The plates were then dried, stained with Coomassie Brilliant Blue, and the percentage of AFP nonreactive with con A was determined by planimetry or by measuring the heights of the two peaks above the AFP line precipitate (Figure 1).

Results

In group \( a \), the 167 samples with a normal concentration of total AFP and a normal outcome of pregnancy, the percentage of con A nonreactive AFP decreased from a median value of 27.4% in the 13th gestational week to 8.5% in the 21st week (Figure 2). A statistically significant (Kruskal Wallis one-way

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analysis of variance, \( p < 0.001 \) average decrease of 1.7% per week was found from the 14th through the 19th week.

Figure 3 shows the percentage of con A nonreactive AFP for the 22 cases (group b) from whom two or more samples of amniotic fluid were obtained during the pregnancy. In all cases the outcome of pregnancy was normal. The amniocenteses were repeated for reasons other than suspicion of fetal malformation. The percentages declined with time in all cases, the average rate of decline for these cases being 2.2% per week.

For group c, the 108 specimens from pregnancies with a normal outcome but with a concentration of total AFP above the 95% reference interval, the results are indicated in Figure 4. A general decrease in the percentage during the observed period is again evident. In seven of these cases the total AFP value (Table 1) exceeded the cut-off concentration proposed by the U.K. Collaborative Study, but in six the con A nonreactive fraction of AFP was well within the normal range (i.e., above the dashed line in Figure 4). This line (i.e., the lower 0.1% limit for normal samples) was calculated by parametric statistics from results for 275 samples, because these samples were found to be log-normal distributed in each week.

We looked for a possible correlation between the total AFP concentration and the con A nonreactive percentage for the 140 samples from normal pregnancies (including eight pairs of twins) collected in the 15th through the 16th gestational week, seeking any association between both of the two variables and gestational age. No statistically significant correlation was found (Spearman rank correlation analysis: \( p > 0.05 \)) (Figure 5). Figure 4 also includes data from 27 pregnancies involving fetal neural tube defect or other fetal abnormality. In all cases the total AFP concentration was above normal (median 180, range 45-600 mg/L), but in five cases (Table 2) it was near the cut-off values proposed by the U.K. Collaborative Study, in one case actually being just below it. The con A nonreactive percentage was below the dashed line of Figure 4 in all cases.

**Discussion**

It has been suggested (5) that in early pregnancy the AFP is produced mainly by the fetal yolk sac. The high con A nonreactive percentage of AFP in amniotic fluid in early pregnancy corresponds to the high percentage found in yolk-sac AFP, whereas the percentage found for fetal AFP is very low (4).

Ruoslahi et al. (5) have recently described a decrease in con A nonreactive fraction of amniotic fluid AFP during gestation. We have confirmed this in the present study for a larger number of cases. They found an average decrease of about 2.4% per week (5), which corresponds to our finding of 1.7% in single samples and 2.2% per week in serial samples. The decrease in percentage of con A nonreactive AFP may be ascribed to several factors, such as the disappearance of the yolk sac between the 10th and 12th gestational weeks, turnover of amniotic fluid proteins as a result of fetal swallowing, and excretion in fetal urine.
We found no correlation between total AFP concentration and the percentage of con A nonreactive AFP. Thus the con A nonreactive fraction seems to be independent of total AFP concentration, at least in specimens from normal pregnancies. In seven unaffected pregnancies the concentrations of total AFP were so high that they seemed to be diagnostic of fetal malformations, and in three of these cases the pregnancy was terminated (Table 1). The percentage of con A nonreactive AFP was within the normal range (shown in Figure 4) in all these cases except one.

In samples from pregnancies involving a neural tube defect or other severe fetal malformation (Figure 4, Table 2) the percentage of con A nonreactive AFP was always low, even in samples with total AFP concentration close to the proposed cut-off values. In one case the AFP concentration was actually below the proposed cut-off value, but the percentage of con A nonreactive AFP strongly suggested a fetal malformation. Therapeutic abortion was carried out and the fetus was found to have gastroschisis.

It has been shown by several groups (4-6) that in fetal malformation with increased AFP into the amniotic fluid the percentage of con A nonreactive AFP is very low, apparently reflecting the composition of AFP in fetal serum and cerebrospinal fluid. In our study, in six of seven cases with increased AFP concentrations that were not due to fetal malformations or to contamination of sample with fetal blood, the percentage of con A nonreactive AFP was normal.

As seen in Figure 4, the clinical usefulness of the con A test seems maximal before the 19th gestational week, but it is still useful thereafter in eliminating nearly all false positives (Table 1). After the 19th gestational week a low percentage of con A nonreactive AFP is of no diagnostic significance, because the percentage of nonreactive AFP can be low in normal pregnancies (see Figure 2).

Two major problems have to be considered in interpreting the results of con A testing. First, the AFP concentration needed to make a diagnosis is not reproducible. This is especially true for the 20th week, when two groups found a marked decrease in AFP concentration in the last half of the week, but in another group it was stable. Second, the percentage of nonreactive AFP is highly dependent on the maternal AFP. In the present study the percentage was 13.8% in the maternal serum and 12.5% in the amniotic fluid, whereas in U.K. studies it was 2.6% in the maternal serum and 2.8% in the amniotic fluid.

**Table 1. Samples with AFP Concentration Exceeding the Cut-Off Value, but No Fetal Malformation**

<table>
<thead>
<tr>
<th>Gestational age, weeks</th>
<th>Total AFP, mg/L</th>
<th>Multiple of median cut-off value</th>
<th>Con A nonreactive AFP, %</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>59</td>
<td>3.1/3.0</td>
<td>12.8</td>
<td>Pregnancy terminated in 22nd week</td>
</tr>
<tr>
<td>16</td>
<td>80</td>
<td>4.0/3.0</td>
<td>6.0</td>
<td>Pregnancy terminated in 21st week</td>
</tr>
<tr>
<td>18</td>
<td>46</td>
<td>3.5/3.0</td>
<td>21.6</td>
<td>Premature delivery in 30th week</td>
</tr>
<tr>
<td>18</td>
<td>42</td>
<td>3.2/3.0</td>
<td>14.9</td>
<td>Cesarean section in 37th week (indication: cesarean in two earlier pregnancies)</td>
</tr>
<tr>
<td>20</td>
<td>41</td>
<td>4.3/3.5</td>
<td>8.7</td>
<td>Cesarean section in 22nd week</td>
</tr>
<tr>
<td>20</td>
<td>34</td>
<td>3.6/3.5</td>
<td>9.4</td>
<td>Cesarean section in 36th week (maternal diabetes mellitus)</td>
</tr>
<tr>
<td>21</td>
<td>35</td>
<td>4.4/3.5</td>
<td>20.2</td>
<td>Gestational age corrected to 17th week</td>
</tr>
</tbody>
</table>

* As proposed by the U.K. Collaborative Study (1).  b 2.1/3.0, after correction

**Table 2. Samples with Borderline Increase in AFP and Pathological Outcome of Pregnancy**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Gestational age, weeks</th>
<th>Total AFP, mg/L</th>
<th>Multiple of median cut-off value</th>
<th>Con A nonreactive AFP, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spina bifida and omphalocele</td>
<td>15</td>
<td>68</td>
<td>3.0/2.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Omphalocele</td>
<td>15</td>
<td>72</td>
<td>3.1/2.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Gastrochisis</td>
<td>18</td>
<td>50</td>
<td>2.9/3.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Spina bifida</td>
<td>19</td>
<td>52</td>
<td>3.5/3.5</td>
<td>5.6</td>
</tr>
<tr>
<td>Anencephaly and spina bifida</td>
<td>22</td>
<td>45</td>
<td>4.3/4.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* As proposed by the U.K. Collaborative Study
AFP results: fetal-blood contamination and gestational-age dependency. If any blood contamination of the amniotic fluid sample is found, the degree and type (Kleihauer test) of contamination should be determined. The total concentration and the percentage of con A nonreactive AFP in amniotic fluid can each be corrected as described by Nørgaard-Pedersen et al. (4). The gestational age dependency of both total AFP concentration and percentage of con A nonreactive AFP has been demonstrated, and thus the gestational age as estimated by ultrasound examination should be carefully noted, especially if there is any discrepancy between calculated and clinically estimated gestational age.

We conclude that determination of the percentage of con A nonreactive AFP in amniotic fluid appears to be a valuable supplementary test in the diagnosis of certain fetal malformations, when there is only a borderline increase in total AFP concentration. Although the percentage varies with gestational age, its estimation still has the potential to decrease substantially the number of false-positive and false-negative conclusions, and renders the determination of total AFP in amniotic fluid less critically dependent on absolute accuracy.

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