Chapter 5
Diagnostic Tests (Invasive Procedures)

Toshiro Ikeda

Abstract Diagnostic tests, which are performed as screening tests in high-risk cases, refer to invasive techniques such as chorionic villus sampling (CVS), amniocentesis, and cordocentesis (percutaneous umbilical cord blood sampling, PUBS).

Advances in diagnostic techniques that have incorporated molecular genetics now allow the diagnosis of most diseases by means of chromosomal or genetic tests rather than by performing histopathological or enzymatic diagnosis. As a result, highly invasive diagnostic procedures are rarely used.

CVS and amniocentesis are the most frequently used diagnostic tests at present, with amniocentesis more commonly performed. However, as CVS is now more commonly performed via transabdominal rather than transcervical procedure, the fetal loss rate has decreased, so the number of CVS procedures is increasing in various regions.

Cordocentesis is also performed in selected cases due to the ability to diagnose fetal anemia and obtain a rapid chromosomal analysis.

In this chapter, we discuss the specific methods of these tests and their advantages and disadvantages.

Keywords Chorionic villus sampling · Amniocentesis · Cordocentesis · Fetal blood sampling · Invasive prenatal diagnostic procedures

Due to the dissemination of noninvasive prenatal genetic testing (NIPT), various screening tests are slowly being phased out of use as diagnostic tests. Furthermore, advances in diagnostic techniques now allow the diagnosis of most diseases by means of chromosomal or genetic tests rather than by performing histopathological or enzymatic diagnosis. As a result, highly invasive diagnostic techniques are rarely

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used. However, retrieval of fetal-derived cells to confirm a diagnosis requires the use of the invasive techniques described in this chapter. The table shows a comparison of frequently used methods.

All of these techniques involve inserting instruments into the pregnant uterus, so aseptic techniques are required. To ensure maximum sterility, the skin and vaginal walls should be adequately disinfected using povidone-iodine or other disinfectants, and the ultrasound probe and ultrasound guide attachment should be covered with a disposable sterile material.

5.1 Chorionic Villus Sampling (Fig. 5.1)

The advantage of Chorionic villus sampling (CVS) is that it can be used to perform confirmatory tests during the earliest stages of pregnancy. This allows couples a great deal of time to make the necessary decisions based on the test outcomes. If couples choose to terminate the pregnancy, it also facilitates the selection of a safer method to do so. The disadvantage is the high miscarriage rate of 1–2%. However, spontaneous miscarriages are common during the period in which CVS is performed, and because the procedure is performed in cases of suspected fetal abnormality, such as increased nuchal translucency, these cases would include fetuses with chromosomal abnormalities or cardiac malformations that would likely have resulted in spontaneous miscarriage. After correcting for these cases, current reports indicate that the increase in the miscarriage rate due to the technique itself is 1 in

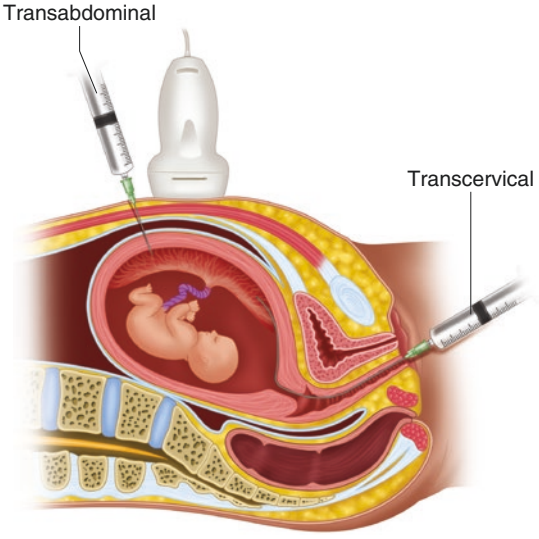

Fig. 5.1 Chollionic villus sampling. (A) Transabdominal, (B) transcervical

370 or lesser, so CVS is as safe as performing amniocentesis. CVS is usually performed via a transcervical or transabdominal approach, with the transabdominal approach becoming popular in recent years due to its increased safety [1, 2].

5.1.1 Approaches for CVS

(a) Transcervical approach

The biggest advantage of the transcervical technique is that it can be performed before gestational week 10, thus allowing collection of the chorionic villi at the earliest stage. Previously, the procedure was performed without ultrasound guidance, but presently, the most popular method involves the insertion of specialized biopsy forceps or catheters via the external cervical os under transabdominal ultrasound guidance, followed by collection of the chorionic tissue. Chorionic villi are abundant during the early weeks of pregnancy, so the tissues can usually be retrieved successfully. Although the procedure has a miscarriage rate of 1–2%, we believe that the miscarriages are primarily caused by bacterial infection. Despite the specification of antibiotics, there has been no obvious decrease in the miscarriage rate.

According to reports, one issue that demands particular caution is the increased rates of fetal limb reduction and oromandibular limb hypogenesis syndrome that are observed when the procedure is performed before 10 weeks [3, 4]. At present, the transabdominal approach discussed below is therefore usually performed from gestational week 10 onward, other than in exceptional cases.

(b) Transabdominal approach

This approach is similar to that used for amniocentesis. However, it is sometimes impossible to perform puncture safely based on the position of the maternal intestines, the placenta, and the umbilical cord attachment site. These issues can sometimes be resolved by either filling or emptying the bladder, although this will only help in ≤10% of patients, so the transabdominal approach must be abandoned in certain cases. When this occurs, it is necessary to determine whether to attempt a transcervical approach or switch to amniocentesis.

The transabdominal approach involves advancing the needle into the chorion and performing aspiration for retrieval. This usually requires the use of an 18 to 21G needle. Various hospitals are devising safer ways to retrieve adequate amounts of chorionic tissue, and recently, the number of hospitals using the double-needle technique to avoid repeated puncture of the uterus has increased. This technique involves puncturing the uterus using a larger 18G needle, then inserting a 20 to 21G needle through the larger needle to perform repeated aspiration to retrieve the required amount of tissue. There are specialized CVS needles that are commercially available, although they may be used in combination with percutaneous transhepatic cholangiography (PTC) needles. A few mL of heparinized saline is drawn up into the syringe that will be used for aspiration, and then the syringe is used to apply

continuous suction while gently moving the needle back and forth to retrieve the required amount of chorionic tissue.

The 18G needles are large, so the more that the angle of insertion into the abdominal wall or uterine surface approaches the horizontal (0°), the more likely there will be resistance to the puncture and an inability to advance the needle in the desired direction. This commonly causes needle bevel position errors, and in most cases, the situation can be resolved by rotating the needle by 180°. The use of any needle that is ≥20G usually necessitates the administration of local anesthesia.

The reason the abdominal method has become more mainstream is due to its lower rate of fetal loss, and there has been no obvious increase in risk as compared to amniocentesis when investigating cases other than high-risk groups, such as patients with increased nuchal translucency.

(c) Transvaginal approach

This approach is still not very popular. The transvaginal approach entails puncture of the uterine corpus under ultrasound guidance using a transvaginal probe, similar to the procedure used for transvaginal oocyte retrieval, and retrieving chorionic tissue. This approach may be required to collect chorionic villous tissue from sites that are difficult to reach via the transabdominal and transcervical approaches [5], although it has not become popular due to its perceived high potential for causing intrauterine infection.

5.1.2 Twin Pregnancies

When dealing with monozygotic (monochorionic) twins, a single CVS procedure may be performed. However, when dealing with dichorionic twins that may be dizygotic, a biopsy needs to be taken from each placenta. If the placentas can be clearly differentiated, then it is possible to perform the procedure on each placenta separately. However, if the placentas lie in close proximity to one another, then amniocentesis should be considered.

5.1.3 Chromosomal Mosaicism

When fetal chromosomal analysis is performed by means of CVS, the cell culture must be divided into two or more flasks to facilitate the determination of mosaics (true/false). True mosaicism is detected in 1–2% of cases, although in most cases, this is confined placental mosaicism, and no definitive statements can be made regarding the mosaicism of the fetus. If it is difficult to make a diagnosis, then amniocentesis must be performed for confirmation. An adequate explanation will need to be provided to the patient before the test is performed.

Conversely, mosaicism may not be detected in the small amount of chorionic tissue that is retrieved from a narrow area. To prevent this, some practitioners advocate advancing the needle to ensure collection of chorionic tissue over a wider area, but this leads to the dilemma of inadvertently increasing invasiveness.

5.1.4 Maternal Cell Contamination

There is a higher rate of maternal cell contamination during CVS than amniocentesis. It is impossible to completely eliminate maternal cells, such as prolapsed membranes, or maternal skin or uterine muscle layers, but it is important to perform irrigation with culture medium, use equipment such as stereoscopic microscopes, and do the utmost to ensure that only villi are collected. To prevent prolapsed membranes from entering the retrieval site, attention must be paid to ensure that the procedure is performed away from the uterine muscle layers.

5.2 **Amniocentesis** (Fig. 5.2)

Amniocentesis is easier to perform and more widely used than CVS. In most cases, puncture is usually performed via a transabdominal approach using a 21 to 25G needle under ultrasound guidance. To minimize the risk of maternal cell

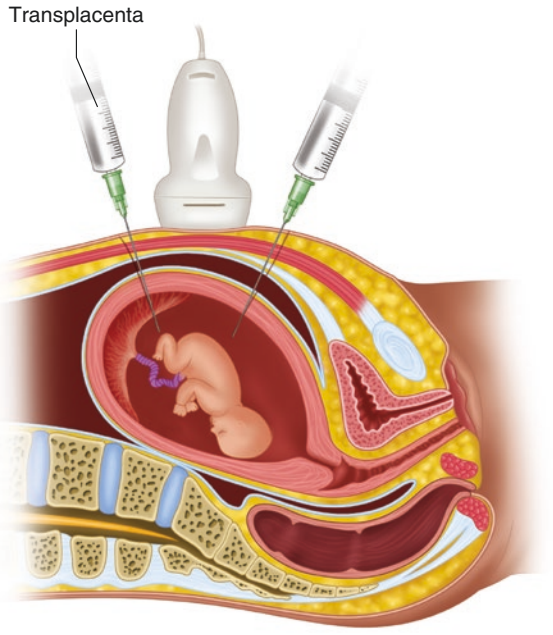

Fig. 5.2
Amniocentesis

contamination, it is preferable to use a specialized amniocentesis needle with a stylet (if one cannot be obtained, then a spinal needle with a stylet or a PTC needle can be used as a substitute). Furthermore, discarding the first 1–2 mL of the aspirate will reduce the number of maternal cells collected.

The procedure can be performed from gestational week 15 onward, although it is increasingly difficult to distinguish between the amnion and chorion from week 16, which significantly reduces the number of puncture attempts. This is why most hospitals perform the procedure from week 16 onward. In addition, in certain cases, despite the ability to see that the needle has entered the amniotic fluid cavity, it may be impossible to perform aspiration. It is not uncommon to be unable to see the needle on ultrasound, but the amnion adopts a tented shape when pressure is applied on the needle. Using a needle that is as sharp as possible and performing the puncture by applying an adequate amount of force at a location where the amnion and chorion have unified reduces this to a significant extent. The needle should be inserted as perpendicularly to the amnion as possible for maximum success.

When performing chromosomal analysis, 10–20 mL of fluid is retrieved, and samples taken for testing mosaicism (true mosaicism, pseudo-mosaicism) should be divided into ≥2 flasks for cell culture.

As discussed in the section on CVS, true mosaicism of the amniotic cells is not necessarily significant for fetal mosaicism, although unlike mosaicism that is detected by means of CVS, confirmatory tests for mosaicism by means of amniocentesis cannot be performed via cordocentesis, which is challenging to perform and a high-risk procedure. Therefore, depending on the case, detailed ultrasound findings and conventional data may be accepted as confirmatory tests.

The rate of fetal loss has been described to be around 0.3%, but the corrected data showed that it was as low as 0.06–0.13% [2, 6, 7].

5.2.1 Placental Puncture

It is best to avoid performing placental puncture to reduce the risk of Rh isoimmunization and mother-to-child transmission of infection, although several reports have indicated that the rate of miscarriage does not change when transplacental puncture is performed. There is no need to avoid this procedure until there is no other option. A considerable amount of caution is required when puncturing the lateral uterus or uterine fundus due to the potential for injury to the uterine artery and vein and subsequent hematoma formation, and the risk of inadvertently puncturing the maternal intestines.

In addition, it is necessary to thoroughly confirm the umbilical cord attachment site before puncture. If the placenta is punctured without considering avoidance of the umbilical cord, devastating consequences may occur if the umbilical cord has a velamentous insertion. The umbilical cord attachment site and any large vessels should also be avoided.

5.2.2 *Twin Pregnancies*

A single puncture is sufficient in the case of monozygotic twins (monochorionic). However, when dealing with dichorionic twins that may be dizygotic, a biopsy needs to be taken from the amniotic fluid cavity of each twin. To puncture each individual amniotic fluid cavity with a high degree of certainty, it is possible to inject dye, such as indigo carmine, before puncturing the first cavity to distinguish it from the second cavity. However, the properties of ultrasonic tomography are so advanced that dye injection is not considered to be necessary in most cases (although it may be necessary in cases of multiple pregnancies with ≥3 fetuses). Particular caution is required to ensure that samples are appropriately interpreted for the intended fetus in the case of multiple fetuses of the same sex.

5.2.3 *Early Amniocentesis*

This term refers to amniocentesis performed prior to gestational week 14. This procedure is not usually performed nowadays due to the ability to perform transabdominal CVS safely as it carries the risk of rupture of the membrane (amniotic fluid leakage), fetal deformities, and miscarriages increases.

5.3 Cordocentesis

Cordocentesis (percutaneous umbilical cord blood sampling, PUBS) is performed under ultrasound guidance to collect blood from the umbilical vein. It is commonly performed using a 21 to 23G needle, but a 25G needle is used in some hospitals to perform the procedure. Reports have indicated that the procedure can be performed before week 14, although it is usually difficult to perform before week 18–20.The fetal loss rate was thought to be high, but recently reported 0.6% [8].

(a) Transplacental puncture of the umbilical cord attachment site (Fig. 5.3)

Transplacental puncture of the umbilical vein may be performed near the umbilical cord attachment site if the placenta is attached along the anterior uterine wall. This form of puncture can be performed with the most ease and greatest certainty, so it is preferred. The disadvantage of this technique is that the maternal blood will mix with the fetal blood, which may promote immunosensitization and decrease test accuracy.

(b) Puncture of the umbilical cord attachment site on the posterior uterine wall (Fig. 5.4)

This technique may injure the fetus and is usually difficult to perform, but if the puncture can be performed safely and only the umbilical vein is punctured, then

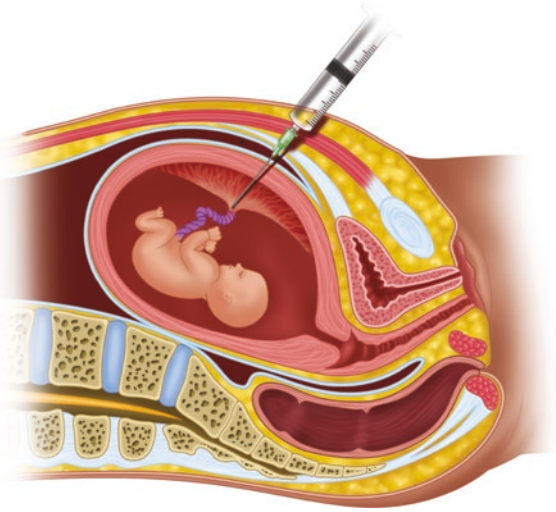

Fig. 5.3 Cordocentesis (anterior placenta)

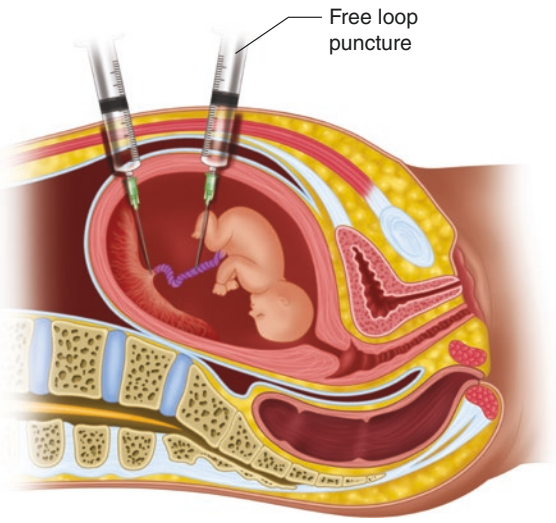

Fig. 5.4 Cordocentesis (posterior placenta)

sensitization is unlikely to occur. As with the other methods, it is important to exercise caution regarding contamination with maternal blood located in the intervillous spaces at sites that are close to the placenta.

(c) Free loop puncture (Fig. 5.4)

The issue of sensitization is associated with placental puncture, but it is not uncommon to be able to avoid the placenta. The objective of this technique is to puncture the umbilical cord (the umbilical vein, as a rule) as it is floating in the amniotic fluid. The free loop will move as soon as it is contacted by the tip of the needle, so the trick of

this technique is to perform the puncture instantaneously as soon as the needle comes into contact with the cord. Provided that there is an obvious separation between the cord and the placenta, fetal blood will be obtained with absolute certainty. However, it is difficult to perform this technique from the second trimester onward.

5.3.1 Confirming That Blood Is of Fetal Origin

It is necessary to rapidly determine whether the blood that has been retrieved is actually of fetal origin. The following methods are useful for detecting erythrocytes of fetal origin.

(a) Blood gas analysis
(b) Erythrocyte morphology (particularly the mean corpuscular volume [MCV] distribution: fetal blood MCV is largely unimodal. If contaminated by maternal blood, there is the simultaneous appearance of an MCV peak that is smaller than that of the fetus.)
(c) Blood type (ABO, RhD)
(d) The Kleihauer-Betke test

So far, the general methods described are summarized in the Table 5.1.

5.4 Other Diagnostic Tests

(a) Coelocentesis

This method involves retrieving coelomic fluid that is present outside the amnion [9]. As its advantages, the procedure can be performed without harming the amnion or chorion, and it can be performed during the early stages of pregnancy. However, this fluid has a high viscosity and cannot be aspirated with needles smaller than 20G. It has not achieved practical application because only a small number of cells are collected, and these cell cultures are difficult. The percentage of contamination by the maternal components is also poorly understood. Preimplantation genetic testing

Table 5.1 Comparisons of common invasive diagnostic tests

		Weeks	Difficulty	Fetal loss[a]
CVS	Transcervical	10~14	Slightly difficult	1~2% (or lesser)
	Transabdominal	10~14	Slightly difficult	−0.11~1.16%
Amniocentesis		15(16~)	Easy	0.06~0.27%
Cordocentesis		18~	Difficult	0.6%~

Although there are various reports on the risks of CVS, it is safer to use the transabdominal approach

[a]Additional risk of fetal loss (exclude spontaneous miscarriage)

(PGT) is usually performed by determining aneuploidy (PGT-A) using next-generation sequencing, and it is expected that this method may be revised in the future.

(b) Fetal muscle biopsy [10]

Reports have indicated that immunohistochemical staining may be used to diagnose suspected cases of Duchenne muscular dystrophy if a biopsy is taken from the fetal gluteal muscle using a needle gun.

(c) Fetal skin biopsy [11]

This is used to diagnose lethal skin diseases. Biopsy forceps are used for this purpose.

(d) Fetal liver biopsy [12]

Reports have indicated that this may be useful in cases of carbamoyl phosphate synthetase deficiency.

(e) Fetal tumor biopsy

5.4.1 Maternal Infections

In the event of antenatal infection with human immunodeficiency virus (HIV) or hepatitis B, or in the case of hepatitis C carriers, it is recommended that noninvasive tests, such as NIPT, perform whenever possible. If invasive tests are required, reports have shown that there is no significant difference in terms of the risk of mother-to-child transmission when amniocentesis is selected over CVS to avoid the placentae in hepatitis B and hepatitis C carriers or patients. Reports have also suggested that the HIV infection rate does not increase as long as patients receive combined antiretroviral therapy, although the evidence for this is insufficient [13]. It is important to explain the risk of inducing fetal infection before performing any test. Ideally, the infection rates for each condition should be explained before the test using the most recent data that is available.

5.4.2 Rh Isoimmunization

After invasive testing is performed on mothers who are Rh(D) negative withs partners who are Rh(D) positive, they will be administered Rh(D) immunoglobulin (RhIG) as prophylaxis against sensitization. If the fetal blood tests and NIPT confirm that the fetus is Rh-negative, then the administration of RhIG is not required, but if the test results are not adequately reliable, then the case must be handled as though the fetus were Rh-positive.

In all cases, the indirect Coombs test and middle cerebral artery peak systolic velocity must be measured periodically after puncture, and the patient should be followed up carefully to ensure that there is no possibility of fetal anemia.

5.4.3 Maternal Risk

Despite being extremely rare, there have been reports of serious maternal complications. These included reports of septicemia that has resulted in maternal death in some cases [14, 15]. These types of complications may be caused by puncture of the maternal intestines when performing transabdominal procedures. Due to the characteristics of ultrasound tomography, it may be challenging to determine whether bowel is lying between the peritoneum and uterus. However, if time is allowed to elapse during the examination, then the peristaltic movements of the bowel will become visible. It is important to perform a thorough examination before puncture to rule out intestinal puncture. There have also been reports of amniotic fluid emboli, but these are even more rare.

Caution is also required during techniques that use the transcervical approach, as this may be associated with the risk of uterine perforation. This is an unavoidable complication, so obtaining appropriate informed consent before the procedure is important.

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