REVIEW

Advances in the Prenatal Diagnosis of Hematologic Diseases

By Blanche P. Alter for the WHO International Registry for Prenatal Monitoring of Hereditary Anemias

The modern era of prenatal testing for hematologic diseases began in 1974. Prior to that time, the only test available consisted of determination of fetal sex following amniocentesis in cases at risk for sex-linked disorders, such as the hemophilias. Methods for obtaining fetal blood in utero in ongoing pregnancies were initiated in 1974, and these developments led to the possibility of prenatal diagnosis of any blood disorder that was expressed in utero and for which the assay could be miniaturized. The first group of diseases for which such testing was utilized was the hemoglobinopathies. Recent advances in molecular biology have now resulted in a return to the use of amniocentesis for many of these prenatal tests (see Orkin for a recent review), although sampling of fetal blood remains the method of choice for detection of hematologic diseases in utero for which deoxyribonucleic acid (DNA) probes are not yet available.

The World Health Organization (WHO) supports an International Registry for Prenatal Monitoring of Hereditary Anemias, which attempts to document all cases examined in utero by fetal blood or DNA studies for hematologic diseases. This Registry is maintained on PROPHET, a computer resource sponsored by the Division of Research Resources of the National Institutes of Health. Analyses of the Registry data are complete for the period of June 1974 through December 1982 and will be referred to here.

Prenatal diagnosis of hemoglobinopathies was last reviewed two years ago, and relevant references may be found there, as well as in a detailed chapter. The previous review cited data from the Registry that ended in March 1981 and included 1,856 cases that had been studied by fetal blood analysis because of the risk of hemoglobinopathies. The current Registry includes 4,133 cases studied for hemoglobinopathies, or more than 2,000 additional cases, as well as more than 300 cases investigated for other hematologic disorders. A total of 4,471 prenatal studies were reported through December 1982. The centers participating in the Registry are listed in Table 1, and the physicians of record at each center are listed in the Acknowledgment section. All but the Scandinavian centers began with examination of fetal blood in cases at risk for thalassemia. Several have now begun to examine the DNA in such cases, which is obtained currently by amniocentesis and, in the near future, will be obtained from chorionic villi. Some centers have also extended their studies of fetal blood to include disorders other than hemoglobinopathies. This review will summarize the major techniques and results reported to the Registry.

Fetal Blood Sampling

Fetal blood can be obtained during the midtrimester by several methods. Most of the procedures are done with local anesthetic. The first technique involves aspiration of blood from the placenta under ultrasound guidance.A A long 20-gauge spinal needle is introduced transabdominally into the placenta, and several small samples of blood are aspirated. The red cell size distribution of the samples is analyzed immediately with a Coulter Channelizer (Coulter Electronics, Hialeah, Fla) to determine the proportion of fetal cells, because fetal erythrocytes have a mean corpuscular volume (MCV) of 140 fl, while adult cells are less than 100 fl. As soon as an adequate sample is obtained, the placentocentesis needle is removed. This method often produces samples of mixed fetal–maternal blood (see Fig 1), which can be enriched in fetal cells (see below), and usually suffice for globin chain synthesis studies. Samples with maternal and/or amniotic fluid contamination are not optimal for determination of fetal blood counts, coagulation factors, or many other examinations.

A more successful technique for obtaining pure fetal blood is fetoscopy. A fiberoptic endoscope (Dyonics Needlescope, Dyonics Inc, Andover, Mass) is inserted transabdominally into the amniotic cavity, and a sample of fetal blood is taken under direct visualization by passing a long 27-gauge needle through the sidearm of the fetoscope cannula. Specimens were initially obtained from fetal vessels on the chorionic plate, which were punctured and bled into the amniotic fluid,
from which the blood was aspirated. Recent modifications have led to blood being drawn directly from the umbilical vein at the placental insertion of the cord.7,8 These samples contain pure fetal blood, with neither maternal blood nor amniotic fluid contamination, and are suitable for all hematologic tests involving cells, serum, or plasma. Figure 1 compares the proportion of fetal cells obtained by several centers by fetoscopy and by placental aspiration. More centers use the former method, and the weighted mean percentage of fetal cells is 96%, compared with 56% by aspiration.

A recent modification has used ultrasound without fetoscopy to obtain pure fetal blood from the umbilical vein and has succeeded in more than 50 cases.9 A 20-gauge long needle was used, which is larger than the 27-gauge needle used through the fetoscope, and the risks to the fetus of this modification are not yet known. Another group has used transvaginal placement of the fetoscope in cases with extensive anterior placentas.10 Other infrequently used approaches have included ultrasound-guided placement of a needle into the hepatic portion of the umbilical vein and ultrasound-guided fetal cardiac puncture.12 The safety and necessity of some of these techniques are open to question.

### Method of Analysis

The proportion of fetal erythrocytes, initially estimated with the Coulter Channelizer, is subsequently confirmed with the acid elution slide test.13 The fetal blood (and a maternal sample as a control) is incubated with 3H-leucine to radiolabel newly synthesized globin chains, which are then analyzed by carboxymethyl cellulose (CMC) chromatography. Details of these techniques are provided elsewhere.14,15 Mutant β-globins, such as S, C, E, O-Arab, and Lepore, are detected because they separate chromatographically from the normal β-chain.

The diagnosis of thalassemia is made when the
normal α- or β-chain is absent or substantially reduced in amount. Cividalli et al. suggested calculation of the ratio of β/γ-globin synthesis. This value increases slowly during the first and midtrimesters and is 0.11 ± 0.05 (mean ± 2 SD) at 16 to 23 weeks in normal fetuses. We found that the β/γ-ratio in fetuses subsequently proven to have β-thalassemia trait was 0.06 ± 0.04. The important aspect is the distinction of homozygotes from heterozygotes. In our experience, β-thalassemia major was diagnosed if the β/γ-ratio was below 0.025. Throughout the world, however, the specific cutoff point ranges from 0.010 (where only β'-thalassemia occurs) to 0.035 (where β'-thalassemia predominates). Each center has determined its own value, depending on the “plusness” of the β-thalassemia in that region and the technical aspects of the separation of β- and γ-globin in its laboratory.

One problem that decreased with increasing obstetric experience is that of impure samples of placental blood. Details of the many approaches that have been used to enrich such samples for fetal red cells are provided elsewhere. In the current WHO Registry, many centers indicated that they now routinely obtain pure fetal samples. Over the preceding years, 1,008 samples required enrichment, and 77% were done with the Ørskov procedure. This is a method for selective hemolysis of adult red cells, which contain carbonic anhydrase, and lyse in the presence of NH₄Cl and NH₄HCO₃. Fetal cells, with low levels of carbonic anhydrase, do not lyse. In the past year, this was the only method used when samples were impure.

Fetal Blood Studies for Hemoglobinopathies

Three thousand nine hundred fifty-nine cases were reported to the WHO Registry from June 1974 through December 1982, in which fetal blood was examined because the fetus was at risk for a hemoglobinopathy. The most recent summary reported in the literature from each center is included in references 19 to 31. The number of cases tested increased significantly each year (Fig 2). Logarithmic transformation of these data results in almost a straight line, with a case number doubling time of approximately 15 months. By the end of 1982, 100 cases were being studied each month worldwide. The disorders for which the fetuses were examined are listed in Table 2. Ninety-two percent were at risk for thalassemias, and the rest were for sickle disorders. Of the thalassemias, 98% were β (β' and β" are combined here, because the specific genetic defect was often unknown), and 60% of the sickle group were at risk for homozygous SS.

The results of the fetal blood studies for hemoglobinopathies are shown in Table 3. Three hundred five of the 3,959 cases required more than one attempt to obtain an adequate sample, but only 54 of the almost 2,000 cases studied in the past two years were in this category, reflecting improved obstetric experience. Three thousand eight hundred eighteen, or 96% of cases, did result in an adequate sample eventually. Of the adequate samples, 25% led to diagnosis of the disease for which the fetus was at risk, which is the proportion expected for autosomal recessive disorders. Of the 961 fetuses found to be affected, only 16 (1.7%) did not request an elective termination.

Errors were reported in 29 cases (0.8% of adequate samples), of which 24 were false negatives. In these, the fetal β/γ-syntethic ratio exceeded the cutoff value for the respective centers. Technical problems with the β- and γ-chain separations on the columns could be implicated in only a few cases. Most of the errors occurred in fetuses with β'-thalassemia major, in whom the β/γ-ratio fell within the heterozygote range. However, the error rates were slightly higher in centers that had studied only small numbers of cases, perhaps indicating some benefit from extensive laboratory

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**Figure 2.** Log plot of cumulative number of cases reported to each Registry. The first Registry ended in December 1978, the second in March 1980, the third in March 1981, the fourth in December 1981, and the fifth in December 1982. (---Δ---) Hemoglobinopathies by fetal blood, (---□---) other diseases by fetal blood, (---○---) hemoglobinopathies by DNA.
Table 3. Fetal Blood Hemoglobinopathy Results, June 1974 to December 1982

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Thalassemias</th>
<th>Sickle</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Total cases</td>
<td>3,636</td>
<td>91.8</td>
<td>323</td>
</tr>
<tr>
<td>Cases with &gt; 1 attempt</td>
<td>291</td>
<td>8.0</td>
<td>14</td>
</tr>
<tr>
<td>No. of adequate samples</td>
<td>3,533</td>
<td>97.2</td>
<td>285</td>
</tr>
</tbody>
</table>

**Affected:**
- Predicted: 900 (25.5%), 323 (8.2%) 961 (25.2%)
- Delivered: 13 (1.4%), 3 (4.9%) 16 (1.7%)

**Errors:**
- False negative: 19 (5%), 5 (24)
- False positive: 5 (0.7%), 0 (5)

**Losses:**
- ≤14 d: 161 (4.4%), 11 (3.4%) 172 (4.3%)
- >14 d in utero: 42 (1.2%), 1 (0.3%) 43 (1.1%)
- Total in utero: 203 (5.6%), 12 (3.7%) 215 (5.4%)
- Term or infant: 27 (0.7%), 2 (0.6%) 29 (0.7%)
- Total overall: 230 (6.3%), 14 (4.3%) 244 (6.2%)
- Maternal complications: 62 (1.7%), 2 (0.6%) 64 (1.6%)
- Prematures*: 169 (6.6%), 13 (5.1%) 182 (6.5%)

Denominators vary throughout the table.
*Corrected for terminations and losses.

experience in the technical aspects, as well as a clearer cutoff point when large numbers of cases had been examined.

The overall fetal loss rate was 5.4%, representing 172 within the first 14 days following fetal blood sampling, and 43 after the first 14 days. An additional 29 infants were lost at term or in the neonatal period, for a total risk of 6.2%. The total loss rate following fetoscopy was 5.4%, compared to 6.3% after placental aspiration. More striking was the observation that the loss rate in cases in which sampling was performed only once was 4.8%, whereas it was 13.6% in the more than 300 cases in which more than one attempt was necessary to obtain an adequate sample. This difference may reflect anatomic difficulties in accessing the fetal circulation, as well as the increased risk of multiple invasive procedures. The loss rate was close to 5% in those centers with a large experience (more than 200 cases), and significantly higher in a few of the centers in which smaller numbers of cases were studied. Thus, the presence of an experienced obstetrical team is clearly important.

Maternal complications, usually leakage of amniotic fluid, occurred in 1.6% of cases, mainly following fetoscopy. This problem may arise because the diameter of the fetoscope cannula, 2.7 mm, is substantially larger than that of the 20-gauge needle used for placental aspiration or for amniocentesis. There were 182 premature infants reported (which is an underestimate, as not all babies were born at the time of the Registry reports). These represent 4.6% of all cases studied, and 6.5% of the cases that were neither lost nor electively terminated. Forty-six babies were reported to have problems at the time of birth. Although the nature of these problems was not always reported, at least three infants had healed superficial needle marks.

In the series reported here, more than 90% of the families benefited from the procedure by having an adequate sample, no fetal loss, and correct information. Prenatal testing for hemoglobinopathies has had a significant impact in several regions with high gene frequencies of β-thalassemia. The birth rate of infants with β-thalassemia major declined to between 10% and 50% of the expected rate in Ferrara, Cyprus, Greece, Sardinia, and among British Cypriots. Thus, fetal blood sampling for prenatal diagnosis of thalassemia has already made a major contribution to the public health problems of those areas.

FETAL DNA FOR HEMOGLOBINOPATHIES

Fetal Tissue Sampling

Midtrimester amniocentesis is a well-established technique, with a fetal risk of < 0.5% above the spontaneous midtrimester loss rate. Twenty milliliters of amniotic fluid obtained at 16 to 20 weeks' gestation contains sufficient fetal amniocytes to provide enough DNA for studies of globin genes. An aliquot of the cells can also be cultured, both for karyotyping and to provide a source of DNA if additional studies are required. The time when the amnio-
centesis is performed and the approximate two weeks required for the analysis result in midtrimester terminations of affected fetuses, as is the outcome when fetal blood sampling is performed.

A new method is now being developed for obtaining fetal tissue, which involves aspiration or biopsy of chorionic villi at eight to 12 weeks' gestation. This is an outpatient procedure and is guided by real-time ultrasound. Sufficient DNA is obtained from the villi (usually 10 to 20 μg) for examination of the globin genes. This method has been reported in nine cases at risk for hemoglobinopathies. In pooled early results from centers just beginning to develop this technique, the loss rate in 113 cases in which samples were obtained for a variety of reasons was cited at 12%. Although higher than that which occurs with amniocentesis or with fetal blood sampling, this rate may decline with obstetric experience. A first-trimester test has obvious psychologic and medical advantages.

Method of Analysis

The recombinant DNA technologies used to detect hemoglobinopathies in utero were reviewed recently in this journal, by Orkin. Globin gene deletion is the cause of many cases of α-thalassemia, ββ′-thalassemia, rare β̄-thalassemia (deletion of the 3′ end), and Lepore. Linked restriction fragment length polymorphisms can be used to detect β̄ and β̄′ genes (HpaI), ββ′-thalassemia in Sardinia (BamHI), and many other β-thalassemias. Extensive and time-consuming family studies are often required to establish these linkages, however. A few DNA point mutations that cause hemoglobinopathies are themselves restriction enzyme cleavage sites, and thus can be diagnosed directly with the appropriate enzymes. Examples of these are the diagnosis of β̄ with Ddel, MstII, or CvnI, all of which cleave at the normal β̄ codon, β̄O-Arab with EcoRI, and a few uncommon β-thalassemia genes with other enzymes. The most useful tool of molecular biology will be the in vitro synthesized oligonucleotides (19-mers), which are specific for each thalassemic mutation. These probes identify either the mutant or the normal β-gene and have been used for prenatal testing of cases at risk for the Sardinian β̄ nonsense mutation. Such methods can identify the most common Mediterranean ββ′-thalassemia due to a mutation in the first intervening sequence. In families in which the specific genetic defect is known, the oligonucleotide method will be the most useful and accurate.

Fetal DNA Studies for Hemoglobinopathies

One hundred seventy-five cases were reported to the WHO Registry to have had DNA studies prior to December 1982, although additional cases had been investigated. Table 4 summarizes the disorders for which the 175 fetuses were at risk. Sixty-eight percent were studied for sickle disorders and 32% for thalassemias. The larger proportion for sickle risks reflects the use of HpaI linkage studies during 1981 and the introduction of MstII in 1981 and 1982.

The results of the DNA studies reported to the Registry are shown in Table 5. Most of the DNA studies used material obtained directly from the uncultured amniotic fluid cells. Twenty-six percent of the fetuses were found to be affected, and there were no errors reported. It should be noted that almost 40% (17/45) of the affected fetuses were subsequently delivered, in contrast to the < 2% (16/961) continuing pregnancies among those where fetal blood sampling was performed. This difference presumably reflects the counseling given to the families, in which fetal blood sampling is cited as having a > 5% fetal loss rate, while amniocentesis has a < 3% loss rate. The difference may include differences in the attitudes of families regarding termination of pregnancy for sickle cell disease versus thalassemia. In the group studied by fetal blood sampling, three of 61 sickle homozygotes were carried to term, compared to 13 of 900 thalassemics. In those analyzed by DNA methods, 15 of 37 sickle and two of eight thalassemia homozygotes were delivered. Thus, the proportion of homozygotes carried to term following DNA analyses was in fact increased in both disease categories. The loss rate in this series was 2.9%. The five cases shown, in fact, include a biased sample. One of these cases required a fetoscopy to provide a definitive diagnosis, and the fetus was lost following that procedure. Two others had histories of multiple miscarriages (one with an incompetent cervix), and one of these plus the fourth had bloody amniotic fluid at the time of the amniocentesis.

The latest methods in molecular biology can thus be used on samples of fetal tissue obtained by amniocentesis or from chorionic villi and should replace fetal blood

<table>
<thead>
<tr>
<th>Disorder</th>
<th>No.</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Thalassemia:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>48</td>
<td>85.7</td>
</tr>
<tr>
<td>β̄</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>α</td>
<td>6</td>
<td>10.7</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>32.0 (of total)</td>
</tr>
<tr>
<td>Sickle:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>109</td>
<td>91.6</td>
</tr>
<tr>
<td>S/β-Thal</td>
<td>4</td>
<td>3.4</td>
</tr>
<tr>
<td>SC</td>
<td>5</td>
<td>4.2</td>
</tr>
<tr>
<td>S/O-Arab</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>68.0 (of total)</td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4. Fetal DNA Testing for Hemoglobinopathies: Fetal Risks, June 1974 to December 1982
Table 5. Fetal DNA Hemoglobinopathy Results, June 1974 to December 1982

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Thalassemias</th>
<th>All Sickles</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Total cases</td>
<td>56</td>
<td>32.0</td>
<td>119</td>
</tr>
<tr>
<td>Affected:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted</td>
<td>8</td>
<td>14.3</td>
<td>37</td>
</tr>
<tr>
<td>Delivered</td>
<td>2</td>
<td>25.0</td>
<td>15</td>
</tr>
<tr>
<td>Errors</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Losses:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤14 d</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>&gt;14 d</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total fetal</td>
<td>3</td>
<td>5.4</td>
<td>2</td>
</tr>
<tr>
<td>Prematures</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

FETAL BLOOD FOR OTHER DISEASES

A large variety of hematologic and nonhematologic disorders can now be detected in fetal blood. Diseases other than hemoglobinopathies were first studied in 1979, and 338 cases were reported to the WHO Registry through December 1982. Of these cases, 243, or 72%, were at risk for coagulopathies (Table 6), mostly the hemophilias. The next largest group was at risk for muscular dystrophies, followed by studies of white cell disorders. For the sex-linked disorders, an amniocentesis was performed first for fetal sex determination. Fetal blood sampling was then only done from male fetuses.

For most of the disorders listed in Table 6, pure fetal blood was optimal, if not absolutely required. Assays of clotting factor activity must be done with pure fetal plasma, because amniotic fluid contains thromboplastic activity. In fact, the need for pure fetal blood for the coagulation diagnoses provided some of the impetus for the development of techniques for sampling from the umbilical vein. VIIIICAg, VIIIIRAg, and IXAg can be measured by immunologic techniques in the presence of amniotic fluid, provided the relative dilution of the fetal plasma is known.

Most of the hemophilia diagnoses were obtained in three centers. Two hundred eight cases were at risk for hemophilia A (factor VIII deficiency), 19 for hemophilia B (factor IX deficiency), and four for homozygous von Willebrand’s disease (inherited as a dominant). There was one error (Table 7), in which a case with hemophilia was not detected in utero. There were seven losses (3%), which indicates that the risk of hemophilia does not increase the risk of fetal loss in utero.

Two fetuses were studied for the possibility of autosomal recessive thrombocytopenias by obtaining platelet counts in fetal blood. One of these fetuses, at risk for thrombocytopenia with absent radii, could have been examined by ultrasound alone. This diagnosis has been reported in the past by means of fetal x-ray studies. Another platelet disorder that has been sought in utero recently is the Wiskott-Aldrich syndrome. This diagnosis was excluded by the findings of a normal fetal blood platelet count and normal mean platelet volume in a male fetus whose affected brother had thrombocytopenia and small platelets.

Red cell disorders other than hemoglobinopathies can be detected in utero. Red cell grouping and typing...
Table 7. Fetal Blood Other Diseases Results, June 1974 to December 1982

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coagulation</th>
<th>WBC + RBC</th>
<th>Muscular Dystrophies</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Total cases</td>
<td>243</td>
<td>71.9</td>
<td>46</td>
<td>13.6</td>
</tr>
<tr>
<td>Cases with &gt;1 attempt</td>
<td>5</td>
<td>2.1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Adequate samples</td>
<td>236</td>
<td>97.1</td>
<td>46</td>
<td>100.0</td>
</tr>
<tr>
<td>Affected:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted</td>
<td>80</td>
<td>33.9</td>
<td>13</td>
<td>28.3</td>
</tr>
<tr>
<td>Delivered</td>
<td>3</td>
<td>3.8</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Errors: negative</td>
<td>1</td>
<td>0.4</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>Losses:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤14 d</td>
<td>4</td>
<td>1.6</td>
<td>2</td>
<td>4.3</td>
</tr>
<tr>
<td>&gt;14 d</td>
<td>3</td>
<td>1.2</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>3.0</td>
<td>3</td>
<td>6.5</td>
</tr>
<tr>
<td>Prematures*</td>
<td>10</td>
<td>6.3</td>
<td>1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

WBC, white blood cells; RBC, red blood cells.
*Corrected for terminations and losses.

Fetal white blood cells can be used to diagnose several types of diseases. The first example involved chronic granulomatous disease, usually an X-linked disorder in which granulocytes and monocytes fail to kill certain types of bacteria due to an inability to generate superoxide radicals. The white cells of affected fetuses were unable to reduce nitroblue tetrazolium on a slide test.\(^{57,58}\) Newburger and Latt noted that samples of mixed fetal-maternal blood could be used, because the male cells could be identified by Y-chromatin fluorescence.\(^{59}\) Three such studies were reported to the Registry. Since adequate numbers of granulocytes are present by 20 weeks' gestation, the diagnosis of neutropenia could also be made, and one such case was studied.

Another major category of white cell disorders involves the immunodeficiency syndromes. Some forms of severe combined immunodeficiency are associated with adenosine deaminase (ADA) deficiency, and one affected fetus was detected by low levels of fetal red cell ADA.\(^{60}\) The risk of fetoscopy can usually be avoided in these cases, because amniotic fluid cell ADA is also reduced in affected cases.\(^{61,62}\) A more general approach to the immunodeficient diagnoses involves determination of the numbers and proportions of fetal lymphocytes and T and B subsets, using fluorescent antibodies and microscopic immunofluorescence assays or a fluorescence-activated cell sorter. Four cases at risk for severe combined immunodeficiency have been reported in the literature (three to the Registry), and one was found to be affected.\(^{63-65}\)

Fetal white cells obtained by fetoscopy can be cultured, thus providing a source of chromosomal analyses by 72 hours, much more rapidly than the two weeks required to culture amniotic fluid cells.\(^{66}\) This is particularly helpful when the amniotic culture fails or the pregnancy is quite advanced when the test is requested. This method was used to diagnose the syndrome of mental retardation associated with fragile X.\(^{67}\) Other chromosome defects would easily be determined from cultured fetal white cells, although in most cases, amniotic cells should suffice (see below). Fetal white cells were used for karyotyping in 35 cases reported to the Registry, and eight were abnormal. Fetal white cells were recently reported to be used to exclude the diagnosis of Hurler’s disease by measurement of leukocyte α-L-iduronidase.\(^{68}\) As with red cells, many enzymes can be measured and diagnoses made depending on the values obtained in relation to normal fetal values.

Fetal serum or plasma is available if pure fetal blood can be obtained. This material was used to measure creatine phosphokinase levels in 49 fetuses at risk for
muscular dystrophies reported to the Registry (Tables 6 and 7), but false negative results were obtained in three cases, and the assay is not considered to be reliable. Current attempts to define restriction enzyme polymorphisms that are closely linked to the genes for the muscular dystrophies will be more valuable than fetal blood sampling.

Fetal serum was used to diagnose Tay-Sachs disease by measurement of hexosaminidase activities, but this is a diagnosis that can be made with amniotic fluid. Fetal plasma was reported to provide the diagnosis of alpha1-antitrypsin deficiency, and the investigators indicated that this was necessary because most of the alpha1-antitrypsin in amniotic fluid is derived from the mother. Such studies were performed in nine cases reported to the Registry.

The WHO Registry data, which extend through December 1982, do not include all of the conditions discussed above, because the use of fetal blood for many other diagnoses is a rapidly growing area. The summarized numbers of cases are small (Table 7). If the cases at risk for muscular dystrophies are excluded, the error rate is <1%, and the fetal loss rate is under 4%. This low loss rate may reflect the fact that most of the nonhemoglobinopathy prenatal testing is performed in only a few of the 11 centers that do any such studies, and these centers have experienced obstetricians.

OTHER HEMATOLOGIC DISEASES DIAGNOSED BY AMNIOCENTESIS

The development of molecular probes for detection of the hemoglobinopathies is advancing rapidly. Cloning of the genes that code for the important coagulation proteins and for red cell enzymes is also underway, and has been accomplished for factor IX, anti-thrombin III, and phosphoglycerate kinase. Prenatal diagnosis of deficiencies for these proteins has not been performed with these probes and awaits determination of whether the diseases are due to gene deletion or whether mutant genes are linked to polymorphic restriction sites. The molecular approach to prenatal diagnosis is under active investigation in many laboratories, for both hematologic and nonhematologic diseases.

Several hematologic diseases can currently be diagnosed by amniocentesis and nonrecombinant DNA technology. One group in this category is the chromosome breakage syndromes, which have autosomal recessive inheritance. The disorder with major hematologic disease in this group is Fanconi’s anemia, in which most patients develop aplastic anemia and/or leukemia in childhood. All patients have increased chromosome breakage, which is further increased following culture of cells with diepoxybutane (DEB), a DNA crosslinker. Twenty-two pregnancies at risk for Fanconi’s anemia have been monitored, and seven affected fetuses were identified by the presence of increased spontaneous and DEB breakage in cultured amniotic fluid cells. Only four of the seven had physical anomalies (two at birth and two of the five terminated), and thus, ultrasound examination alone would not have been diagnostic. One very recent case had Fanconi’s anemia excluded in the first trimester by the observation of no increase in breakage following culture of chorionic villi alone or with DEB.

Other chromosome breakage syndromes have an increased incidence of neoplasia. Patients with ataxia telangiectasia have immunodeficiencies and lymphoid malignancies. One fetus was found to be affected, based on finding a clastogenic factor in the amniotic fluid that produced chromosome breaks in normal lymphocytes, as well as increased breakage of the fetal amniotic cells plus a translocation involving chromosome 14 in those cells. Patients with Bloom’s syndrome show growth retardation and develop leukemia and other malignancies at an early age. Cultured cells have increased breakage plus very high numbers of sister chromatid exchanges (SCEs). Several pregnancies at risk have been monitored by ultrasound and amniotic cell SCE, but all were normal. Xeroderma pigmentosum is characterized by skin sensitivity to sunlight and ocular and cutaneous malignancies. Cells from such patients fail to repair ultraviolet irradiation-induced damage. Three pregnancies have been reported to be monitored for xeroderma pigmentosum, and two fetuses were found to be affected. All of these conditions in which amniotic fluid cultures were assayed might be detected in cultured chorionic villi.

Amniotic cells have been used to identify disorders affecting cellular enzymes. One example is glucose phosphate isomerase deficiency, which results in severe congenital nonspherocytic hemolytic anemia. A fetus at risk was diagnosed in utero by assay of the amniotic cell enzyme level, and then was treated immediately following birth by exchange transfusions. Severe methemoglobinemia due to cytochrome b reductase deficiency was detected in amniotic fluid cells. Both of these enzyme deficiency disorders could presumably have been diagnosed from studies of fetal blood or from chorionic villi, if sufficient material were available.

Porphyria can also be diagnosed by analysis of enzymes in amniotic cells. Sassa et al diagnosed autosomal dominant acute intermittent porphyria due to deficiency in uroporphyrinogen I synthetase, and Deybach et al excluded autosomal recessive congenital erythropoietic porphyria by observing normal levels of uroporphyrinogen III cosynthetase.
Properties of white cells that are expressed in amniotic cells can be used to diagnose nonhematologic conditions as well. Pollack et al used HLA typing of amniotic fibroblasts to determine paternity, assess whether fetuses could be bone marrow transplant donors or recipients, and diagnose HLA-linked diseases, congenital adrenal hyperplasia due to 21-hydroxylase deficiency, and complement C4 deficiency. Forest et al also used HLA linkage, combined with amniotic fluid hormone levels, to diagnose congenital adrenal hyperplasia in utero. HLA typing can also be performed on white cells obtained from fetal blood and perhaps on chorionic villi cells as well.

It should be stressed that the reliability of prenatal testing for many of the hematologic and nonhematologic disorders discussed here awaits the test of time. The numbers of cases studied are often small. The experience with muscular dystrophy (Table 7) has shown that in utero tests may not be error-free. The proper role of prenatal testing requires a reliable assay that distinguishes affected from unaffected fetuses; such assays are still being developed for some of the conditions summarized in this review.

**SUMMARY**

Prenatal diagnosis of hematologic diseases can now be performed with fetal blood, fetal amniotic fluid cell DNA, and fetal chorionic villi DNA. Some hemoglobinopathies can be detected by all three methods, and the choice will depend on the available obstetric and laboratory techniques, as well as the time of presentation of the pregnancy. Hopefully, further development of molecular probes and techniques will soon expand the options to all of the globin disorders.

Detection of coagulation disorders in utero currently requires samples of pure fetal blood. Gene cloning is accomplished for some (factor IX and antithrombin III) and is underway for others (factor VIII), and further investigation is necessary to determine whether deficiencies in these gene products are due to gene deletion or to mutant genes linked to polymorphic restriction enzyme sites of diagnostic use. Thus, molecular biology may be applied to prenatal diagnosis of the clotting problems, but this has not yet been accomplished.

Disorders affecting the number and/or function of erythrocytes, leukocytes, and platelets can be diagnosed by analysis of fetal blood. Blood samples will continue to be required until more is known about the molecular biology of hematopoiesis.

Syndromes that can be diagnosed by chromosome studies should be revealed in cultures of amniotic fluid cells, fetal blood lymphocytes, and chorionic villi cells. Cultured cells can be examined for karyotypes, Y-chromatin, spontaneous or induced chromosome breakage, DNA repair, SCEs, and translocations. The techniques for culturing amniotic cells and fetal blood white cells are established, and those for growing cells from chorionic villi are improving rapidly. Direct preparations of cells from villi only may suffice for some of the above analyses.

The study of hematologic disease in utero has thus come full circle, from the use of amniotic cells to determine the sex in X-linked disorders, to fetal blood sampling for the analysis of gene products, then back to amniocentesis for DNA, and now earlier in gestation to chorionic villi. All of this has occurred in less than ten years, and it is anticipated that developments in the next ten years will be equally dramatic. The future should bring all prenatal testing into the first trimester, use molecular probes, and provide for both early diagnosis and early treatment of genetic hematologic disease.

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**REFERENCES**

33. NICHD National Registry for Anomiaicitess Study Group: Mid trimester amniocentesis for prenatal diagnosis. JAMA 236:1471, 1976
42. Chang JC, Golbus MS, Kan YW: Antenatal diagnosis of sickle cell anaemia by sensitive DNA assay. Lancet 1:463, 1982
82. Auerbach AD, Fergamast E, Alter BP: Unpublished observations
85. Ramsay CA, Colotar TM, Blunt S, Pawsy SA, Giannelli F:


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