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Prenatal Diagnosis

From the Editor and Authors:

The Spring 2001 *Genetic Drift* provides an update on prenatal screening and diagnostic techniques currently in use, and a brief preview of those on the horizon. Since our last update on prenatal diagnosis in 1994, there have been significant advances in many areas of prenatal diagnosis, including some of the topics discussed in this issue: single gene disorders, diagnostic fetal ultrasound, and preimplantation genetic diagnosis. Given the rapid advances in prenatal diagnosis, primary care providers are encouraged to develop a relationship with local experts in maternal-fetal medicine and clinical genetics, who can be consulted as questions inevitably arise.

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In This Issue

Overview

Many of the advances in diagnostic studies utilizing molecular genetics (i.e., on DNA), biochemical markers (i.e., on body fluids and tissues), and cytogenetics (i.e., on chromosomes, including fluorescence in-situ hybridization [FISH]) have applications in prenatal diagnosis. Hence, it is anticipated that the demand for prenatal diagnostic procedures will increase. With the improvements in preimplantation diagnosis and targeted fetal ultrasound, prenatal diagnosis is already no longer limited to amniocentesis, chorionic villus sampling (CVS), and percutaneous umbilical blood sampling (PUBS).

Primary care providers will be called upon to make referrals to a prenatal diagnosis center, which should employ individuals who have expertise in a number of areas. There should be experts in prenatal ultrasound utilizing high quality imaging equipment, individuals who are competent in performing prenatal diagnosis techniques including CVS, amniocentesis, and PUBS, and individuals who are qualified to pro-

vide genetic counseling who have Internet access to current information on prenatal genetics and testing laboratories. A medical geneticist consultant should also be available. There are a number of prenatal diagnostic services available throughout the mountain states region, which can be found in the Genetic Services Directory at www.mostgene.org.

Prenatal Diagnosis of Single Gene Disorders

The number of genetic disorders that can be diagnosed prenatally is constantly expanding, and Internet resources should be consulted for the most up-to-date information. In many instances, a diagnosis is based on molecular techniques, which can identify specific mutations. Examples include autosomal recessive disorders such as cystic fibrosis, X-linked disorders such as Factor VIII deficiency (Hemophilia A), and autosomal dominant disorders such as Huntington's Disease. Other disorders, such as Smith-Lemli-Opitz syndrome, an autosomal recessive disorder caused by abnormal cholesterol synthesis, can be diagnosed by biochemical methods from cells obtained by chorionic villus sampling or amniocentesis. The purpose of this section is to review the general approach to prenatal diagnosis of any genetic condition.

The first step: Recording the family history

Ideally, the process begins by obtaining a detailed family history prior to conception. If a positive family history of a specific disorder is identified, a referral to a genetic specialist may be appropriate to confirm the diagnosis. If both partners are of an ethnic group known to have a high carrier frequency for a particular disease, carrier testing is indicated. For example, carrier testing for the following seven conditions is offered to parents of Ashkenazi Jewish descent: Tay-Sachs disease, Canavan disease, cystic fibrosis, Gaucher disease, Bloom syndrome, Fanconi's anemia (Group C) and Niemann-Pick disease (Type A).

For several reasons, the best time to offer carrier testing is also prior to conception. First, decisions about whether to conceive or to use gamete donors may depend on an accurate risk assessment. Second, being told that one is a carrier of a genetic disorder may have emotional consequences, and these may be exaggerated if a current pregnancy is involved. Third, the time needed to obtain the results of carrier testing can be lengthy. For an autosomal recessive disorder, it may be more cost-effective to test only one partner rather than the couple. If the tested individual is not a carrier, then the pregnancy is essentially not at risk. However, if a second trimester pregnancy is involved, time may be a constraint, and both parents will need to be tested simultaneously.

Genetic counseling can be beneficial for the couple concerned about genetic risks to their offspring. In many cases, when a genetic disorder has been diagnosed in a family member, the couple imagines their risk to be much higher than it actually is.

Example: Ms. L had a brother and a sister with cystic fibrosis (CF). Her brother is deceased, and her sister is awaiting a lung transplant. She and her husband are both Caucasians of Northern European descent. What is their risk of having a child with CF? Ms. L has a risk of ⅔ to be a carrier, and Mr. L has a risk of 1 in 25, if there is no history of CF in his family. CF is inherited as an autosomal recessive disorder, so the overall risk of having an affected child is ⅔ x 1/25 x ¼, or 1/150. While this risk is approximately 17 times higher than the general population risk for Caucasian couples with no family history of CF, the risk is still less than 1%. In this case, it makes sense to offer CF mutation analysis to Mr. L first, if time is not critical. If no mutation is identified, his carrier risk is reduced to less than 1 in 200, and the overall risk of having an affected child drops to less than 1 in 1200.

For some genetic conditions, such as Charcot-Marie-Tooth disease, more than one inheritance pattern has been described. Pedigree analysis can sometimes help to identify the mode of inheritance in a particular family.

Confirming the diagnosis in the affected individual

Another critical step in the process is obtaining medical records, to confirm the diagnosis in the affected relative. Many specialized referral laboratories require that documentation of the diagnosis accompany the specimen being sent for prenatal diagnosis. For example, if the diagnosis involves a metabolic disease, the enzyme levels in the affected child and the carrier parents may provide information that would be helpful in interpreting the results from the prenatal sample.

The lab that made the diagnosis of the affected individual would be the best lab to test the prenatal specimen or perform carrier tests on other family members. This is not always possible, since insurance companies may dictate where a sample must be sent.

Determining whether prenatal diagnosis is possible

After the genetic diagnosis has been confirmed and the risk has been assessed, the next step is to determine whether prenatal testing is available. A very valuable resource is GeneTests™, formerly called Helix, at www.genetests.org. This is a comprehensive database of genetic laboratories funded by the National Library of Medicine of the National Institutes of Health, and the Maternal and Child Health Bureau. It is available without cost to any health professional, although one must register as a user in order to access the information.

GeneTests™ lists over 700 genetic diseases and over 450 laboratories that provide DNA and other genetic testing. One can search by disease name, and a list of labs performing testing will appear. The listing will indicate whether testing is for research only, clinical, and/or prenatal. A contact person is also listed for each lab, so that the specific case can be discussed before sending a sample. For instance, the cost and turn-around time may differ between two labs testing for the

same disease. GeneTests™ also offers a genetic clinic directory, which can be searched by location or type of clinic.

It is important to note that there are still many recognized single gene disorders for which no carrier testing or prenatal diagnosis is currently available. Since change occurs so rapidly in this field, families should be encouraged to regularly contact their genetics center to see if advances have occurred for the disorder in question. This is especially true if they are considering a pregnancy.

When and how to proceed with prenatal diagnosis?

Once it has been established that prenatal diagnosis is an option for a pregnant woman, the next question concerns the gestational age at which the diagnosis can be made. If a DNA test is available, usually CVS will be the preferred method to obtain the sample. When both parents are carriers of a recessive disease, they have a 25% risk with each pregnancy. The slightly increased risk of fetal loss with CVS, as compared to amniocentesis, is usually acceptable to them in order to obtain a result in the first trimester. As discussed elsewhere in this publication, CVS is usually performed at 10-13 weeks from the last menstrual period. However, if the pregnancy is beyond the first trimester, amniocentesis remains a viable option. Patients need to be informed about when to expect results, since some testing may take several weeks.

Non-invasive techniques

If no biochemical or molecular diagnosis is available, it is important to determine whether any anatomic features of the disorder could possibly be identified by fetal ultrasound, and at what gestational age one would first look. In such cases, serial ultrasound examinations may be required. For example, ultrasound has been useful in the prenatal diagnosis of alpha-thalassemia in at-risk fetuses (Lam, et al., 1999).

Analysis of maternal urine has shown promise in evaluating for Smith-Lemli-Opitz syndrome, an autosomal recessive disorder of cholesterol metabolism. When a woman is carrying an affected fetus, disease-specific sterols are present in her urine (Shackelton, et al., 1999).

Practical considerations

Preparations must be made before offering prenatal diagnosis for rare disorders, as the process can be time-consuming. Special arrangements must be made in advance with the referral laboratory. Proper pre-authorization from the insurance company should be sought, recognizing that carrier testing is often denied as a covered benefit, even when clearly indicated. The shipping and handling requirements may be complicated, as when the sample must be shipped on dry ice. The lab may also require special consent forms.

For all these reasons, it is important to work with a genetic consultant when an at-risk couple is identified. Failure to do the necessary groundwork has resulted in situations where a physician has performed an amniocentesis on a patient, only to learn that there is no prenatal diagnosis available for the disorder in question.

Non-invasive Prenatal Diagnostic Techniques

Maternal serum marker screening for Down syndrome

Prenatal screening for Down syndrome is constantly being refined. Initially, the screen was limited to the health care provider's consideration of the mother's age at delivery, once the association between advanced maternal age (i.e., maternal age 35 or older at delivery) and increased risk for Down syndrome was recognized. Asking 'How old are you?' completed the screening process. In the late 1970's a collaborative study in the United Kingdom was published which associated elevated maternal serum alpha-fetoprotein (MSAFP) with an open neural tube defect in the fetus. Serendipitously, an association between low MSAFP and Down syndrome pregnancies was recognized, and by 1986 MSAFP alone was being utilized to achieve the detection of 30% of Down syndrome fetuses in women under the age of 35. By 1988, a complex algorithm using maternal age, and the materum serum analytes AFP, human chorionic gonadotropin (hCG), and unconjugated estriol (uE3) was developed which achieved a 60 - 80% Down syndrome detection rate. In 1993-1994, multiple marker serum screening for all pregnant women under the age of 35 years was recommended by the American College of Obstetricians and Gynecologists and the American College of Human Genetics. It has become standard of care to offer multiple marker serum screening for pregnant women under the age of 35 years at delivery and amniocentesis to those 35 years or older.

Improvements in screening the fetuses of low risk pregnant women for Down syndrome are constantly being made. For example, new analytes in the second trimester serum, as well as first trimester serum and urine are being investigated, and currently various combinations of 2, 3, or 4 serum markers are offered. The variety of screening profiles available can make choices difficult for patients and primary care providers. Seeking advice from a prenatal diagnosis center is recommended. Ultrasound assessment of the fetus, including nuchal skin fold thickness, has also been proposed as a variable to adjust Down syndrome risk.

Individual laboratories offer different multiple marker screens, for which they have determined their own detection rate and initial positive rate (also called false positive rate). Hence each laboratory provides its own risk figure for a given sample, based on the analytes and information provided. It is important to recognize that all screening analyses serve only to adjust the risk to the pregnant woman, and definitive diagnosis of Down syndrome can be made only by chromosome analysis of fetal tissues obtained by CVS, amniocentesis, or PUBS.

It is a challenge for health care providers to choose the most appropriate Down syndrome screening assessment for their patients. It would exceed the scope of this publication to address the specifics of each analyte combination, risk algorithm, risk cutoff, detection rate, and goal for the various screening profiles that are available. It is recommended that providers learn about the specific laboratory studies available to their patient population.

In multiple marker screening, the "bottom line" maternal risk calculation for Down syndrome must start from accurate patient personal information in order for the interpretation to be valid. Each piece of information about the patient and each of the laboratory's analyte levels has equal weight in the algorithm used to calculate the Down syndrome risk. The Foundation for Blood Research and the College of American Pathologists recommend that the patient report should include:

1. The gestational age (GA)
2. How the GA was calculated (last menstrual period, ultrasound, physical exam, etc.)
3. Patient's age at delivery
4. The age-related Down syndrome risk
5. The patient's weight, race, diabetic status
6. The multiple of the median (MoM) values for each analyte
7. Down syndrome risk based on the above

As an added service, many laboratories also provide a written interpretation and/or suggested clinical actions based on the interpretation. One limitation of multiple marker screening is that Down syndrome risk for pregnancies with multiple fetuses cannot be assessed.

The variety of available screening profiles is constantly expanding. The best use of this tool requires that the health care providers confirm patient information on the report and understand the specific screening parameters used by the laboratory. Each laboratory has a responsibility to provide information about their screening program, educate clients as needed, and discuss report specifics when indicated.

In summary, multiple marker screening is a valuable tool to assess the risk of Down syndrome in a given pregnancy, but the accuracy of any approach depends on accurate pregnancy dating and patient information as well as the reliability of the screening parameters utilized by a particular laboratory. Again, the definitive diagnosis of Down syndrome in the second trimester is made by chromosome analysis of amniocytes and the multiple marker screening should not be mistaken for a diagnostic test.

Fetal ultrasound

Medical sonography has benefited from rapid advances in technology, which provide better images at earlier gestational ages. Many women have an ultrasound examination in the second trimester, as a screen for structural malformations. There is increasing evidence that ultrasound markers can be used to adjust the risk that the fetus has Down syndrome. This is particularly true when the ultrasound findings are combined with the results of the multiple marker biochemical screen in maternal serum. It is anticipated that these noninvasive tools can be used to assess a patient's specific risk and to help her make informed decisions about whether to have amniocentesis.

Analogous to maternal serum marker screening, ultrasound identification of a fetal structural abnormality provides information to adjust the risk for a fetal chromosome abnormality. For example, identification of a fetal cardiac anomaly should lead to careful fetal evaluation for other malformations using targeted (formerly referred to as high-resolution) ultrasound. Fetal echocardiography may also be useful in delineating the cardiac defect. Amniocentesis is usually offered if a major fetal structural anomaly is present.

More controversial is how to evaluate less specific ultrasound findings, which have been associated with chromosome abnormalities such as Down syndrome. These include but are not limited to nuchal edema or translucency, hyperechoic bowel, pyelectasis, choroid plexus cysts, and short humerus and femur lengths. In some cases, the ability to assess the fetal markers is limited by the position of the fetus, the body habitus of the patient, or the gestational age.

As with any screening method, there will be false positives and false negatives. There does not appear to be a consensus about how to counsel a patient when the biochemical multiple marker screen and the evaluation of ultrasound markers give conflicting risk assessments.

Chromosome analysis of fetal cells remains the only definitive way to diagnose abnormal chromosome constitution prenatally. Patients who opt for targeted ultrasound and biochemical markers should be informed that normal screens might falsely reassure them. However, for patients who are having great difficulty deciding whether to proceed with amniocentesis, this may be a preferred first step. Also, for women unwilling to accept even a small risk of pregnancy loss from amniocentesis, this approach may provide some helpful information.

There is active research into the use of biochemical markers and ultrasound markers in the first trimester to refine a patient's risk for carrying a fetus with Down syndrome. If this is proven to be reliable, it would have significant benefit to patients because it would allow earlier diagnosis than the second trimester studies currently in use.

Invasive Prenatal Diagnostic Techniques

Amniocentesis

Amniocentesis was first used for prenatal diagnosis in the 1950's. The first case of prenatal diagnosis of trisomy 21 was reported in 1968. Since then the role of amniocentesis has greatly expanded to include the diagnosis of chromosome abnormalities, biochemical disorders, and a number of single

gene disorders. In the US, more than 15,000 amniocentesis procedures are performed annually in the second trimester. The desire for earlier prenatal diagnosis has led to an increase in the numbers of both early amniocentesis and CVS procedures performed.

Standard amniocentesis is routinely performed at 15 to 16 weeks gestation. Early amniocentesis is done between 10 and 14 weeks. First, an ultrasound examination is performed to assess fetal viability and number, gestational age, fetal anatomy, and placental location. Next, an optimal pocket of amniotic fluid is identified, ideally avoiding the fetus, placenta, and umbilical cord. Approximately 20 milliliters of fluid are collected with second trimester amniocentesis and approximately 1 milliliter of fluid per week gestation is removed with early amniocentesis. When performing amniocentesis on multiple gestation pregnancies, it is imperative to properly identify the fetuses. One should attempt to describe the location of the fetuses at the time of the procedure by tracing the umbilical cords to their placentas. Attempts should be made to describe any other ultrasound features that may help to identify the fetuses. If the results of the amniocentesis are abnormal, it will be necessary to correctly identify the fetus with the abnormal result.

Complications/risks of amniocentesis

Several large prospective trials have been undertaken to establish the safety of second trimester amniocentesis. All of these studies were nonrandomized and performed in the 1970's before ultrasound guidance was routinely used. Although the exact risk associated with amniocentesis is controversial, it is not a completely innocuous procedure and can result in a spontaneous abortion. Factors that have been reported to be associated with increased rates of fetal loss include a large number of needle insertions, using a needle greater than 18 gauge, and discolored amniotic fluid. Although earlier data suggested that placental perforation was associated with fetal loss, more recent data have not confirmed this.

Other reported procedure-related complications include leakage of amniotic fluid, vaginal bleeding, amnionitis, and needle puncture of the fetus. Leakage of amniotic fluid occurs in approximately 1-2% of women after undergoing amniocentesis. In most cases the fluid loss is minimal and resolves within several days. Vaginal bleeding may occur in 2-3% of cases and is self-limiting in most cases. Intra-amniotic infection following amniocentesis occurs in approximately 0.1% of cases. With the use of continuous ultrasound guidance, needle puncture of the fetus is usually avoidable.

Amniocentesis can potentially lead to future reproductive complications secondary to sensitization to Rh and other antigens. Rh immune globulin prophylaxis should be administered to Rh-negative patients. However, this will not protect women from being sensitized to more rare antigens such as Kell.

Early amniocentesis

Early amniocentesis, performed before 14 to 15 weeks gestation, potentially provides fetal chromosome results much earlier than second trimester amniocentesis. Studies have shown that the chromosome study results from early amniocentesis are as accurate as those obtained in the second trimester. In contrast to CVS, potential advantages associated with early amniocentesis include the use of a familiar technique that is widely available, the ability to assess amniotic fluid alpha-fetoprotein measurement, and reduction of maternal cell contamination. Lastly, the likelihood that an abnormal chromosome result is due to chromosomal mosaicism confined to the placenta is reduced. This is because the chorionic villi, while of fetal origin, can contain abnormal chromosomes found only in the placenta and not in the fetus. The technique for early amniocentesis is similar to the technique used for second trimester amniocentesis. Early amniocentesis may be technically more difficult to perform because the amnion and chorion, which must be pierced by the needle, are often still separated by the extraembryonic coelom until 14 weeks gestation. This may result in tenting and stretching of the amniotic membranes and prevent access to the amniotic cavity.

Another difficulty encountered in early amniocentesis may be that the placenta is so extensive that access to the optimal pocket of fluid may require a transplacental approach. At this time, there has not been an observed increased rate of complications associated with transplacental early amniocentesis.

A number of studies have been conducted to evaluate the safety of early amniocentesis. An increased rate of post-procedure amniotic fluid leakage has been reported, which was associated with an increased incidence of talipes equinovarus. Based on the information available, it appears that early amniocentesis at 11 weeks through 12 weeks, 6 days is associated with an increased risk of fetal loss and talipes equinovarus. Thus, amniocentesis is not usually performed before 13 weeks gestation unless there are special circumstances. There is currently not enough data available to reach conclusions regarding the safety of early amniocentesis between 13 weeks and 14 weeks, 6 days gestation.

Chorionic villus sampling (CVS)

Chorionic villus sampling (CVS) provides the ability to obtain fetal tissue from the developing trophoblast for diagnostic studies in the first trimester. The chorion frondosum, which contains the most mitotically active cells, is the area that is sampled. The indications for CVS are similar to those for amniocentesis as the specimens can be evaluated for fetal chromosome constitution as well as other molecular or biochemical studies. However, neural tube defects and other structural malformations cannot be detected with CVS. CVS is usually performed at 10-13 weeks gestation and can be accomplished using either a transcervical or transabdominal approach. Prior to the procedure, an ultrasound is performed to assess fetal viability, gestational age, and placental position. The fetal nuchal translucency may be measured as well.

Transcervical CVS was first described in the late 1960's, prior to the introduction of ultrasound guidance in 1979. The procedure involves passing a polyethylene catheter with a malleable obturator through the cervix to the thickest part of the placenta using ultrasound guidance. Placental trophoblast is then aspirated through the catheter into a 20 milliliter

syringe that contains tissue culture medium.

Transabdominal CVS was first described in 1984. A needle is placed through the long axis of the placenta under ultrasound guidance. The stylet is withdrawn from the needle, a syringe containing tissue culture medium is attached to the hub of the needle and suction is applied as the needle is moved up and down through the placenta until an adequate amount of tissue is obtained. Following the CVS procedure the sample should be inspected to ensure that an adequate sample of chorionic villi has been obtained.

In most cases, physician or patient preference will dictate which method is used, and either procedure is usually successful. The techniques have been shown to be equally safe and efficacious provided that the operator is experienced with both approaches.

In approximately 3-5 percent of cases, clinical circumstances will support one approach over the other. Absolute contraindications to the transcervical approach include active cervical or vaginal infections such as herpes, gonorrhea, or chlamydia. Known maternal blood group sensitization is a contraindication for either approach.

Complications/risks of chorionic villus sampling (CVS)

The National Institute of Child Health and Human Development (NICHD) sponsored a seven center nonrandomized study that evaluated the safety and efficacy of transcervical CVS. Although it did not reach statistical significance, the loss rate associated with CVS was 0.8% greater than amniocentesis. There was an increased loss rate associated with procedures in which more than one attempt was made, particularly those requiring three or four passes.

There have been a number of reports suggesting that CVS may cause limb reduction defects. Since the initial report, there have been many other publications both supporting and refuting this association. It appears as though there may be an association of transverse limb defects with CVS procedures performed very early in gestation. There also may be an association of limb reduction defects with less experienced operators.

Based on the data available, it appears that there is a slightly higher risk of pregnancy loss associated with CVS compared to second trimester amniocentesis. The data would suggest that CVS performed after 10 weeks by an experienced operator is not associated with an increased incidence of limb defects compared to the general population. It is important that women are educated regarding the potential benefits and risks including a slightly higher loss rate compared to second trimester amniocentesis and the transverse limb defect controversy. CVS procedures should not be performed prior to 10 weeks gestation.

Percutaneous umbilical blood sampling (PUBS)

The first report of an ultrasound guided percutaneous technique (i.e., through the mother's skin by needle puncture) to enter the umbilical cord appeared in 1983. The ability to obtain fetal blood samples opened up many new diagnostic possibilities. For example, the fetal blood can be used to diagnose hemoglobinopathies and fetal infection, to obtain rapid chromosome analysis, to evaluate fetal hydrops and fetal acid-base status in growth restriction, and to diagnose erythroblastosis fetalis. Treatment of fetal anemias can be accomplished by direct intravascular transfusion by the percutaneous route.

PUBS involves obtaining a sample of fetal blood by placing a needle into the umbilical vein, most often where it inserts into the placenta and is least mobile. While fetal blood sampling has been obtained from the umbilical artery, the umbilical vein is preferred because it is larger and is less likely to cause a fetal bradycardia when punctured. Once the needle has been guided into the umbilical vein, fetal blood is aspirated into a syringe. In order to confirm that the sample is fetal in origin, the mean corpuscular volume (MCV) of the sample should be assessed. The MCV of a sample of fetal blood should be above 100 fL.

Complications/risks of percutaneous umbilical blood sampling (PUBS)

The most critical factor related to the safety of PUBS is operator experience. Reported fetal loss rates for PUBS are 7.2% (96/1328) overall, and 3% in a low risk group (20/660). In addition to fetal loss, other complications associated with PUBS include bleeding from the puncture site in the umbilical cord, cord hematomas, transient fetal bradycardia, infection, and feto-maternal hemorrhage. Bleeding from the cord puncture site is the most common complication and is usually self-limited. Fetal bradycardia, which lasted for a short time in the majority of cases, occurred after 9% of the procedures. In a report on diagnostic cordocenteses and intravascular transfusions, bleeding was observed from the umbilical cord puncture site in 29% of cases. Although the duration of bleeding was significantly longer after arterial puncture than after puncture of the umbilical vein, the blood loss was not clinically significant in any of the cases. The incidence of a clinically significant fetal bradycardia was 6.6%.

Clinical uses of PUBS

Based on the available data, it is clear that PUBS is a riskier procedure than CVS or amniocentesis. Currently, PUBS is offered only where there is no alternative to achieve a timely diagnosis. Fortunately, there have been major inroads in cytogenetic, molecular, and ultrasound techniques that have diminished the need for fetal blood-based diagnosis. For example, the use of PUBS for rapid fetal chromosome analysis for trisomies 13, 18, and 21 has been largely replaced by fluorescence in-situ hybridization (FISH) techniques applied to amniocytes, which can provide chromosome results within 48 hours. Placental biopsy carries a very low risk and villi obtained throughout gestation can be processed within 48 hours. Performing PCR (polymerase chain reaction)-based assays on amniotic fluid specimens can make a number of fetal viral diagnoses. Lastly, Doppler studies have supplanted

the need to sample the fetal circulation for assessment of fetal acid-base status.

Nonetheless, it is clear that PUBS has played a major role in the history of prenatal diagnosis and it is still the method of choice for lifesaving diagnostic and therapeutic measures in erythroblastosis fetalis. Other potential uses for PUBS include assessment of the fetal platelet count in cases of alloimmune thrombocytopenia and in idiopathic thrombocytopenia purpura.

Preimplantation Genetic Diagnosis

There are an increasing number of genetic disorders that can be diagnosed by direct DNA analysis, such as cystic fibrosis. For couples at high risk for having affected offspring, the options for prenatal diagnosis were previously limited to analyzing fetal DNA samples obtained by chorionic villus sampling or amniocentesis. However, couples then face the dilemma of whether or not to interrupt the pregnancy if the genetic abnormality is present. In some cases this may not be a viable option for religious or personal reasons.

Preimplantation genetic diagnosis provides an alternative approach. The procedure samples DNA from embryos that are derived from the in vitro fertilization of the mother's eggs by the father's sperm. Following several embryonic cell divisions, one or more cells are removed from the developing embryos and the DNA amplified and analyzed for the genetic abnormality. The results can often be obtained within 12-24 hours. Embryos without the genetic defect are then transferred into the mother's uterine cavity to develop into a normal pregnancy.

Research techniques for early genetic diagnosis in humans were initiated in the UK in the late 1980's. The first report of the successful application of this technique came from the Hammersmith Hospital, London, which currently is the center with the highest number of births following preimplantation diagnosis. Over 30 pregnancies have now been reported globally, including the US. The conditions diagnosed include cystic fibrosis, Tay-Sachs disease, hemophilia A, and Fragile-X syndrome. Only a few centers worldwide are offering preimplantation diagnosis. The number of successful cases is small enough that this method of diagnosis is still considered to be experimental. Efforts continue to be focused on improving methods to obtain an accurate diagnosis from only one or two cells. Techniques are now available to screen for more than one condition simultaneously, however the accuracy of these modifications needs to be tested further. Although there is certainly a demand for this approach, it will continue to be available only in select specialized institutions with advanced in vitro fertilization and molecular biology laboratories.

The Future of Prenatal Diagnosis

It is likely that future advances in ultrasound and other technology will continue to increase the number of options for less invasive and thus less risky prenatal diagnostic procedures. Already, some conditions that were first diagnosed using fetoscopy (high risk visualization of the fetus with an endoscope) may now be diagnosed using ultrasound, in some cases as early as the first trimester.

Isolation of fetal cells from the maternal circulation represents a promising approach to noninvasive prenatal diagnosis. An international multicenter trial sponsored by the National Institutes of Health is currently being conducted to assess the diagnostic efficacy of using fetal cells in maternal blood to detect fetal chromosome disorders. Preliminary data suggest that the sensitivity is 40-50%.

There have been significant advances in screening for Down syndrome in the first trimester using nuchal translucency and measurement of serum pregnancy-associated plasma protein-A (PAPP-A) and free beta-hCG levels. Detection rates for Down syndrome have been estimated to be between 72 and 87%. If first trimester noninvasive screening techniques prove accurate, it is possible that patients with reassuring screening results will elect not to proceed with invasive prenatal diagnosis procedures. Alternatively, if patients at increased risk for fetal chromosome disorders have reassuring first trimester screening, they may choose to delay prenatal diagnosis until the second trimester and avoid the potential increased risks associated with CVS and early amniocentesis compared to second trimester amniocentesis.

It is important to maintain a close working relationship with your local genetic center offering prenatal diagnosis to assist you in offering your patients the most up-to-date information in this rapidly changing field. With the sequencing of the human genome nearly completed, we will see continued expansion in the number and types of tests available. Information on services available in the mountain states region can be obtained via the Mountain States Genetics Network website (www.mostgene.org).

References

(extensive references available upon request)

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Timetable for Prenatal Diagnostic/Screening Procedures

10 – 13 weeks	Chorionic villus sampling (CVS)
10 – 14 weeks	Early amniocentesis
15 weeks – term *Optimum* 15 weeks - 20½ weeks*	Standard amniocentesis
15 – 21 weeks *Optimum** 16 weeks - 18½ weeks*	Multiple marker screening *(MSAFP + additional markers)*
18 weeks – term *Optimum* 17½ - 24 weeks*	Targeted ultrasound
18 weeks – term *Optimum* 18 - 24 weeks*	Fetal echocardiography
18 weeks – term	Percutaneous umbilical blood sampling (PUBS)

**Optimum times for procedures are dependent on maternal habitus.*
*** Optimum times for serum tests are based on best times for neural tube defect detection.*

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