Amniotic Fluid Testing

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Giving birth to a healthy baby is truly a miracle. There are numerous things that can go wrong in a pregnancy that can be detrimental or affect the quality of life of a fetus. During pregnancy, a prenatal care physician offers a variety of testing that is available to detect fetal abnormalities. Several options in testing exist for the mother and physician. Some are more invasive than others; therefore, the risks involved are different. Some are screening tests, while others are diagnostic. Amniotic fluid testing, using fluid extracted by amniocentesis, is used for specific diagnosis of several genetic abnormalities and is an important source of information to physicians about the health of the fetus.

To fully understand the procedure of amniocentesis an overview of the production and components of amniotic fluid is useful. In the beginning of pregnancy, amniotic fluid is made by the amnion and later is supplied by the kidneys and lungs. The normal volume for amniotic fluid is 500-1500 mL at full term. A constant temperature and cushioning are provided to the fetus through amniotic fluid. The composition of amniotic fluid varies greatly throughout gestation. At its earliest, it is primarily composed of maternal serum. As the fetal kidneys develop, urea, creatinine, and uric acid, in addition to small amounts sodium and osmolality are detected. Also, phospholipids along with steroid and protein hormones are found (Burtis & Ashwood, 2001). Karyotyping of the fetal chromosomes is facilitated by cells shed from the amnion, fetal skin, bladder, gastrointestinal tract, and tracheobronchial tree around the sixteenth week of gestation (Burtis & Ashwood, 2001; “CDC publishes,” 1995). If the fetus is under stress, meconium, a stool-like substance, may be noticed in the amniotic fluid as a green hue (Burtis & Ashwood, 2001).

Amniocentesis procedures are usually performed in a physician’s office and the sample is sent to a clinical laboratory for testing. To extract the fluid, a physician uses a fine gauge needle to puncture the mother’s abdomen through to the amniotic sac in regions that are unoccupied by the fetus and withdraws approximately ten mL of fluid (Burtis & Ashwood, 2001). Anesthesia is generally not needed (Michigan State University, n.d.); however, the mother may complain of mild cramping (Himes, 1999). As mentioned above, ultrasonography is used simultaneously to produce real-time images to help in guiding the needle (Burtis & Ashwood, 2001). If amniocentesis is performed on an Rh negative woman, she should receive an injection of Rh immunoglobulin to avoid possible immunization of the mother (Roberts, 1998). After extraction, transferring the sample to a brown sterile tube is necessary to prevent the breakdown of bilirubin. In addition, if fetal lung maturity is to be assessed, the sample must be iced during transport (Burtis & Ashwood, 2001).

After the sample has been collected, it is sent to the laboratory where it is logged in and the testing procedures begin. First, the amniotic fluid is centrifuged. Subsequently, a medical technologist is able to assess whether or not the sample has the five percent fetal cell material that is necessary to continue testing. If the cell count is adequate, most of the supernatant is decanted and used for other testing including alpha-fetoprotein and lecithin/sphingomyelin ratio. The remainder is resuspended with growth media and placed into three flasks. Different growth media may be placed in each respective flask to increase the chances of growth. Carbon dioxide is then added and a 37°C incubation period of six days begins. If, after this time, enough growth is not available, a food source is added until sufficient growth occurs. The average time needed for cell growth is five to nine days, a much decreased interval from ear-
lier days. After this, the amniocytes are ready for chromosomal studies or karyotyping (Rapp, 1999). Karyotyping requires that cell growth be halted in the metaphase stage of mitosis (Verma et al., 1998). These cells are centrifuged, the supernatant is removed, and the cell buttons are resuspended. A hypotonic solution is added to lyse the cells. Following another centrifugation and addition of fixative, the chromosomes are applied to glass microscope slides and dried. The integrity of the suspension and chromosome spread is evaluated at this point. The slides are stained and dried. Chromosomes now appear to have distinct dark, light, and intermediate gray bands that are used for judging normalcy and abnormality of chromosomes (Rapp, 1999).

Counting the number of chromosomes present is the first action in analysis (Rapp, 1999, 1999). Karyotype images are created using chromosome karyotyping analysis. Usually, chromosomes are organized from longest to shortest. By arranging the chromosomes, anomalies are more readily seen (Xiong, Wu, & Castlemen, n.d.). Twenty cells must be counted. If there is an abnormality, thirty cells are analyzed. At thirty cells, the diagnosis is confirmed. Several other investigative circumstances may arise and a protocol exists for each one. Finally, abnormal slides are always inspected by additional personnel to confirm the diagnosis (Rapp, 1999).

In addition to chromosome studies, other components of the amniotic fluid are tested as well to determine the health of the fetus. Determining fetal lung maturity is important in detecting Respiratory Distress Syndrome (RDS) and aids the physician in making the decision to facilitate labor. To do this the lecithin/spingomyelin (L:S) ratio is used. A ratio of 2:1 or greater indicates that the fetal lungs should be mature enough to function outside the uterine environment. The most widely used quantitative technique to determine fetal lung maturity is fluorescence polarization. Bilirubin in the amniotic fluid may also be measured to indicate hemolytic disease of the newborn (HDN). The most common occurrence of HDN is with Rh negative mothers and Rh positive babies. Bilirubin is measured to determine the severity of the disease. It is measured by using absorption spectrophotometry. Finally, levels of alpha-fetoprotein and acetylcholinesterase in the amniotic fluid can be measured to detect neural tube defects (NTD) (Burtis & Ashwood, 2001).

Two distinct types of amniocentesis exist—early and midtrimester. While the amniocentesis procedure and testing for type are essentially the same, the separation between the two is important. Midtrimester, or standard amniocentesis (SA) has been available for prenatal testing since the 1970’s (Verma et al., 1998). In the 1980’s, earlier diagnosis became desirable; therefore, testing became available for early amniocentesis (EA) (Mennuti, 1996). For example, an advantage to earlier diagnosis is that treatment may be available, but if the diagnosis is delayed, the damage may be irreversible. SA is offered after the fifteenth week of gestation, while the time span for EA varies. Any time from nine to fifteen weeks is accepted as EA (Himes, 1999). Testing for genetic disorders using cytogenetics with EA is equivalent to SA; however, the success rate of using EA to detect NTD by levels of alpha-fetoprotein and acetylcholinesterase is not established (Kuller & Laifer, 1995). Increased risks associated with EA have been studied. In a study described by Himes in the Journal of Perinatal and Neonatal Nursing (1999), the women randomly selected for the EA had a higher rate of fetal loss of 1.7% and an increased rate of amniotic fluid leakage than did the SA participants. A very significant increase of talipes equinovarus (club foot) was noted in the EA group (Sundberg et al., 1997). In addition, orthopedic and respiratory problems have been shown to occur after birth in EA patients. These problems may be linked to the time period during which the fluid is collected which is during limb and lung development (Himes, 1999). An additional study by Kuller & Laifer discussed in American Family Physician (1995) supported these results. In addition to amniotic fluid leakage, vaginal bleeding was more prominent in patients undergoing EA. This study found that there was a higher rate of pregnancy loss in EA during the thirty days immediately following the procedure, but after this period the outcome of pregnancy was essentially equivalent among the two groups (“Complications,” 1996).

Why would a physician risk these adverse effects? Amniotic fluid testing is used to inform the family and their physician about the health of the fetus. The main reason mothers undergo amniocentesis is to diagnose or rule out Down syndrome. In addition to this, other genetic anomalies such as trisomy 13 and trisomy 18 are detectable. All three of these disorders have increased rates of occurrence with increased maternal age (Mennuti, 1996). Neural tube defects can also be detected using amniotic fluid (Stevenson et al., 2000). High levels of AFP and acetylcholinesterase are detected in the presence of a NTD (Kuller & Laifer, 1995). Also, if an intra-amniotic infection is present, amniotic fluid may be cultured to identify the causative organism (Blanco, 2000). There is a host of other conditions detectable by amniotic fluid testing (Himes, 1999). A perhaps overlooked reason for prenatal diagnosis is the bonding of parents and child at birth. Parents can be more prepared and adapted which facilitates a more natural bonding process (Scioscia, 2001; Kuller & Laifer, 1995).

In some cases, treatment may be available for some conditions detected. An example of a condition that can be improved with treatment is the abnormal steroids produced in a female fetus with congenital adrenal hyperplasia. Because of the abnormal
Steroids, females may exhibit male secondary sex characteristics. However, this condition can be avoided if it is recognized by early prenatal diagnosis (Himes, 1999). For treating fetuses with Severe Combined Immunodeficiency Disorder (SCID), an experimental procedure using in utero transplantation of bone marrow cells has been performed in Italy with some success. This may be a promising treatment for SCID infants in the future (Wengler et al., 1996). Stem cell therapy is another experimental therapy that may be used for treatment in utero in the future (Himes, 1999).

A more debatable use of amniotic fluid testing is elective abortion. This can be considered an advantage or disadvantage. In these cases, if parents are given a diagnosis of a genetic condition affecting their baby, they may decide to abort the pregnancy. This has potential to evolve a society where parents may be able to abort a pregnancy on the basis of smaller or unimportant traits (Stoller, 1997).

An additional use of amniocentesis besides diagnostic purposes is for therapy. An example where therapeutic amniocentesis is advantageous is twin-twin transfusion syndrome (TTTS). TTTS occurs in identical twins who share a placenta. In this condition, there is an imbalance of blood flow between the two fetuses due to deformities in the vasculature connecting the fetuses. Therefore, amniotic fluid volume varies between the two sacs along with blood volume. One fetus has too much blood and amniotic fluid, while the other does not have enough. This results in differences in fetal maturity. Amniocentesis may be used several times during the pregnancy to increase survival rate of both twins. This procedure results in the mother’s abdomen becoming less distended and, the volume in the two amniotic sacs becomes more equivalent. Other procedures may be employed if the problem is not remedied (Farley, Cox, & Long, 2000).

While the benefits of amniocentesis are encouraging, certain dangers are associated with this procedure. One of the main reasons patients decide to bypass the procedure is the risk of fetal loss (Mennuti, 1996). For standard amniocentesis the rate of fetal loss is 0.5% (Mennuti, 1996; Stoller, 1997). Some problems that could result in fetal loss are the puncturing of a blood vessel (Collins, 1999) or compression of the uterine cavity due to the removal of amniotic fluid (Saura, Taine, Guyon, Mangione, & Horowitz, 1998). However, included in this percentage are patients who lose pregnancies due to other problems not related to the procedure (Mennuti, 1996). In addition to fetal loss, club foot is increased in pregnancies that underwent early amniocentesis. In contrast, the incidence of birth defects is not increased in standard amniocentesis (Himes, 1999).

Because of the risk, amniocentesis is not recommended to everyone for prenatal diagnostic testing. Some reasons for considering amniocentesis include increased maternal age, genetic conditions carried by parents, abnormal ultrasound, abnormal triple screen, and taking harmful medications (MSU, n.d.).

In addition to the risks involved, amniotic fluid karyotype testing does include pitfalls. The culture may fail to grow or there may be contamination due to maternal cells (Verma et al., 1998). Alternatives are available for prenatal diagnosis. Some are less invasive, others are not. All options must be considered.

One such option is the triple screen in which the mother’s blood is the specimen tested (Mennuti, 1996). The triple screen is performed during fifteen and twenty weeks gestation (Kuller & Laifer, 1995) and is often used on mothers who are younger than thirty-five years. Levels of alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG), and unconjugated estriol (uE3) are measured (Mennuti, 1996). An elevated AFP is indicative of an open neural tube defect. However, other factors may cause this elevation such as multiple fetuses, vaginal bleeding, fetal ventral wall defects, and fetal death. An elevated AFP should be investigated further. An ultrasound provides important information that may answer questions. Different levels of AFP are normal for different gestational ages; therefore, the physician must accurately determine the age of the fetus or if there are multiple fetuses present. After the ultrasound, the physician and mother may decide to pursue amniotic fluid testing (Kuller & Laifer, 1995). A low AFP is indicative of Down syndrome. AFP is a more sensitive marker for NTD than for Down syndrome. Down syndrome may be indicated by low levels of uE3 and elevated levels of HCG which is the most sensitive marker. Other abnormalities, such as trisomy 18 and abdominal wall defects, can be detected by the triple screen (Mennuti, 1996). However, the triple screen is only a screening tool and should not be considered diagnostic (MSU, n.d.). A risk ratio may be calculated using the levels of each marker in combination with maternal age to determine the need for amniocentesis (Kuller & Laifer, 1995).

Another option is ultrasound. This can be used to identify structural problems (Kuller & Laifer, 1995). However, sex chromosome aneuploidies cannot be detected (Mennuti, 1996). In addition, certain markers may be visible on the ultrasound in Down syndrome (Kuller & Laifer, 1995). The main advantage with ultrasound is that it is deemed completely safe with no side effects. However, as with the triple screen, ultrasonography is only a screening tool and not diagnostic (MSU, n.d.). Ultrasonography has additional advantages such as determining gestational age and fetal number, in addition to aiding in amniocentesis as mentioned earlier (Kuller & Laifer, 1995).

A more invasive procedure is chorionic villus sampling (CVS). This procedure, like early amniocentesis, was developed in the 1980’s for earlier diagnosis (Mennuti, 1996). CVS can be done at ten to twelve
weeks of gestation. NTD and abdominal wall defects are undetectable with this test. Complications include vaginal bleeding and cramping. Also, terminal transverse limb reduction is a more serious problem that has been related to CVS (Himes, 1999). Rate of fetal loss is slightly higher than in SA at 1.0 to 1.5% (Mennuti, 1996). Amniotic fluid testing is considered to produce more reliable results with less potential fetal damage (Himes, 1999).

An additional option is polymerase chain reaction amplification (PCR). This is an alternative to routine amniotic fluid testing in diagnosing Down syndrome. There are advantages to this procedure. Amniotic fluid is required for this procedure but in a smaller amount. In PCR, the results are available the same day of the procedure, whereas routine amniotic fluid testing takes several days for a result. PCR is also more accurate and the results are easy to read. In the future, PCR may be extended to detect other genetic abnormalities as well. Therefore, it may be chosen over traditional amniotic fluid testing for prenatal diagnostic screening (Verma et al., 1998).

The science of prenatal diagnosis has changed dramatically over the last fifty years. Families and physicians no longer have to go into the delivery room blind. All of these technologies discussed, such as amniotic fluid testing and ultrasonography, have improved the survival rate of fetuses. Because of early detection, the physicians have the information they need to decide what is best for the mother and the baby. One can only hope that in the future these will be improved further so as to decrease the risk of fetal loss and congenital defects even more.

References


Michigan State University prenatal genetics clinic. (n.d.)


Questions for STEP Participants

Answer questions only on the official STEP answer sheet. If you do not have the official STEP answer sheet, a year’s supply can be obtained (at no cost), simply by writing to: STEP Program Answer Sheets, American Medical Technologists, 710 Higgins Road, Park Ridge, IL 60068-5765, or by fax: 847/823-0458, or by e-mail: paula.simoncini@amt1.com

In addition to marking your answers, be sure to include all the required information on the answer sheet and a processing fee of $3.00 per article.

In the following, choose the one best answer for each question.

1. Amniotic fluid is NOT produced by the:
   - A. kidney
   - B. liver
   - C. lungs
   - D. amnion

2. A green discoloration to an amniotic fluid specimen may be due to:
   - A. meconium
   - B. hemolysis
   - C. hyperlipidemia
   - D. excess placental steroids

3. Amniotic fluid testing would be of little or no value in:
   - A. determining renal sufficiency
   - B. detecting chromosomal abnormalities
   - C. investigating for possible neural tube defects
   - D. evaluating cases of hemolytic disease of the newborn

4. Which of the following lecithin:sphingomyelin ratios would indicate fetal lung immaturity?
   - A. 1.0
   - B. 2.0
   - C. 3.0
   - D. 4.0

5. The MOST debatable use of amniotic fluid testing is in the area of:
   - A. Down syndrome
   - B. elective abortion
   - C. neural tube defects
   - D. severe combined immunodeficiency disease

6. Amniocentesis may be used as a therapeutic technique in:
   - A. diagnosis of Down syndrome
   - B. prevention of neural tube defects
   - C. quantitation of bilirubin in Rh incompatibility
   - D. treatment of twin-twin transfusion syndrome

7. Which of the following problems is least likely to be associated with amniocentesis?
   - A. Liver failure
   - B. Fetal demise
   - C. Talipes equinovarus
   - D. Respiratory disorders

8. A problem associated with amniotic fluid karyotyping is contamination of the fluid with:
   - A. fetal urine
   - B. maternal cells
   - C. excessive lipids
   - D. placental steroids

9. Amniotic fluid should be transported and stored in a dark container when the evaluation includes:
   - A. creatinine
   - B. L:S ratio
   - C. bilirubin
   - D. alpha fetoprotein

10. Which of the following is NOT a component of the triple screen?
    - A. Alpha fetoprotein
    - B. Unconjugated estriol
    - C. Lecithin:sphingomyelin ratio
    - D. Human chorionic gonadotropin