DEGUM Recommendations on Diagnostic Puncture in Prenatal Medicine

Empfehlungen der DEGUM zu diagnostischen Punktionen in der Pränatalmedizin

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ABSTRACT

Diagnostic puncture (amniocentesis, chorionic villus sampling, and fetal blood sampling) is an essential part of prenatal diagnostics and the only established and sufficiently scientifically evaluated possibility of diagnosing genetic diseases from pregnancy-specific cells. The number of diagnostic punctures in Germany, as in other countries, has fallen significantly. This is largely due to the introduction of first-trimester screening with further detailed ultrasound examination of the fetus and the analysis of cf-DNA (cell-free DNA) from maternal blood (noninvasive prenatal test – NIPT). On the other hand, knowledge about the incidence and appearance of genetic diseases has increased. The development of modern molecular genetic techniques (microarray and exome analysis) makes a differentiated investigation of these diseases increasingly possible. The requirements for education and counseling regarding these complex correlations have thus increased. The studies performed in recent years make it clear that diagnostic puncture performed in expert centers is associated with a low risk of complications. In particular, the procedure-related miscarriage risk hardly differs from the background risk for spontaneous abortion. In 2013, the Section of Gynecology and Obstetrics of the German Society for Ultrasound in Medicine (DEGUM) published recommendations on diagnostic puncture in prenatal medicine [1]. The developments described above and new findings in recent years make it necessary to revise and reformulate these recommendations. The aim of this review is to compile important and current facts regarding prenatal medical puncture (including technique, complications, genetic examinations). It is intended to provide basic, comprehensive, and up-to-date information on diagnostic puncture in prenatal medicine. It replaces the publication from 2013 [1].

ZUSAMMENFASSUNG

Diagnostische Punktionen (Amniozentese, Chorionzottenbiopsie und Fetalblutentnahme) sind ein wesentlicher Bestandteil der Pränataldiagnostik und die einzige etablierte und wissenschaftlich ausreichend evaluierte Möglichkeit der Diagnostik genetischer Erkrankungen aus schwangerschaftsspezifischen Zellen. Die Anzahl diagnostischer Punktionen in Deutschland ist, wie in anderen Ländern, deutlich gesunken. Dies ist maßgeblich auf die Einführung des Ersttrimester-Screenings mit weiterführender detaillierter Ultraschalluntersuchung des Fetus und die Analyse von cf-DNA (cell-free DNA) aus maternalem Blut (sogenannter „Nicht Invasiver Pränataler

Test“ – NIPT) zurückzuführen. Andererseits sind die Erkenntnisse über die Inzidenz und das Erscheinungsbild genetischer Erkrankungen gestiegen. Die Entwicklung moderner molekulargenetischer Techniken (Mikroarray- und Exom-Analyse) macht eine differenzierte Untersuchung dieser Erkrankungen mehr und mehr möglich. Die Anforderungen an Aufklärung und Beratung über diese komplexen Zusammenhänge sind dadurch wesentlich höher geworden. Die Studien der letzten Jahre machen deutlich, dass diagnostische Punktionen, die in Expertenzentren durchgeführt werden, mit einem niedrigen Risiko für Komplikationen assoziiert sind. Insbesondere der eingriffsbedingte Abort unterscheidet sich kaum vom Hintergrundrisiko für einen Spontanabort. Die Sektion Gynäkologie und Geburtshilfe der Deutschen Gesellschaft für Ultraschall in der Medizin (DEGUM) hat im Jahr 2013 Empfehlungen zu diagnostischen Punktionen in der Pränatalmedizin publiziert [1]. Die oben geschilderten Entwicklungen und neuen Erkenntnisse der letzten Jahre machen eine Revision und Neuformulierung dieser Empfehlungen nötig. Ziel dieser Übersicht ist eine Zusammenstellung wichtiger und aktueller Fakten zu pränatalmedizinischen Punktionen (u. a. Technik, Komplikationen, genetische Untersuchungen). Sie soll der grundlegenden umfassenden und aktuellen Information über diagnostische Punktionen in der Pränatalmedizin dienen. Sie ersetzt die Publikation von 2013 [1].

Introduction

Diagnostic puncture is an essential part of prenatal diagnostics and makes it possible to acquire cells from the fetus and placenta so that they can be examined with respect to the relevant medical issue (microscopic and molecular karyotyping, molecular genetic analysis of monogenic diseases, infections, hematological diagnostics, etc.). Puncture is currently the only established and sufficiently scientifically evaluated option for diagnosing genetic diseases based on pregnancy-specific cells.

In 2013, the Section of Gynecology and Obstetrics of the German Society for Ultrasound in Medicine (DEGUM) published recommendations on diagnostic puncture in prenatal medicine [1].

The developments and new findings in recent years make it necessary to revise and reformulate these recommendations. The acceptance and number of diagnostic punctures in Germany, as in other countries, has fallen significantly largely due to the introduction of first-trimester screening and the analysis of cf-DNA (cell-free DNA) from maternal blood (noninvasive prenatal test – NIPT) (▶ **Table 1**). According to data from the National Association of Statutory Health Insurance Physicians, the total number of amniocentesis and chorionic villus sampling (CVS) procedures in Germany in the period from 2003 to 2020 in relation to the number of births decreased from 8.3 % to 1.5 %. The number of amniocentesis procedures decreased significantly more than the number of chorionic villus sampling procedures. Fetal blood sampling (FBS) comprises only a small portion of punctures (326 in 2019).

The decreased demand for diagnostic puncture is due to changes in diagnostic options. Today, puncture is primarily performed due to abnormal sonographic findings in the first, second, and third trimester as well as due to first-trimester screening and cf-DNA analysis results requiring clarification.

On the other hand, numerous studies and meta-analyses were able to show that the risk of miscarriage after diagnostic puncture is very low at expert centers and does not differ from the natural risk of miscarriage [2, 3, 4, 5].

In addition, knowledge of the incidence and the clinical picture of genetic diseases that can be diagnosed after puncture using molecular-genetic techniques (microarray and exome analysis) and karyotyping has increased exponentially in recent years. Many of these diseases are not detected by ultrasound or currently available cf-DNA tests. The requirements for the informed consent discussion and counseling regarding these complex correlations have thus increased significantly.

The term “invasive diagnostic testing”, which was commonly used for a long time to refer to amniocentesis, CVS, and FBS, has had a negative connotation for a few years. Instead, the term “diagnostic puncture” is used in the following and is intentionally juxtaposed with the term “NIPT” (noninvasive prenatal testing). In this text NIPT refers to cf-DNA analysis (cell-free DNA analysis) [6].

▶ **Table 1** Number of diagnostic punctures in prenatal medicine calculated according to the uniform value scale and live births per year in Germany (source: statistics of the National Association of Statutory Health Insurance Physicians and the Federal Statistical Office of Germany).

Fee schedule item	2003	2013	2015	2017	2019	2020
01781 – amniocentesis	54,393	17,809	12,330	9,265	7,163	7,182
01787 – CVS	4,493	4,611	4,101	4,112	4,084	4,284
Total	58,886	22,420	16,431	13,377	11,247	11,466
Births	706,721	682,069	737,575	784,901	778,100	773,100
Percentage of amniocentesis + CVS Per birth	8.3 %	3.3 %	2.3 %	1.7 %	1.4 %	1.5 %

The recommendations provided in the following are intended to provide information about all relevant aspects of diagnostic puncture in prenatal medicine.

The goal of this review article is to provide important and current facts regarding puncture in prenatal medicine (techniques, complications, genetic testing). It is intended to provide basic, comprehensive, and up-to-date information on diagnostic puncture in prenatal medicine and is written for physicians and other persons who provide medical care for pregnant women and do not have their own puncture experience.

According to the authors of this article, there is no similar up-to-date compendium of diagnostic puncture procedures in prenatal medicine for colleagues receiving puncture training.

Physicians with puncture experience will find here the currently valid rules for puncture, the current complication numbers, and an overview of the options for genetic testing.

These guidelines for diagnostic puncture in prenatal medicine replaces the version from 2013 [1].

Gestational age

Chorionic villi sampling: starting at 11 + 0 gestational weeks **Amniocentesis:** starting at 15 + 0 gestational weeks (or only if there is fusion of amnion and chorion, i. e., possibly also later). **Cordocentesis**: starting at 20 + 0 gestational weeks, or earlier in exceptional cases [7].

All techniques can be used starting at the gestational ages mentioned above over the entire course of the pregnancy. The following aspects must be taken into consideration:

In the case of puncture of the placenta in the second and third trimesters (placental biopsy), fewer villi than in the first trimester are usually aspirated and less mitotic activity is seen in the individual villi. Evaluation is thus more difficult due to the changes in the differentiation of the villi [8]. Therefore, after 20 + 0 weeks, cordocentesis should be considered.

However, it should always be taken into consideration that the selected puncture method can vary based on indication. Therefore, in cases of doubt, the method should be selected in consultation with a human genetic council.

Potential indications and possible laboratory tests

The indications for amniocentesis (AC) and CVS are largely the same (▶ **Table 2**). In addition, hematological features of the fetus (e. g. hemoglobin and platelet count) can be examined with FBS.

Today, it is possible to detect approximately half of complex malformations between 11 and 14 gestational weeks [11, 12].

Due to the strong association between fetal malformations and genetic diseases, additional attempts are being made to detect these earlier and with greater frequency.

In the case of fetal malformations and intrauterine growth restriction with malformations, pathological karyograms are seen in 9–30 % of cases with conventional cytogenetic examination depending on the indication [13, 14]. Even if the classic trisomies 21, 18, and 13 comprise the majority of cases, other chromosomal abnormalities must also be taken into consideration.

▶ **Table 2** Possible indications for diagnostic puncture according to [6] and [1] * See below for explanation.

1. Increased risk for fetal chromosomal aberration or monogenic disease
▪ Fetal deformities
▪ Growth restriction (particularly early)
▪ Increased risk after first-trimester screening
▪ Increased nuchal translucency *
▪ Abnormal biochemical findings in first-trimester screening PAPP-A < 0.2 MoM or f-ßHCG < 0.2 or > 5 MoM [9, 10]
▪ Abnormal cf-DNA screening findings
▪ Chromosomal aberrations in the parents
2. Increased risk for a known familial genetic disease
▪ Familial genetic diseases with known mutations
▪ Genetic metabolic diseases
▪ Prior pregnancies with genetic abnormalities
▪ Carrier status of the pregnant woman for a disease with X-chromosome inheritance
▪ Carrier status of both parents for an autosomal-recessive genetic disease
3. Diagnosing an infection
▪ Detection of viral, bacterial, and parasitic diseases
4. Wishes of the pregnant woman
▪ Fear of genetic disease of the fetus

Therefore, in a large study including approximately 130,000 fetuses with unremarkable ultrasound, pathological karyograms were seen in 1.6 % of cases after CVS or amniocentesis, with trisomies of chromosomes 13, 18, and 21 being seen in 49 % of cases and other chromosomes being affected in 51 % of cases [15].

Cytogenetic testing has been increasingly supplemented in recent years by quickly developing molecular genetic methods and has even been replaced in some cases.

The resolution of karyotyping using a microscope is 5–10 megabases (Mb) but is less than 100 kilobases (Kb) in microarray analysis (comparative genomic hybridization). In this way the diagnosis of numeric aberrations (other than triploidy) as well as the detection of submicroscopic chromosomal imbalances (microdeletions and duplications, known as pathological copy number variations – CNVs) are possible.

Although it is recommended in numerous countries to perform microarray analysis as the first examination (first tier test) after prenatal diagnostic puncture [16], Germany requires a sequential approach (karyogram before microarray analysis).

In the case of mental retardation, autism, epilepsy, dysmorphic syndromes, and other abnormal findings, pathological CNVs (microdeletions and duplications) are found postnatally by microarray in up to 15 % of cases.

In the case of abnormal ultrasound findings in fetuses with a normal karyogram, the findings of the microarray analysis are abnormal in 6–8 % of cases and in approximately 1 % of fetuses with unremarkable ultrasound [17, 18, 19, 20].

A CNV does not always represent a pathological change. CNVs are categorized as “benign CNV” (benign polymorphism), “probably benign CNV”, “pathological CNV”, “CNV of unclear clinical relevance”, and “probably pathogenic CNV”. The following methods

are used to differentiate a CNV: Evaluation of the genes in the region, comparison with databases containing mapping of known CNVs, examination of the parents with respect to a “de novo” change, and comparison with the fetal phenotype.

The Danish Fetal Medicine Study Group showed that, in the case of an indication for diagnostic puncture for a risk of ≥ 1:300 for trisomy 21 and ≥ 1:150 for trisomy 13 and 18, approximately 5 % of pregnant women are offered puncture, with a detection rate of > 90–95 % for all chromosomal aberrations being achieved. Analyses of subgroups showed that that rate of pathological karyograms and CNVs is higher particularly in the case of isolated abnormal biochemical values in first trimester screening (see ▶ **Table 2**) [21, 22].

In the case of a nuchal translucency of > 3.5 mm and normal karyogram, CNVs are found by microarray in 5–13 % of cases [23, 24].

Maya et al. found an increasing number of pathological CNVs (1.7 %, 6.5 %, and 13.8 %) as a function of the nuchal translucency (up to 2.9 mm, 3.0–3.4 mm, and > 3.4 mm) [25].

Numerous complex malformations can be caused by monogenic diseases or single-gene mutations, e. g., in skeletal dysplasia and other syndromes like Meckel-Gruber syndrome. In these cases, corresponding molecular genetic testing can be performed via next generation sequencing (NGS). NGS panels are compilations of clinically relevant genes for a certain clinical picture that are examined in parallel during molecular genetic testing via next generation sequencing (NGS). Small multi-gene panels are differentiated from large multi-gene panels (clinical exome, clinical exome sequencing), exome sequencing (whole exome sequencing), and sequencing of the whole genome (whole genome sequencing).

A change from microarray analysis to exome sequencing is currently taking place here.

These examinations are increasingly included in the workup of abnormal sonographic findings in Germany. The catalog of indications is currently changing so that the method and the scope of genetic analysis should be discussed in the individual case with the human genetic center performing the analysis.

A method-specific feature of the genetic result of CVS is the fact that 1–2 % of cases of mosaicism that are diagnosed are limited to extraembryonic tissue in approximately 80 % of cases (confined placental mosaicism: CPM) [26]. The clinical effects of CPM with respect to reduced placental function, intrauterine growth restriction, and unfavorable pregnancy outcome, as described in several studies [27, 28, 29] but not in others [30, 31] or only for trisomy 16 [32], currently cannot be conclusively evaluated. True fetal mosaicism is found in 20 % of cases [33].

In some cases, e. g. in mosaicism, the results of CVS must be compared to additional diagnostic tests, ultrasound examinations, or amniocentesis in order to verify the results and to allow additional diagnostics, e. g. determination of the exact loss/gain in deletions/duplications via microarray analysis; diagnosis of a uniparental disomy, e. g., in placental trisomy 15, from trophoblast cells (direct preparation). The possibility of CVS results requiring clarification should be thoroughly discussed prior to the examination in the informed consent discussion with the patient. Similar findings after amniocentesis are rarer but must also be clarified.

Preparation

Prior to puncture, the patient history must be taken, the pregnant woman must be examined for puncture risk factors, an informed consent discussion and genetic counseling must be provided in accordance with the German Genetic Diagnostics Act and the Pregnancy Conflict Act, and the pregnancy must be examined on ultrasound.

Ultrasound examination checks the following: Vitality of the fetus (fetal heart rate), fetal biometry (verification of gestational age), placenta location, amount of amniotic fluid, determination of the suitable puncture site, in the case of amniocentesis: amnion-chorion fusion or separation.

If this has not yet been performed, a differentiated sonomorphological examination (detailed diagnosis) adapted to gestational age should be performed.

Additional factors that must be taken into consideration prior to diagnostic puncture:

- Rhesus status: In RhD-negative mothers and RhD-positive fetuses proven by cf-DNA analysis or in the case of a lack of a fetal cf-DNA rhesus test, anti-D prophylaxis must be administered after the procedure according to the valid regulations at the time of publication.
- A general check of HIV, HBV, and HBC status is not recommended prior to puncture and should only be performed in high-risk groups or in suspicious cases. The risk of vertical transmission of HIV infection due to amniocentesis can be lowered by HAART (Highly Active Anti-Retroviral Therapy).
- The transmission of HBV does not seem to be increased in HBeAg-negative pregnant women. There is only minimal data regarding HBC infections that tends to show that amniocentesis does not increase the risk of transmission. There is no corresponding data for CVS [7].
- Diagnostic puncture in pregnant women with infections and in those in whom vertical transmission through puncture is possible should only be performed at expert centers with experience with such infections in pregnancy.
- Puncture-related antibiotic prophylaxis is not currently recommended [7].

General principles (amniocentesis, chorionic villi sampling, and cordocentesis)

1. Generous disinfection of the skin in the region in which the procedure will be performed and a sterile approach are required.
2. Amniocentesis does not require local anesthesia [7]. Local anesthesia can be used for CVS due to the larger needle size but is not absolutely necessary based on the experience of the authors of this publication.
3. CVS, amniocentesis, and FBS are performed under continuous ultrasound guidance and typically "free hand", i. e., without puncture aids. The needle is guided in the longitudinal

direction in the acoustic window. The entire needle should be displayed on ultrasound during the puncture procedure.

Chorionic villus sampling

Chorionic villus sampling is largely performed using a transabdominal approach but can also be performed using a transcervical approach depending on the position of the placenta or the anatomical position of the uterus (retroflexio uteri). The complication rate for transcervical access compared to transabdominal puncture is not significantly higher [34]. However, transcervical chorionic villus sampling is more technically challenging and difficult to learn. Therefore, transabdominal chorionic villus sampling is the method of choice.

For transabdominal CVS, various needles can be used: 18 to 21-gauge needle or more rarely 18/21-gauge double needles.

For transcervical CVS, biopsy forceps inserted through the cervical canal or a biopsy catheter with a guidewire can be used [7].

Suction is created with a syringe filled with culture medium mounted on the needle. The needle is then moved slowly forward and backward under sonographic guidance in the chorion thereby aspirating chorionic villi. The chorionic plate must not be damaged since this can result in a miscarriage. During puncture, the villi must be drawn into a tube containing sodium-heparin to avoid blood coagulation at the villi.

Amniocentesis

The puncture needle is inserted under sonographic guidance through the mother's abdominal wall and the uterus into the amniotic cavity and amniotic fluid is aspirated. The first milliliter of aspirated amniotic fluid is discarded to reduce the risk of contamination with the mother's cells. Paraplacental access is the method of choice. In the case of a complete anterior placenta and transplacental puncture, the placental umbilical cord insertion or vessels of the chorionic plate are not damaged.

In the case of chorioamniotic separation, it is recommended to delay amniocentesis either until a later time when the amnion and chorion are fused or to select placentacentesis.

A 20- to 22-gauge needle is recommended for amniocentesis.

Cordocentesis

Cordocentesis is performed with a transabdominal approach under continuous ultrasound guidance preferably with a 20- to 22-gauge needle. The needle is advanced through the mother's abdominal wall and uterine wall into the umbilical vein. Transplacental access to the umbilical vein in the case of an anterior or lateral placenta is technically easiest. Puncture of the fetal umbilical cord insertion, the intrahepatic part of the umbilical vein, or a free umbilical cord loop is also possible but technically more demanding due to fetal movement among other things. Umbilical cord bleeding occurs more frequently and for longer periods after puncture of a free loop [35].

In comparison, significantly shorter puncture times were seen in puncture of the placental umbilical cord insertion, while a significantly lower rate of maternal blood contamination was seen in puncture of a free cord loop [36].

Whether the cord insertion, the intrahepatic part of the umbilical vein, or a free cord loop is punctured depends mainly on the location of the placenta, the position of the fetus, and thus on the accessibility of the umbilical vein.

In the case of puncture of the placental cord insertion, it is recommended to verify the fetal origin of the blood. This is performed by analyzing the concentration of the fetal hemoglobin – HbF – or by determining the mean corpuscular erythrocyte volume – MCV.

Diagnostic puncture in multiples

Puncture in multiples should only be performed by experts and centers with a high level of experience.

One advantage of CVS compared to amniocentesis in multiples is that the procedure is performed earlier in the pregnancy. Thus, in the case of a pathological result with indication for a selective reduction, this can also be performed at an earlier point in time and with a lower risk of miscarriage [37].

The planning of diagnostic puncture in multiples requires determination of the chorionicity and amnionicity.

Each placenta must be precisely assigned to the corresponding fetus. The acquired sample must be able to be clearly assigned to the respective fetus and labeled accordingly.

Amniocentesis in dichorionic twins can be performed as a single or multiple puncture. In the case of a single puncture, after puncture and aspiration of the amniotic fluid from the first amniotic cavity, the needle is advanced through the separating wall into the second amniotic cavity where the second amniotic fluid sample is collected in a second syringe. In the case of multiple puncture, two needles (or a number of needles corresponding to the number of multiples) are used and the amniotic cavities are punctured separately. The decision as to which method is used is based on the experience of the person performing the procedure and also depends on the anatomic conditions.

In the case of monochorionic-diamniotic twins, puncture of one amniotic sac can be sufficient. In the case of discordant biometric or sonoanatomic results, puncture of both amniotic sacs is recommended [7].

When performing CVS for dichorionic multiples, it must be ensured that chorionic villi are acquired from each placenta. The puncture sites should be able to be definitively assigned to the respective chorion.

In the case of monochorionic multiples, CVS can be performed as a single puncture of the shared chorion. In the case of discordant biometric or anatomical findings, amniocentesis with separate puncture of the two amniotic sacs can be offered. Alternatively, in such cases, CVS can be performed as a multiple puncture of the chorion in the vicinity of the placental cord insertions.

After puncture (amniocentesis, CVS, FBS)

The fetal heart rate and the amount of amniotic fluid are checked and documented. This examination can be repeated several days after the puncture.

Although physical rest is typically recommended for 24–48 hours, this is not based on evidence. The administration of tocolytic

substances after puncture does not have a clear benefit with respect to preventing complications [7].

Patients should be advised to seek medical attention if experiencing symptoms like lower abdominal pain, amniotic fluid leakage, or fever.

Complications

Maternal complications

Maternal complications after diagnostic puncture are extremely rare and are usually limited to pain at the puncture site, small hematomas in the abdominal wall, and circulatory dysregulation [38, 39].

Severe maternal complications (sepsis) have been reported in individual cases and can be caused by puncture of the maternal bowel [7].

Injury to the fetus

Injury to the fetus is very rare with continuous ultrasound monitoring during the procedure [40]. However, injury is possible if the needle is not completely visible. Therefore, the entire needle must be visible during the puncture.

Although contact between the fetus and the needle is possible during proper performance of the procedure under continuous ultrasound guidance, it has only been reported in individual case reports as a minor superficial skin lesion [7, 41, 42, 43].

Leakage

Transient leakage of amniotic fluid can occur as a result of amniocentesis. This is usually temporary and ceases spontaneously. Expectant management results in a live-birth rate of over 90 %. Leakage thus has a significantly better prognosis than spontaneous premature membrane rupture [44, 45].

Since leakage is rare, there are no recommendations for management equivalent to those for spontaneous premature membrane rupture. Clinical practice is an approach adapted from spontaneous premature membrane rupture. However, there is no evidence for this.

Puncture-related miscarriage

The calculation of the risk of miscarriage after diagnostic puncture is based on the probability of natural loss of a pregnancy, i. e., spontaneous abortion (background risk). The probability of a spontaneous abortion is primarily dependent on gestational age. Further factors that increase the risk for spontaneous abortion are maternal characteristics, e. g., maternal age, preexisting conditions, and obesity [46]. Pregnancy-related factors, like fetal anomalies and genetic aberrations of the fetus, also increase the risk for miscarriage. Some laboratory parameters are indicative of an increased miscarriage rate. A lower concentration of PAPP-A is associated with an increased risk for spontaneous abortion [41, 46, 47, 48, 49, 50], (▶ **Table 3**).

The individual background risk greatly influences the possibility of a miscarriage after diagnostic puncture. When evaluating the puncture-related miscarriage risk, the a-priori risk for a spontaneous abortion must therefore be taken into consideration. Cohorts with and without diagnostic puncture with a comparable a-priori risk are ideally compared to one another.

The study data on miscarriages after amniocentesis, CVS, and FBS varies due to this individual background risk but also due to study-specific factors (including the completeness of the data regarding the further course of the pregnancy, presence of control groups, randomization, duration of follow-up, time of the procedure, comparison of low-risk and high-risk groups for chromosomal aberrations and other genetic diseases, inclusion or disregarding of the background risk).

▶ **Table 3** Risk factors for miscarriage [7, 43, 46, 49, 51].

Maternal
▪ Vaginal bleeding before or during puncture/hematoma (contraindication for puncture)*
▪ Symptomatic vaginal infection (contraindication for puncture)
▪ Hypertension
▪ Obesity
▪ Multiparity (more than 3 births)
▪ Prior history of 3 or more abortions
▪ Nicotine abuse
Pregnancy-specific
▪ Abnormal sonographic finding
▪ (increased NT, fetal malformation, growth retardation)
▪ Chromosomal aberration
▪ Abnormal serum screening (elevated AFP, low PAPP-A)

* In the case of prior acute or transient vaginal bleeding or vaginal infection, puncture should be delayed by 2–4 weeks depending on the course.

Amniocentesis and chorionic villi sampling

Current publications since 2015 show that the miscarriage risk after amniocentesis and CVS performed in expert centers is not significantly higher than the spontaneous abortion rate [2, 3, 5].

In the Danish national cohort study by Wulff et al. in 2016 [3], 5,072 CVS procedures and 1,809 amniocentesis procedures were analyzed using propensity score matching in a total of 147,987 pregnant women after first-trimester screening. The miscarriage risk was not higher after diagnostic puncture than in the control group.

A similar result was seen in a meta-analysis by Akolekar et al. (2015) [2], including 21 studies (14 amniocentesis studies and 7 CVS studies) each with at least 1000 punctures published after the year 2000. The procedure-based weighted miscarriage risk was 0.11 % for amniocentesis and 0.22 % for CVS.

A current meta-analysis, an update of the publication by Akolekar in 2015 [2], analyzed 12 controlled studies including a total of 63,723 amniocentesis procedures (control group 330,469 without amniocentesis) and 7 studies with a total of 13,011 CVS procedures (control group 232,680 without CVS) [5]. The weighted miscarriage rates were 0.3 % (amniocentesis) and 0.2 % (CVS). Analysis of studies on pregnant women with a comparable risk

profile showed procedure-related miscarriage rates of 0.12 % (amniocentesis) and 0.11 (CVS). This study emphasizes the influence of the comparison of inhomogeneous study groups on miscarriage rates after amniocentesis and CVS.

The ACOG (American College of Obstetricians and Gynecologists) included the results of the current studies on miscarriage rates after amniocentesis and CVS (0.11 % for amniocentesis, 0.22 % for CVS) in the Practice Bulletin published in 2016 [51].

A retrospective study by Gil et al. [50] analyzed the procedure-related miscarriage risk after CVS with propensity score matching. The authors conclude that the procedure-related risk is low in the low-risk collective for aneuploidy and comparable to that of the group without CVS. Since pregnancy-related and demographic characteristics affect the procedure-related risk, these should be taken into consideration when counseling pregnant women (see ▶ **Table 3**).

Older studies from the 1970 s and 1980 s specifying a miscarriage risk of 0.5–1 % [52, 53, 54, 55] no longer correspond to current conditions.

Today, diagnostic puncture in prenatal medicine is performed under continuous ultrasound guidance. Consequently, the rate of procedure-related miscarriage is significantly lower [40] and so is the rate of fetal injuries and blood in the aspirated specimen.

The image quality of current ultrasound devices has improved significantly, consequently allowing puncture with higher precision.

In addition, the current criteria for ruling out puncture, e. g. bleeding, are stricter.

In summary, based on the data from the more recent literature regarding the miscarriage rate after amniocentesis or CVS, the following can be stated:

- In centers with a high level of puncture experience, the procedure-related miscarriage risk after diagnostic puncture is not statistically significantly different from the rate of spontaneous abortion (0.11 % for amniocentesis, 0.22 % for CVS).
- The results from more recent literature must be correctly included when counseling pregnant women on prenatal diagnostics so that they can make informed decisions.
- Examiners performing diagnostic puncture should have an overview of the further course and outcome of the pregnancy (follow-up) so that this information can be used as the basis for the counseling of pregnant women (see the section on quality control).
- During the informed consent discussion for pregnant women, special factors, like fetal anomalies, chorioamniotic separation, bleeding, retrochorionic hematomas, etc. should be taken into consideration as risk factors (see ▶ **Table 3**).

Fetal blood sampling

The miscarriage risk after FBS has been examined in multiple studies. There may be a higher miscarriage risk after FBS than after amniocentesis and CVS. The published miscarriage rates are between 0.4 % and 1.4 % [56, 57, 58, 59].

A current study retrospectively analyzes 6290 FBS procedures and shows a procedure-related increase in the miscarriage rate of 0.6 % compared to a control group (1.6 % vs. 1.0 %). The authors of this study define transplacental puncture, prolonged bleeding (> 1 minute), and fetal bradycardia (fetal heart rate < 100/min, > 1 minute) as risk factors for miscarriage [60].

Further consequences of FBS can be umbilical cord bleeding and fetal bradycardia. Both complications usually resolve spontaneously [56].

However, the comparability of studies on complications after FBS is limited by the low number of cases and the heterogeneity of the study collectives and the indications.

Since FBS is restricted to several centers in Germany, pregnant women requiring FBS are also informed with respect to the center-specific outcome. According to the authors of this article, the complication rate after FBS is lower than described in the literature.

Multiples

Studies on miscarriage rates in multiples have an inhomogeneous result compared to current studies on singleton pregnancies. In addition, there is little data examining the procedure-related risk of miscarriage in the context of background risk. However, the results of current studies indicate that the procedure-related risk of miscarriage is not or is only minimally higher than the background risk [61, 62, 63, 64].

Multiple randomized studies hypothesize that puncture in the case of multiples is not associated with a higher miscarriage rate [65, 66, 67].

A meta-analysis of 16 studies including 3419 twin pregnancies with amniocentesis and 2517 without amniocentesis did not show a significant difference between pregnancies with and without amniocentesis. The pooled miscarriage rate in both groups was 2.4 % [62].

A multicenter study that used multivariate regression to examine the relationship between CVS and miscarriage in twin pregnancies showed double the risk for miscarriage in the group with CVS compared to the group without CVS [68]. The authors attribute the increase in the miscarriage risk after CVS primarily to the influence of different factors and not to the procedure itself. These factors are: maternal obesity, monochorionicity, biometric discordance between twins, and increased NT.

One study including 8581 twin pregnancies with 445 CVS procedures using propensity score matching also shows that the procedure-related risk of miscarriage is largely dependent on risk factors. These are mainly the factors that are also the indication for CVS [69]. In comparison to groups with a low risk for spontaneous abortion, the authors of this multicenter study found an increase in the rate of miscarriage of 3.5 % after CVS.

The selection of the puncture method (single or multiple puncture) does not seem to affect the miscarriage rate [70, 71].

Alloimmunization

According to older studies, feto-maternal bleeding after amniocentesis and CVS can trigger alloimmunization against fetal blood group antigens in approx. 1 % of cases [55, 72].

Rh-negative women pregnant with RhD-positive fetuses who did not receive anti-D prophylaxis were examined in a Danish

cohort study. There was a very low rate of immunization (none in 189 amniocentesis procedures and 1 in 543 CVS procedures) [73].

Nonetheless, anti-D prophylaxis is currently recommended after puncture when the fetal RhD status is positive or unknown.

Only in the case of an RhD-negative partner and reliably verified paternity can anti-D prophylaxis be omitted. In these cases, the blood group of the partner should be documented.

Unsuccessful puncture

In the case of unsuccessful amniocentesis ("dry tap"), puncture at another location can be performed. However, more than two punctures per session are not recommended due to the significant increase in the risk of miscarriage [74]. It is recommended to stop the procedure after two unsuccessful puncture attempts and to refer the pregnant woman to a facility with greater puncture experience.

Further complications

Further extremely rare complications include amnion separation, bleeding into the amniotic cavity, and formation of a retrochorionic hematoma.

Informed consent

The currently valid legal requirements must be taken into consideration. Puncture with the goal of analyzing genetic properties of the fetus is subject to the law on genetic testing in humans (German Genetic Diagnostics Act) dated 7/31/2009.

According to the Patients' Rights Act that went into effect in 2013, pregnant women have a right to comprehensive information about all available and necessary examinations, diagnoses, and treatments. The content of the law regarding the prevention and management of pregnancy conflicts (Pregnancy Conflict Act) must be taken into consideration.

Documentation

Documentation of diagnostic puncture should include the following information:

- Findings from which the indication for diagnostic puncture arises
- Documentation of the informed consent discussion prior to puncture including the written informed consent of the pregnant woman for the examination
- Documentation of the ultrasound examination prior to the procedure (see above)
- Documentation of the procedure: instrument being used, puncture site, number of punctures, sample amount, appearance of the amniotic fluid sample
- Documentation of the vitality of the fetus and the amniotic fluid amount after the procedure and possible indications of early complications (see above)
- Documentation of anti-D prophylaxis (incl. the lot number)
- Documentation of the procedure in the maternity passport
- Documentation of consent to participate in a genetic study in accordance with the German Genetic Diagnostics Act.

Quality control

The goal of each diagnostic puncture in prenatal medicine is to acquire an adequate amount of the material needed to answer the particular medical question and to prevent complications. This can only be ensured if the examiner is highly qualified.

There is an association between the complication rate after prenatal diagnostic puncture and the examiner's experience measured based on the number of procedures performed annually [75]. However, the number of procedures needed to ensure adequate quality varies greatly in the literature. It is currently not possible to specify an evidence-based minimum number of procedures to be performed annually for quality assurance since the relevant data in the literature varies greatly [76, 77, 78, 79, 80]. According to the recommendations of the Royal College of Obstetricians and Gynecologists (RCOG), at least 30 procedures per year with continuous review are required. The RCOG requires at least 100 procedures per year for experienced examiners [81].

Training and specialist training

Diagnostic puncture training should begin with model/simulator training in which the needle is guided in the ultrasound window so that the entire needle up to the tip remains visible and the intended target is reliably reached.

Once model-based training has been mastered, clinical training should begin with "simple" amniocentesis procedures.

This includes procedures performed in geriatric pregnancies (e. g. amnion drainage), procedures in posterior placenta, and procedures in the case of a sufficient amount of amniotic fluid.

The number of procedures needed to master the procedure varies in the literature and ranges between 30 and 400. However, no improvement is able to be identified after 100 procedures [78, 80, 81].

Conflict of Interest

The authors declare that they have no conflict of interest.

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