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GUIDELINES

ISUOG Practice Guidelines: invasive procedures for prenatal diagnosis

Clinical Standards Committee

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INTRODUCTION

The aim of this document is to describe the main aspects of invasive fetal procedures for prenatal diagnosis. Technical issues, clinical indications, diagnostic capabilities and possible complications are considered in light of the available literature. In this new era dominated by cell-free fetal DNA (cffDNA) testing, the number of invasive procedures for fetal testing is decreasing dramatically and this is having a considerable impact on clinical practice. This Guideline summarizes current information regarding when, how and why practitioners perform invasive procedures for prenatal diagnosis. Details of the grades of recommendations and levels of evidence used are given in Appendix 1.

1. AMNIOCENTESIS

- Amniocentesis should be performed at or beyond 15 + 0 completed weeks of gestation (**GRADE OF RECOMMENDATION: A**).
- A 20–22-G needle should be inserted transabdominally under continuous ultrasound guidance (**GRADE OF RECOMMENDATION: B**).
- Needle entry through the placental cord insertion site must be avoided and, if technically feasible, avoidance of the placenta is preferable, especially in Rhesus-negative women (**GRADE OF RECOMMENDATION: C**).
- The frequency of maternal cell contamination increases with the presence of blood-stained amniotic fluid and when the operator is less experienced. To minimize contamination with maternal cells, the first 2 mL of fluid should be discarded (**GRADE OF RECOMMENDATION: C**).

Amniocentesis refers to transabdominal aspiration of amniotic fluid from the uterine cavity. This procedure has been performed since 1970[1].

Technique

A 20–22-G needle should be inserted transabdominally under continuous ultrasound guidance[2–5]. Firm entry is suggested to prevent tenting of the amniotic membrane[3] (**EVIDENCE LEVEL: 1–**). A small ($n = 200$) randomized controlled trial (RCT) comparing 20-G and 22-G needles for amniocentesis showed that intrauterine bleeding rates were similar (4/100 *vs* 8/100), but the larger-caliber needle (20-G) was associated with faster fluid retrieval[6] (**EVIDENCE LEVEL: 2+**). A retrospective study ($n = 793$) reported similar fetal loss rates with 20-G (1.57%), 21-G (1.47%) and 22-G (1.61%) needles[7].

The impact of transplacental needle passage has been studied in retrospective cohorts. The rates of fetal loss were similar when using a transplacental and a transmembrane approach, but transplacental passage was

associated with increased rates of bloody tap[8–11]. Nevertheless, it is currently recommended that needle entry through the placental cord insertion site be avoided and, if technically feasible, avoidance of the placenta is preferable (especially in Rhesus-negative women)[2–7,12] (**EVIDENCE LEVEL: 1+**).

Once the needle has reached the amniotic cavity, the inner stylet is removed and 15–30 mL fluid (depending on the indication) is aspirated. Fluid aspiration may be performed by the operator, by an assistant or by using a vacuum device[3,13].

Maternal cells can be detected in amniotic fluid samples, and older reports stated that about one in two samples may contain more than 20% maternal cells, this proportion being 50% or more in bloody samples[14]. In a retrospective study of 150 samples, factors associated with high contamination rates were placental penetration (6.0% *vs* 1.0%), two passes (27.5% *vs* 2.0%) and operator inexperience[15]. The frequency of maternal cell contamination was reported to be much lower (0.35%) in a more recent series of 6332 samples[16]. To minimize contamination with maternal cells, it is recommended that the first 2 mL of fluid should be discarded[17] (**EVIDENCE LEVEL: 2+**).

Timing

The safety and diagnostic reliability of early ($< 14+0$ weeks) *vs* midtrimester ($> 15+0$ weeks) amniocentesis was studied in RCTs in the 1990s. Although a smaller trial ($n=695$) indicated similar rates for total pregnancy loss (7.8% *vs* 7.4%) and fetal congenital defects (2.4% *vs* 2.6%)[18,19], a much larger multicenter RCT ($n=4374$) showed that early ($11+0$ to $12+6$ weeks) amniocentesis was associated with a significantly higher rate of total fetal losses (7.6% *vs* 5.9%), fetal talipes (1.3% *vs* 0.1%) and post-procedure amniotic fluid leakage (3.5% *vs* 1.7%), compared with midtrimester ($15+0$ to $16+6$ weeks) amniocentesis[20,21]. This may be due to the presence of the extraembryonic celom in the first trimester or the reduced amount of amniotic fluid in the amniotic cavity. As a result of these concerns, scientific and professional bodies currently recommend that amniocentesis should be performed at or beyond $15+0$ weeks of gestation[2,17,22] (**EVIDENCE LEVEL: 1+**).

Laboratory aspects

Failure of amniocyte culture is reported after 0.1% of procedures. Blood-stained amniotic fluid and late gestational age at amniocentesis increase the risk of culture failure[17]. Amniotic cell mosaicism is seen in 0.25% of procedures[17]. In these cases, genetic counseling is recommended and, dependent on the result, fetal blood sampling (FBS) may be indicated to exclude a true fetal mosaicism[17]. The risk for culture failure also increases with advanced gestational age. A retrospective study of amniocentesis after 28 gestational weeks reported a 9.7% culture failure rate[23] (**EVIDENCE LEVEL: 2++**).

Complications

- For women undergoing amniocentesis, the additional risk of fetal loss in comparison with controls has been reported to vary from 0.1% to 1%, with recent reports being closer to the lower limit (**GRADE OF RECOMMENDATION: B**).
- The risk of membrane rupture after amniocentesis is 1–2%; the prognosis in these cases may be better than that in cases of spontaneous preterm prelabor rupture of the membranes (PPROM) (**GRADE OF RECOMMENDATION: B**).
- Fetal injury and serious maternal complications are rare events (**GRADE OF RECOMMENDATION: D**).
- Experience and familiarity with amniocentesis may decrease the risk of procedure-related fetal loss. Multiple attempts, blood-stained amniotic fluid and the presence of fetal abnormalities may increase the risk for fetal loss. The effect of other risk factors is less consistent (**GRADE OF RECOMMENDATION: C**).

Fetal loss

Most of the data for fetal loss rate after amniocentesis are derived from observational studies. There is only one RCT, the Danish one of 1986, in which 4606 low-risk pregnant women were randomized to either amniocentesis or expectant management. The fetal loss rate was 1.7% in the amniocentesis group *vs* 0.7% in the control group, yielding a 1.0% net procedure-related risk[12] (**EVIDENCE LEVEL: 1+**). Several observational studies that followed reported lower or higher risks, and a recent meta-analysis calculated that the weighted pooled procedure-related risk of miscarriage for amniocentesis is 0.11% (95% CI, −0.04 to 0.26%)[24] (**EVIDENCE LEVEL: 2++**). A review from Denmark of 147 987 invasive procedures, published in 2016, reported a rate of miscarriage of 0.56% within 28 days and a risk of stillbirth of 0.09% within 42 days after amniocentesis[25] (**EVIDENCE LEVEL: 2++**).

Amniotic fluid leakage

The risk of amniotic fluid leakage following amniocentesis is increased up to 24 weeks of gestation. Its occurrence is reported to vary between 1 and 2%[17,19,26]. However, in women with amniotic fluid leakage after amniocentesis, spontaneous sealing of the membranes is commonly observed and, compared with cases of spontaneous rupture of membranes at the same gestational age, the risk of perinatal loss is substantially lower[27] (**EVIDENCE LEVEL: 2++**).

Chorioamnionitis

The risk of chorioamnionitis and uterine infection after genetic amniocentesis is low ($< 0.1\%$)[17].

Needle injury

The occurrence of needle injury to the fetus is extremely rare[17]. Sporadic injuries have been reported in older case reports, particularly those using unguided procedures, and included ocular trauma[28], cutaneous injuries (dimpling and scarring)[29,30], tendon trauma[29], trauma in the fetal vessels[31] and brain injury (including porencephaly)[32,33] (**EVIDENCE LEVEL: 3**).

Maternal complications

Severe maternal complications related to amniocentesis, including sepsis or even death, have been reported in a very small number of cases[34–38]. These events may be caused by inadvertent puncture of the bowel. Moreover, microorganisms can colonize ultrasound gel and probes and pose a risk of maternal infection[2] (**EVIDENCE LEVEL: 3**).

Risk factors for complications

Lower fetal loss rates have been documented if more than 100 procedures are performed per annum[2] (**EVIDENCE LEVEL: 2+**). A higher number of attempts (three or more punctures) increases the risk of fetal loss. If more than two punctures are necessary, it has been suggested to delay the procedure by 24 hours[3,22].

The presence of fetal structural anomalies is itself associated with a higher background risk of miscarriage, and this risk is further increased following amniocentesis[22]. A bloody or discolored (i.e. brownish) specimen may reflect current intra-amniotic bleeding and is consistently reported to herald a higher risk of post-procedural fetal loss. This seems to be due to the association of intra-amniotic bleeding with underlying placental disorders[22,39] (**EVIDENCE LEVEL: 2+**). Expert opinion suggests that an operator's competence should be reviewed when loss rates exceed 4/100 consecutive amniocenteses[2,40] (**EVIDENCE LEVEL: 2+**).

Several risk factors have been suggested to increase the risk of fetal loss following amniocentesis, although their association has not been proven consistently. Included in this group of plausible risk factors are[22,41,42]: uterine fibroids; Müllerian malformations; chorioamniotic separation; retrochorionic hematoma; previous or current maternal bleeding; maternal body mass index $> 40\,kg/m^2$; multiparity (> 3 births); manifest vaginal infection; history of three or more miscarriages (**EVIDENCE LEVEL: 2+/2–**).

2. CHORIONIC VILLUS SAMPLING (CVS)

- Chorionic villus sampling (CVS) should be performed after 10 + 0 gestational weeks (**GRADE OF RECOMMENDATION: A**).
- CVS can be performed transabdominally or transcervically, according to the operator's experience, preference or placental location.
- There are no RCTs on the fetal loss rate after CVS compared with no CVS, but observational trials indicate that it may be quite low, ranging from 0.2 to 2% (**GRADE OF RECOMMENDATION: B**).
- The risk for miscarriage after CVS appears to decrease with increasing experience. Repeated needle insertions and gestational age < 10 weeks increase the risk of fetal loss (**GRADE OF RECOMMENDATION: B**).

CVS is the withdrawal of trophoblastic cells from the placenta. This procedure was first described in China in the mid-1970s[43] and introduced into clinical practice in the early 1980s[44].

Technique

The needle should be inserted into the placenta under continuous ultrasound guidance. Generally, this is achieved either by the free-hand technique or using a biopsy adaptor. As data comparing the safety or efficiency between these two methods are lacking, the choice should be made according to operator experience or preference[2,45].

Access to the placenta may be transabdominal or transcervical. A RCT in 3873 women with singleton pregnancy (gestational age range, 7–12 weeks, but most > 10 weeks) showed that the fetal loss (2.3% *vs* 2.5%) and successful sampling (95% *vs* 94%) rates were similar between the two methods[46] (**EVIDENCE LEVEL: 1+**).

Transabdominal approach. Local anesthesia may be applied for transabdominal CVS[2] (**EVIDENCE LEVEL: 4**). A single needle of 17–20 G or a two-needle set of outer 17/19 G and inner 19/20 G may be used[47] (**EVIDENCE LEVEL: 1–**). Once the needle has reached the target within the placenta, between one and 10 back-and-forth movements are performed, while the vacuum is maintained and samples are aspirated either manually by an assistant or with a vacuum adaptor[3,45,48].

Transcervical approach. Biopsy forceps are inserted transvaginally through the cervical canal to the trophoblastic area, or a catheter with plastic or metal stylet under syringe aspiration may be used[3]. A RCT of 200 women undergoing CVS between 10 + 0 and 12 + 6 weeks reported comparable placental trauma and effectiveness between biopsy forceps and catheter techniques (**EVIDENCE LEVEL: 1–**); however, the former method was preferred by both operators and patients[49].

The amount of villi obtained in the sample must be checked visually. A minimum amount of 5 mg villi in each sample is required to achieve a valid result[3]. Sampling failure is reported to occur in 2.5–4.8% of procedures[2,45].

Timing

CVS should not be performed before 10 + 0 completed weeks of gestation, due to the higher risk of fetal loss and complications before this time[2,17]. Reports from the early 1990s highlighted an increased incidence of limb reduction/oromandibular hypoplasia in fetuses which underwent CVS earlier than 10 weeks of gestation, compared

with the general population. There remains insufficient evidence to refute or confirm causation with confidence. The limbs and mandible seem to be more susceptible to vascular disruption before 10 weeks[3,50,51] (**EVIDENCE LEVEL: 3**).

Laboratory aspects

Failure of the cytotrophoblastic culture is reported to occur after fewer than 0.5% of procedures in which at least 5 mg chorionic villi are obtained[49]. In some of these cases, maternal decidual cell contamination occurs; this can be reduced by separating maternal decidual cells and blood from chorionic villi under a dissection microscope[52] (**EVIDENCE LEVEL: 2–**). Placental cell mosaicism is seen in 1% of procedures[17]. In these cases, genetic counseling is recommended and amniocentesis may be indicated to differentiate true fetal mosaicism from confined placental mosaicism[17].

Complications

Fetal loss

No RCTs comparing CVS *vs* no testing are available, so the entire evidence regarding the procedure-related risk of miscarriage comes from retrospective cohort studies.

For women undergoing CVS, the additional risk of fetal loss in comparison with controls has been reported to vary between 0.2% and 2%[2,24]. This risk appears to be lower in experienced centers and to decrease with increasing experience, ranging between 1/150 and 1/500[2,53]. A retrospective study from the Danish registry, of 31 355 cases undergoing CVS, reported a total fetal loss rate of 1.9% after CVS (*vs* 1.4% after amniocentesis); the miscarriage rate was correlated inversely with the number of procedures performed in a department and it was 40% higher for departments performing fewer than 1500 procedures, compared with those performing more than 1500, annually[40] (**EVIDENCE LEVEL: 2++**). An update in 2016 of the same database reported practically no impact of CVS on fetal loss rates (risk of miscarriage, 0.21% at 21 days after CVS)[25] (**EVIDENCE LEVEL: 2+**). This result is similar to the findings of a large retrospective study comparing the miscarriage rate in 5243 women who underwent CVS (2.7%) with that of 4917 controls (3.3%)[54]. According to a recent meta-analysis, the rate of fetal loss after CVS does not appear to be increased significantly in comparison with the non-exposed population (pooled risk < 24 weeks, 0.22% (95% CI, −0.71 to 1.16%))[24]; this estimate does not incorporate the 2016 Danish report[25] (**EVIDENCE LEVEL: 2++**).

The fetal loss rate after transcervical CVS was reported to be 2.5% in a retrospective series of 1251 procedures[55], and very similar miscarriage rates (2.5% *vs* 2.3%) were reported in a large RCT comparing transcervical with transabdominal CVS[46] (**EVIDENCE LEVEL: 1+**). One randomized study compared transabdominal CVS with second-trimester amniocentesis and found no significant difference in the total pregnancy loss between the two procedures (6.3% *vs* 7%; relative risk (RR), 0.90 (95% CI, 0.66–1.23))[56] (**EVIDENCE LEVEL: 1–**). However, a meta-analysis of four randomized trials showed that, compared with second-trimester amniocentesis, transcervical CVS carries a significantly higher risk of total pregnancy loss (RR, 1.40 (95% CI, 1.09–1.81)) and spontaneous miscarriage (RR, 1.50 (95% CI, 1.07–2.11))[57].

Vaginal bleeding

Vaginal bleeding is reported to occur in 10% of cases[52,53]. Its occurrence seems more frequent after the transcervical (up to 30% of cases) than after the transabdominal approach[52] (**EVIDENCE LEVEL: 2–**).

Uncommon complications

The risk of amniotic fluid leakage following CVS is exceedingly rare, occurring after < 0.5% of procedures[52] (**EVIDENCE LEVEL: 2–**). Clear figures on the risk of pregnancy loss in such cases are scarce. The risk of chorioamnionitis and uterine infection after CVS is extremely small (1–2/3000)[52] (**EVIDENCE LEVEL: 2–**). No cases of septic shock or maternal death following CVS have been reported.

Association with pre-eclampsia and intrauterine growth restriction

There have been some reports associating CVS with development of pre-eclampsia later in pregnancy, possibly due to placental damage, but these findings have not been consistent across studies and a meta-analysis failed to show an association[58] (**EVIDENCE LEVEL: 2+**). Similarly, a case–control study did not detect an association between CVS and impaired fetal growth; in the regression analysis the higher incidence of pre-eclampsia in the CVS group was due to maternal and fetal confounders (e.g. low pregnancy-associated plasma protein-A (PAPP-A), increased uterine artery resistance)[59] (**EVIDENCE LEVEL: 2+**).

Risk factors for complications

Lower fetal loss rates have been documented if more than 100 procedures are performed per annum[2]. Expert opinion suggests that an operator's competence should be reviewed when loss rates exceed 8/100 and sampling failure exceeds 5/100 consecutive CVS[2].

In a large retrospective study, factors associated with increased risk for miscarriage after CVS were African-American maternal race, at least two aspirations/needle insertions, heavy bleeding during CVS, maternal age younger than 25 years and gestational age at CVS < 10 weeks[54] (**EVIDENCE LEVEL: 2++**). The presence of fetal structural anomalies and increased nuchal translucency thickness (NT) are associated with a higher

background risk of miscarriage[2]. This risk is further increased following CVS. Lower levels of PAPP-A in maternal serum have also been suggested to herald a higher risk of fetal loss after CVS. This seems to be due to the association of low PAPP-A levels with placental disorders[60] (**EVIDENCE LEVEL: 2++**).

There are a number of factors which may plausibly increase the risk of fetal loss following CVS, although this association has not been proven consistently. Included in this group are[3,22]: fibroids; advanced maternal age; uterine malformations; chorioamniotic separation; retrochorionic hematoma; previous or current maternal bleeding; retroverted uterus; post-procedure persistent fetal bradycardia (**EVIDENCE LEVEL: 2–**).

3. FETAL BLOOD SAMPLING (FBS)

- FBS should be performed transabdominally after 18 + 0 weeks, using a 20–22-G needle under ultrasound guidance.
- The most common indications for FBS are investigation of chromosomal mosaicism after amniocentesis and hematological assessment of the fetus.
- Factors associated with increased risk of fetal loss after FBS include fetal structural defects (including hydrops), intrauterine growth restriction (IUGR) and, possibly, gestational age < 24 weeks (**GRADE OF RECOMMENDATION: B**).

There are several reported approaches to the umbilical vein for FBS, including cordocentesis (at the placental cord insertion site or a free loop) and puncture of the intrahepatic portion of the vein via the fetal liver. The term 'cordocentesis' refers to the ultrasound-guided puncture of the umbilical cord (umbilical vein), for either diagnostic (FBS) or therapeutic (intrauterine transfusion or drug instillation) purposes. The first series describing experience with FBS was published in 1987[61]. FBS should be performed beyond 18 + 0 completed weeks of gestation, as the risk of fetal loss is increased before this stage[62].

Technique

A 20–22-G needle is introduced transabdominally under continuous ultrasound guidance and inserted into the umbilical vein. The free-hand technique is used more commonly, although the use of a needle guide is preferred by some. If the placenta is anterior, a puncture of the cord at the level of placental insertion is suggested; if the placenta is posterior, a free loop of the cord or the intra-abdominal portion of the umbilical vein is sampled[62] (**EVIDENCE LEVEL: 4**).

Once the needle appears to have reached the target, flushing with saline may be used to confirm its correct position. Care should be taken to avoid the umbilical arteries. Aspiration by syringe is attempted by an assistant or the operator until blood is obtained in the sample. Origin of the blood should be confirmed by microscope (automated blood analyzer) to assess the mean corpuscular cell volume, or using a rapid acidification test (i.e. Kleihauer Betke or Apt test)[62].

The intrahepatic vein has been proposed as an alternative site when cord access is difficult or sampling fails at the placental cord insertion site[63]. Additional advantages of FBS at the intrahepatic vein include absence of cord complications, reduced risk of fetal blood loss and feto-maternal hemorrhage, and certainty of the fetal origin of the sample.

Fetal loss

The risk of fetal loss after FBS is between 1% and 2%[64–66]. In a large retrospective study of 1821 women who had undergone successful FBS, the procedure was associated with a 3.2% risk of fetal loss *vs* 1.8% for matched controls, yielding a net loss rate of 1.4%[64] (**EVIDENCE LEVEL: 2++**).

Factors associated with increased risk of fetal loss after FBS include fetal anomalies, IUGR and gestational age < 24 weeks. A small retrospective study found that the fetal loss rate was 14% (4/29) in fetuses with structural defects and 25% (9/36) in fetuses with hydrops, compared with only 1% (1/76) in fetuses with normal ultrasound findings[65] (**EVIDENCE LEVEL: 2++**). A similar, but much larger ($n = 1878$), retrospective study also reported increased fetal loss rates for fetuses with severe IUGR (8.9%) or structural abnormalities (13.1%), compared with 1% for fetuses with normal ultrasound findings[66] (**EVIDENCE LEVEL: 2++**). In addition, a large retrospective series of 2010 procedures indicated that the FBS-related loss rate may be higher before 24 weeks compared with after 24 weeks (2.7% *vs* 1.9%)[67] (**EVIDENCE LEVEL: 2++**).

This procedure should be performed only by experienced operators. Although there are no specific data, the risk of complications or sampling failure is expected to decrease with increasing experience of the operator.

4. ELIGIBILITY FOR INVASIVE PRENATAL DIAGNOSIS

- Detailed counseling should precede any invasive procedure, covering the expected benefits, risks and technical aspects of the test.
- Currently valid indications for invasive prenatal testing include increased risk for fetal chromosomal abnormality, increased risk for hereditary genetic or metabolic disease and increased risk for some perinatal infections.

Prior to a prenatal diagnostic procedure, pretest counseling of the couple is required. This may be carried out by the specialist in obstetrics or in fetal medicine who performs the procedure or by a geneticist or a dedicated midwife (**EVIDENCE LEVEL: 4**). The following issues should be presented and discussed[2]: benefits and risks of

invasive prenatal diagnosis *vs* screening[17,22]; differences between CVS and amniocentesis in terms of accuracy of results, complications and different timing and type of termination of pregnancy in case of abnormal results[22]; national and locally estimated risks of procedure-related pregnancy loss; accuracy and limitations of the particular laboratory test(s) being performed, with information on the rate of inconclusive results and reporting times; method of communication of results; indications for seeking medical advice following the test; the need for anti-D passive immunization post-procedure if the woman is Rhesus negative and non-immunized[2,22]. At the end of this detailed informative process, written consent should be obtained from the woman[2].

Indications for amniocentesis or CVS

The following are currently considered valid indications for invasive prenatal diagnosis by amniocentesis or CVS: increased risk of fetal aneuploidy, increased risk for a known genetic or biochemical disease of the fetus, maternal transmittable infectious disease and, under certain circumstances, maternal request.

Increased risk of fetal aneuploidy (EVIDENCE LEVEL: 4)

The increased risk may derive from a screening test (first-trimester combined test; cffDNA test/non-invasive prenatal test (NIPT); second-trimester biochemistry, such as triple or quadruple test); abnormal ultrasound findings (fetal structural anomaly commonly associated with chromosomal abnormality); obstetric history (previous fetus or child affected by aneuploidy) or family history (parental carrier of chromosomal balanced translocation or inversion, parental aneuploidy or mosaicism for aneuploidy)[17].

Advanced maternal age (> 35 years) alone should not be considered an indication, although in some countries it is still among the accepted criteria for invasive testing[4,17].

Conception by assisted reproductive technique in itself is not considered a valid indication for invasive prenatal diagnosis. However, in pregnancies achieved by intracytoplasmic sperm injection because of oligospermia, the prospective parents should be informed that there is an increased risk of chromosomal anomalies in the sperm causing infertility which may be transmitted to male offspring.

Increased risk for a known genetic or biochemical disease of the fetus[17] (EVIDENCE LEVEL: 4)

The increased risk may derive from: a family hereditary disease with a known mutation or biochemical change; male fetus and carrier status of pregnant woman for a disease with X-chromosomal inheritance; carrier status of both parents for an autosomal recessive disorder.

Maternal transmittable infectious disease[17] (EVIDENCE LEVEL: 4)

In the case of maternal primary infection or seroconversion involving toxoplasma, cytomegalovirus or rubella, prenatal invasive testing may be indicated to confirm or exclude transmission of the infection to the fetus.

Maternal request (EVIDENCE LEVEL: 4)

Maternal request as a standalone criterion is not generally considered a valid indication for invasive prenatal diagnosis, though under exceptional circumstances, for example when there is acute parental anxiety, and after extensive counseling, the fetal medicine specialist may permit this.

Indications for FBS (EVIDENCE LEVEL: 4)

The commonest indications for FBS are investigation for chromosomal mosaicism after amniocentesis or hematological assessment of the fetus (quantification of fetal anemia or platelet/lymphocyte count)[17,62].

In current practice, the following indications have become extremely rare, having been replaced largely by CVS and amniocentesis[17,62]: full karyotype; blood type or platelet antigen status; genetic testing; infection; plasma or serum studies (e.g. metabolites, hormones).

5. CHECKLIST BEFORE AND AFTER THE PROCEDURE

- The Rhesus status of the mother and the presence of alloantibodies in the serum should be checked before performing a prenatal invasive procedure; prophylactic anti-D immunoglobulin should be given to non-sensitized women within 72 h post-procedure unless the alleged father of the fetus is proven also to be Rhesus negative.
- Universal maternal screening for blood-borne viruses (hepatitis B & C virus (HBV & HCV); human immunodeficiency virus (HIV)) is not recommended.
- Antibiotic prophylaxis before an invasive procedure is currently not recommended.
- The main principles of asepsis need to be observed while performing an invasive procedure.
- A detailed report regarding the procedure must be provided to the managing healthcare provider.

Maternal blood group testing and Rhesus prophylaxis (EVIDENCE LEVEL: 2+)

All current guidelines recommend testing of women for their Rhesus status and for presence of alloantibodies before invasive procedures[68]. Rhesus prophylaxis is strongly recommended after an invasive procedure in non-sensitized Rhesus-negative women with a Rhesus-positive partner (unless the fetus has been found to be Rhesus negative by cffDNA testing of maternal

serum). A single intramuscular dose of anti-D antibodies in fixed preparation is commonly used[68]. In a prospective series of 361 Rhesus-negative women who underwent amniocentesis, did not receive anti-D prophylaxis and delivered a Rhesus-positive infant, five (1.4%) yielded a positive anti-D antibody result; none of these infants had clinical consequences[69]. The corresponding rate in a series of 115 women was 3.4%; one of these four infants required two exchange transfusions but was reported to be developmentally normal at the age of 2 years[70]. Nevertheless, anti-Rhesus prophylaxis after amniocentesis has been recommended since the late 1970s[71], and in a series of 944 Rhesus-negative women who received anti-D immunoglobulin, no case of Rhesus sensitization occurred[72].

Maternal screening for blood-borne viruses

The risk of viral transmission to the fetus through invasive testing is negligible and is probably limited to those pregnant women with high viral load[73].

Antibiotic prophylaxis

There is only one RCT on the prophylactic administration of antibiotics (azithromycin) before amniocentesis ($n = 34\,923$): a lower rate of procedure-related miscarriage (0.03%) and PPROM (0.06%) was observed in the azithromycin group ($n = 21219$) *vs* the no-intervention group (0.28% and 1.12% respectively, $n = 12\,529$)[74] (**EVIDENCE LEVEL: 1–**). However, the publication of this study triggered a scientific and legal dispute[75–77] and its results should be interpreted with caution. A much smaller ($n = 1744$) retrospective study did not detect differences in fetal loss rate between patients treated with prophylactic antibiotics (amoxicillin/clavulanic-acid or azithromycin, rate 1.3%) and untreated women (1.2%)[78] (**EVIDENCE LEVEL: 2++**). There are insufficient high-quality data to evaluate the effect of antibiotic prophylaxis before an invasive procedure[79], and its use is currently not endorsed by scientific bodies.

Ultrasound (pre- and post-procedure) (EVIDENCE LEVEL: 4)

Before subjecting a woman to an invasive procedure, the following items should be checked by ultrasound: number of fetuses and viability; placental location; amount of amniotic fluid; gestational age[3]. Ultrasound examination is also performed commonly after an invasive procedure to check the fetal heart rate, placenta (presence of hematoma) and amount of amniotic fluid. This can be done immediately or some days later, depending on local policy[22].

Asepsis (EVIDENCE LEVEL: 4)

The main principles of asepsis need to be observed while performing an invasive procedure to minimize the risk of fetomaternal infection. The use of a tray with sterile gloves, gauze pads, forceps and needles is recommended[3]. Before transabdominal CVS, amniocentesis or FBS, the abdominal skin needs to be cleaned with antiseptic solution (chlorexidine or iodine) and subsequently covered with a sterile drape. The use of a sterile bag to enclose the probe is commonly adopted. Alternatively, the probe may be disinfected. The use of separate sterile gel is strongly recommended to avoid bacterial contamination. Before transcervical CVS, a sterile speculum is inserted and both cervix and vaginal walls are cleansed with an antiseptic solution[2,3,5].

Local anesthesia

A recent Cochrane meta-analysis pooled the results of five RCTs evaluating different methods of analgesia for amniocentesis; there was no randomized trial for CVS. It was concluded that, in general, there is only minor pain during amniocentesis and therefore there is no evidence to support the use of analgesia[80] (**EVIDENCE LEVEL: 1+**). Before transabdominal CVS, local anesthetic can be used to reduce the discomfort of the patient caused by the larger needle size[2,3,80]. In a recent UK survey, 89% of operators reported the use of local anesthesia at CVS[47] (**EVIDENCE LEVEL: 3**). Before FBS, the use of local anesthetic may be considered in order to reduce the risk of maternal movements during the procedure[62]. The use of local anesthetic before transcervical CVS has not been reported.

Reporting (EVIDENCE LEVEL: 4)

A detailed report regarding the procedure must be given to the patient and to her healthcare provider. The following data should be included: indication for invasive diagnosis[2]; ultrasound findings prior to the procedure[2]; procedure description: instrument used, puncture site, number of punctures, quantity of sample, appearance of the amniotic fluid (in case of amniocentesis); viability of the fetus, appearance of the placenta and amniotic fluid volume after the procedure[2]; Rhesus status and prophylaxis[2]; laboratory exams requested (conventional G-banded karyotype and/or quantitative fluorescence polymerase chain reaction (QF-PCR)/fluorescence *in-situ* hybridization (FISH) with or without microarray)[2].

Post-procedure instructions (EVIDENCE LEVEL: 4)

Limiting physical activity for 12–24 h is optional as there is no evidence of clinical benefit. No particular pharmacological treatment is widely recommended although the use of paracetamol (acetaminophen) may be considered soon after the procedure in case of substantial abdominal discomfort[3]. Administration of progesterone or tocolytic drugs (i.e. terbutaline) following amniocentesis or CVS has not been demonstrated to yield a clear benefit in terms of relevant clinical outcome[79]. Post-test genetic consultation is recommended only in cases of abnormal result[17] (**EVIDENCE LEVEL: 4**).

6. TYPES OF GENETIC TESTING: WHAT TO LOOK FOR

The following laboratory testing may be carried out on the fetal sample obtained by the invasive procedure: full karyotype, rapid testing, molecular diagnosis of chromosomal imbalances and diagnosis of monogenic disease.

Full karyotype (EVIDENCE LEVEL: 4)

The conventional method for karyotype analysis is metaphase analysis of cultured amniocytes or that of placental mesenchymal cells obtained from amniocentesis or CVS, respectively. The results are available in 2 weeks. In contrast, metaphase analysis of fetal lymphocytes obtained from cordocentesis is available in 2–5 days. Following CVS, direct analysis of cytotrophoblastic metaphases is feasible and may be achieved within 5 days[17].

Rapid testing (EVIDENCE LEVEL: 4)

Rapid testing, such as QF-PCR (or, more rarely, FISH), may be carried out on villi or amniotic fluid to test for specific chromosomes (21, 13, 18, X, Y). These tests provide results in 1–2 days and are commonly employed following a screen-positive result or in fetuses with ultrasound findings or markers of common aneuploidies[17]. In some settings, the use of QF-PCR has replaced the full karyotype. However, inaccuracies of the rapid testing results (false positive or false negative) are reported occasionally. On this basis, abnormal rapid testing should be confirmed by metaphase culture or should be associated with ultrasound anomalies before making clinical decisions regarding continuation of the pregnancy[81]. The right to terminate the pregnancy following a sole abnormal rapid testing result varies across different healthcare systems and is based on local policy.

Molecular diagnosis of chromosomal imbalances

Microarray techniques (e.g. array comparative genomic hybridization (aCGH)) were introduced recently into the field of prenatal diagnosis. These methods are able to detect submicroscopic chromosomal deletions and duplications (copy number variants (CNV))[17]. Different platforms are available, including genome-wide (10–400-Kb resolution), targeted (i.e. prenatal 'bacterial artificial chromosome (BACS)-on-beads' (BoBs)) and mixed arrays. In the first large study comparing microarrays with karyotype for prenatal diagnosis, it was found that the former could detect clinically relevant aberrations in 6.0% of fetuses with normal karyotype and structural defects and in 1.7% of those undergoing invasive testing for advanced maternal age or positive screening results[82]. Several studies have followed and pooled incremental diagnostic yields of 7.0% and 5.0% were reported with use of aCGH in fetuses with congenital heart defects or increased NT, respectively[83,84] (**EVIDENCE LEVEL: 2++**).

Currently, the use of these techniques is recommended in cases of fetal structural anomalies[82] or NT > 3.5 mm in the first trimester[83,84]. Among these groups of pregnancies, an increased rate of pathological CNV in comparison with conventional analysis is yielded by the use of microarray. However, their use in an unselected population is strongly debated due to the difficult interpretation and counseling in cases of variants of unknown significance (VOUS). The possibility of not reporting VOUS in order to overcome the issue of counseling prospective parents in the context of uncertain and probably irrelevant findings has been proposed by some[6] (**EVIDENCE LEVEL: 4**).

Diagnosis of monogenic disease

Invasive procedures may be used in the prenatal diagnosis of any monogenic disease whose molecular defect is well known or has been characterized previously (**EVIDENCE LEVEL: 4**).

7. MATERNAL INFECTION

- The risk for vertical transmission of HBV after amniocentesis does not appear to be increased in HBeAg-negative women.
- The risk for vertical transmission of HIV does not appear to be increased in women receiving combined highly active antiretroviral therapy (HAART).
- It is prudent that in any case of maternal HBV, HCV or HIV infection, non-invasive testing is preferred; whenever amniocentesis is performed, every effort should be made to avoid the placenta.

In women with chronic infection, transplacental needle insertion during amniocentesis should be avoided. In general, the rate of fetal transmission seems to depend on the maternal viral load[85].

Hepatitis B virus (HBV)

A study comparing the vertical transmission rates in infants of HBsAg-positive mothers who had or had not undergone amniocentesis found that the amniocentesis group had a higher transmission rate overall (6.35% *vs* 2.53%). Transmission rates did not differ between amniocentesis and control groups when the viral load was low, but they were very high in the amniocentesis group (50%) for viral loads ≥ 7 $\log_{10}$ copies/mL[85] (**EVIDENCE LEVEL: 2++**).

The rate of fetal transmission does not seem to be increased in HBsAg-positive HBeAg-negative women in comparison with controls (1.5–3%), while the risk is probably increased compared with controls in HBeAg-positive patients. The protective role of immunoprophylaxis or antiviral therapy before the procedure has not been explored in these cases[86,87] (**EVIDENCE LEVEL: 2++**).

Although data are limited, particularly with respect to the potentially increased risk for HBeAg-positive women, the Society of Obstetricians and Gynaecologists of Canada currently recommends that every effort should be made to avoid inserting the needle through, or very close to, the placenta[73].

Hepatitis C virus (HCV)

Few data are available on the rate of maternal–fetal transmission of HCV during amniocentesis, although fetal infection rates have been shown to be similar in cases with HCV-positive mothers who have not undergone amniocentesis[17].

Human immunodeficiency virus (HIV)

Amniocentesis was a major risk factor for vertical HIV transmission in the pre-antiretroviral drugs era. A retrospective study on 553 infants of HIV-1-positive women reported that amniocentesis was an independent risk factor for vertical transmission, increasing the risk approximately four-fold (odds ratio, 4.1 (95% CI, 2.1–9.5))[88] (**EVIDENCE LEVEL: 2+**).

The introduction of combined antiretroviral therapy (c-ART) changed this picture radically. A Spanish study compared the outcomes of 366 HIV-positive mothers before and after 1997, when antiretroviral therapy was widely implemented: the rates of vertical transmission in women who had undergone amniocentesis and those who had not were 30% (3/10) and 16.2% (40/247), respectively, before 1997, while the corresponding rates decreased to 0% (0/18) and 3.7% (3/81) after 1997[89] (**EVIDENCE LEVEL: 2+**). Similarly low rates were reported after 1997 in an Italian (3.3%)[90] and a French (0%)[91] study. Furthermore, a multicenter French study highlighted the superiority of HAART (transmission rate, 0%) over zidovudine alone (transmission rate, 6.1%) or no treatment (transmission rate, 25.0%) in HIV-positive women undergoing amniocentesis[92] (**EVIDENCE LEVEL: 2++**).

In HIV-infected pregnant women, fetal transmission does not seem to be increased in those undergoing amniocentesis compared with controls not undergoing the procedure if the viral load is low, if the patient was on c-ART before conceiving or if the viral load is high but c-ART was started at least 2 weeks before amniocentesis[90,93].

According to the Society of Obstetricians and Gynaecologists of Canada, for women not on c-ART, the risk of vertical transmission is increased by amniocentesis. When possible, c-ART should be initiated and the procedure postponed until the viral load is undetectable[73]. Similar to HBV and HCV, every effort should be made in HIV-positive mothers to avoid inserting the needle through, or very close to, the placenta[73].

The risk of HBV, HCV or HIV vertical transmission following CVS or cordocentesis has not yet been investigated thoroughly[73].

8. MULTIPLE PREGNANCY

- The rates of fetal loss after CVS and amniocentesis appear to be similar in twin pregnancies (**GRADE OF RECOMMENDATION: C**).

In multiple pregnancy it is preferable that invasive procedures are carried out by a specialist who is able to perform selective termination[17]. Data regarding the risk of miscarriage related to the procedures come from retrospective cohort studies, as no RCTs are available.

Amniocentesis in twins

Several retrospective studies have assessed the miscarriage rate after amniocentesis in twins. Among the most recent, a Canadian case–control study reported a 3.0% loss rate after amniocentesis, compared with 0.8% in controls[94]; a Spanish series reported 2.7% *vs* 2.6% loss[95] and an American study reported a loss rate of 3.2% *vs* 1.4%[96] (**EVIDENCE LEVEL: 2+**). A meta-analysis summarizing the data reported a pooled 3.07% pregnancy loss rate, and a 2.54% loss rate before 24 weeks; for case–control studies, the pooled loss rates for twin pregnancies undergoing amniocentesis and for control twins were 2.59% *vs* 1.53% (RR, 1.81 (95% CI, 1.02–3.19))[97]. No difference was found between single *vs* double uterine entry[97] (**EVIDENCE LEVEL: 2++**).

CVS in twins

The data for CVS are even more limited in twins. The aforementioned meta-analysis[97] reported a pooled loss rate of 3.84% after CVS in twins. No significant differences were found between transabdominal and transcervical approaches, use of a single-needle system *vs* a double-needle system and single uterine entry *vs* double uterine entry[97] (**EVIDENCE LEVEL: 2++**). There have been no significant differences in loss rates reported between CVS and amniocentesis in retrospective studies comparing the two methods. A study including data from the years 1984–1990 reported a 3.2% loss rate after CVS *vs* 2.9% after amniocentesis[98]. Similar data were reported in a more recent study, with loss rates of 3.85% and 4.0% after CVS and amniocentesis, respectively[99] (**EVIDENCE LEVEL: 2+**). There are insufficient data to compare the loss rate of CVS with the background risk in twins.

Higher-order pregnancies

Data regarding the risk of miscarriage related to invasive procedures among higher-order multiple gestations are lacking.

Chorionicity and mapping

Before performing an invasive procedure in multiple gestations, it is critically important that chorionicity and

placentation are mapped accurately and twins labeled (with diagrams), and that it is noted whether the sex is discordant[3,100,101].

Technique of amniocentesis in twins

The technique of amniocentesis in twins varies according to chorionicity[98,101].

Amniocentesis in dichorionic twins

In a dichorionic twin pregnancy, sampling of both amniotic sacs is recommended. With the two-puncture technique (one per sac) there is a small (1.8%) risk of sampling the same sac twice[101]. To overcome this problem, a dye (indigo carmine) may be instilled in the first sac in dubious cases or in high-order multiple pregnancy. The use of methylene blue as dye has been abandoned due to an increased risk of fetal anomalies (jejunal atresia)[102,103] (**EVIDENCE LEVEL: 2+**). The single-puncture technique with intertwin membrane passage is an alternative option. In this case, the first 1–2 mL of amniotic fluid sampled after intertwin membrane passage should be discarded to avoid contamination from the first twin[101]. The risk of fetal loss has not been shown to be increased with the two-puncture compared with the single-puncture technique[99] (**EVIDENCE LEVEL: 2+**).

Amniocentesis in monochorionic diamniotic twins

In monochorionic diamniotic twin gestation, sampling of a single sac is warranted when chorionicity has been determined clearly at ultrasound prior to 14 weeks and fetal growth and anatomy are concordant. If this is not the case, double sampling should be considered[101] (**EVIDENCE LEVEL: 4**). A two-sampling approach may also be considered after *in-vitro* fertilization (IVF) or in the case of discordant anomaly/growth (small risk of heterokaryotype). If sampling of two sacs is clinically indicated, the two-puncture technique is recommended to avoid iatrogenic monoamnionicity[101] (**EVIDENCE LEVEL: 4**).

Technique of CVS in twins

The sampling technique of CVS in multiple gestations should also be tailored to the chorionicity[97].

CVS in dichorionic twins

In dichorionic twins undergoing transabdominal CVS, either two separate punctures, one at each trophoblastic area, or a single-puncture technique sampling the two placentae in sequence (double needle with single outer of 18–19 G and two different inners of 20 G, one for each placenta), may be performed. In transcervical CVS, two biopsies, one at each placental site, are warranted[101] (**EVIDENCE LEVEL: 4**). Sampling error or inaccurate sampling are reported to occur in 3–4% of cases[101]. Cross-contamination of chorionic tissue with coexistence of cells from different placentae in the same sample is described in 1% of CVS in twins[104]. To reduce the risk of unreliable or inaccurate results, placental sampling near the cord insertion and avoidance of the area around the dividing membrane is recommended. Alternatively, a combination of transabdominal and transcervical approaches may be considered (**EVIDENCE LEVEL: 4**).

CVS in monochorionic twins (EVIDENCE LEVEL: 4)

In monochorionic twins, a single-sampling approach around the amniotic equator is warranted. A shift to amniocentesis with a two-sampling approach must be considered after IVF or in the case of discordant anomaly/growth (due to the small risk of heterokaryotype in these cases)[101].

9. THROMBOPROPHYLAXIS BEFORE INVASIVE PROCEDURES

There are no available data regarding the discontinuation of thromboprophylaxis before fetal invasive procedures. Recommendations may be derived from studies carried out on other types of percutaneous invasive procedures, including liver biopsy. Regarding prophylactic dosage of aspirin and low-molecular-weight heparin, discontinuation before the procedure does not seem justified clinically. However, withholding a single dose of heparin seems advisable[105,106].

10. AUDIT

Each examiner should carry out his/her own quality control by collecting the following parameters: number of interventions performed per year; number of samples with insufficient material; number of samples with bloody amniotic fluid; number of interventions with more than one puncture and number of punctures; pregnancy outcome (including number of miscarriages and their time interval following the procedure, leakage, premature delivery, rupture of membranes); other pregnancy complications[22].

11. TRAINING

Training for invasive procedures should begin on a model/simulator, to practice maintenance of the needle path within the ultrasonic window, so that the entire needle remains visible at all times to ensure safety. Clinical training should begin with 'simple' amniocentesis (i.e. posterior placenta and with adequate amount of amniotic fluid) or CVS (i.e. easily accessible placenta) or in women undergoing termination of pregnancy where this is allowed. The minimum number of procedures that it is necessary for an operator to perform, in order to optimize their competence in doing them safely, varies widely in the literature (from 45 to 300). However, according to most, no further improvement is expected[2] after 100 procedures performed independently.

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REFERENCES

1. Sarto GE. Prenatal diagnosis of genetic disorders by amniocentesis. *Wis Med J* 1970; **69**: 255–260.
2. Royal College of Obstetricians & Gynaecologists. *Amniocentesis and Chorionic Villus Sampling*. Green-top Guideline No. 8, June 2010.
3. Wilson RD, Davies G, Gagnon A, Desilets V, Reid GJ, Summers A, Wyatt P, Allen VM, Langlois S; Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada. Amended Canadian guideline for prenatal diagnosis (2005) change to 2005–techniques for prenatal diagnosis. *J Obstet Gynaecol Can* 2005; **27**: 1048–1062.
4. Tabor A, Alfirevic Z. Update on procedure-related risks for prenatal diagnosis techniques. *Fetal Diagn Ther* 2010; **27**: 1–7.
5. Cruz-Lemini M, Parra-Saavedra M, Borobio V, Bennasar M, Goncé A, Martínez JM, Borrell A. How to perform an amniocentesis. *Ultrasound Obstet Gynecol* 2014; **44**: 727–731.
6. Athanasiadis AP, Pantazis K, Goulis DG, Chatzigeorgiou K, Vaitsi V, Assimakopoulos E, Tzevelekis F, Tsalikis T, Bontis JN. Comparison between 20G and 22G needle for second trimester amniocentesis in terms of technical aspects and short-term complications. *Prenat Diagn* 2009; **29**: 761–765.
7. Uludag S, Aydin Y, Ibrahimova F, Madazli R, Sen C. Comparison of complications in second trimester amniocentesis performed with 20G, 21G and 22G needles. *J Perinat Med* 2010; **38**: 597–600.
8. Giorlandino C, Mobili L, Bilancioni E, D'Alessio P, Carcioppolo O, Gentili P, Vizzone A. Transplacental amniocentesis: is it really a higher-risk procedure? *Prenat Diagn* 1994; **14**: 803–806.
9. Bombard AT, Powers JF, Carter S, Schwartz A, Nitowsky HM. Procedure-related fetal losses in transplacental versus nontransplacental genetic amniocentesis. *Am J Obstet Gynecol* 1995; **172**: 868–872.
10. Marthin T, Liedgren S, Hammar M. Transplacental needle passage and other risk-factors associated with second trimester amniocentesis. *Acta Obstet Gynecol Scand* 1997; **76**: 728–732.
11. Seeds JW. Diagnostic mid trimester amniocentesis: how safe? *Am J Obstet Gynecol* 2004; **191**: 607–615.
12. Tabor A, Philip J, Madsen M, Bang J, Obel EB, Nørgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1986; **1**: 1287–1293.
13. Calda P, Brestak M. Amniovacucentesis vs standard syringe technique for amniocentesis: experience with 1219 cases. *Am J Obstet Gynecol* 2009; **201**: 593.
14. Nuss S, Brebaum D, Grond-Ginsbach C. Maternal cell contamination in amniotic fluid samples as a consequence of the sampling technique. *Hum Genet* 1994; **93**: 121–124.
15. Hockstein S, Chen PX, Thangavelu M, Pergament E. Factors associated with maternal cell contamination in amniocentesis samples as evaluated by fluorescent in situ hybridization. *Obstet Gynecol* 1998; **92**: 551–556.
16. Welch RA, Salem-Elgharib S, Wiktor AE, Van Dyke DL, Blessed WB. Operator experience and sample quality in genetic amniocentesis. *Am J Obstet Gynecol* 2006; **194**: 189–191.
17. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 88, December 2007. Invasive prenatal testing for aneuploidy. *Obstet Gynecol* 2007; **110**: 1459–1467.
18. Johnson JM, Wilson RD, Winsor EJ, Singer J, Dansereau J, Kalousek DK. The early amniocentesis study: a randomized clinical trial of early amniocentesis versus midtrimester amniocentesis. *Fetal Diagn Ther* 1996; **11**: 85–93.
19. Wilson RD, Johnson J, Windrim R, Dansereau J, Singer J, Winsor EJ, Kalousek D. The early amniocentesis study: a randomized clinical trial of early amniocentesis and midtrimester amniocentesis. II. Evaluation of procedure details and neonatal congenital anomalies. *Fetal Diagn Ther* 1997; **12**: 97–101.
20. Randomised trial to assess safety and fetal outcome of early and midtrimester amniocentesis. The Canadian Early and Mid-trimester Amniocentesis Trial (CEMAT) Group. *Lancet* 1998; **351**: 242–247.
21. Farrell SA, Summers AM, Dallaire L, Singer J, Johnson JA, Wilson RD. Club foot, an adverse outcome of early amniocentesis: disruption or deformation? CEMAT. Canadian Early and Mid-Trimester Amniocentesis Trial. *J Med Genet* 1999; **36**: 843–846.
22. Kähler C, Gembruch U, Heling KS, Henrich W, Schramm T; DEGUM. [DEGUM guidelines for amniocentesis and chorionic villus sampling]. *Ultraschall Med* 2013; **34**: 435–440.
23. O'Donoghue K, Giorgi L, Pontello V, Pasquini L, Kumar S. Amniocentesis in the third trimester of pregnancy. *Prenat Diagn* 2007; **27**: 1000–1004.
24. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015; **45**: 16–26.
25. Wulff CB, Gerds TA, Rode L, Ekelund CK, Petersen OB, Tabor A; Danish Fetal Medicine Study Group. The risk of fetal loss associated with invasive testing following combined first trimester risk screening for Down syndrome – a national cohort of 147 987 singleton pregnancies. *Ultrasound Obstet Gynecol* 2016; **47**: 38–44.
26. Philip J, Silver RK, Wilson RD, Thom EA, Zachary JM, Mohide P, Mahoney MJ, Simpson JL, Platt LD, Pergament E, Hershey D, Filkins K, Johnson A, Shulman LP, Bang J, MacGregor S, Smith JR, Shaw D, Wapner RJ, Jackson LG. Late first-trimester invasive prenatal diagnosis: results of an international randomized trial; NICHD EATA Trial Group. *Obstet Gynecol* 2004; **103**: 1164–1173.
27. Borgida AF, Mills AA, Feldman DM, Rodis JF, Egan JF. Outcome of pregnancies complicated by ruptured membranes after genetic amniocentesis. *Am J Obstet Gynecol* 2000; **183**: 937–939.
28. Cross HE, Maumenee AE. Ocular trauma during amniocentesis. *Arch Ophthalmol* 1973; **90**: 303–304.
29. Epley SL, Hanson JW, Cruikshank DP. Fetal injury with midtrimester diagnostic amniocentesis. *Obstet Gynecol* 1979; **53**: 77–80.
30. Cambiaghi S, Restano L, Cavalli R, Gelmetti C. Skin dimpling as a consequence of amniocentesis. *J Am Acad Dermatol* 1998; **39**: 888–890.
31. Sepúlveda W, Quiroz V, Fernández R. [Trauma of the fetal vessels during amniocentesis]. *Rev Chil Obstet Ginecol* 1984; **49**: 99–103.
32. Eller KM, Kuller JA. Porencephaly secondary to fetal trauma during amniocentesis. *Obstet Gynecol* 1995; **85**: 865–867.
33. Squier M, Chamberlain P, Zaiwalla Z, Anslow P, Oxbury J, Gould S, McShane MA. Five cases of brain injury following amniocentesis in mid-term pregnancy. *Dev Med Child Neurol* 2000; **42**: 554–560.
34. Okyay RE, Gode F, Saatli B, Guclu S. Late-onset maternal mortality after amniocentesis. *Eur J Obstet Gynecol Reprod Biol* 2011; **158**: 367–368.

35. Bodner K, Wierrani F, Bodner-Adler B. Maternal sepsis due to *Clostridium perfringens* after 2nd-trimester genetic amniocentesis. *J Obstet Gynaecol* 2011; **31**: 339–340.
36. Pinette MG. Maternal death after second-trimester genetic amniocentesis. *Obstet Gynecol* 2005; **106**: 409.
37. Elchalal U, Shachar IB, Peleg D, Schenker JG. Maternal mortality following diagnostic 2nd-trimester amniocentesis. *Fetal Diagn Ther* 2004; **19**: 195–198.
38. Plachouras N, Sotiriadis A, Dalkalitsis N, Kontostolis E, Xiropotamos N, Paraskevaidis E. Fulminant sepsis after invasive prenatal diagnosis. *Obstet Gynecol* 2004; **104**: 1244–1247.
39. Hess LW, Anderson RL, Golbus MS. Significance of opaque discolored amniotic fluid at second-trimester amniocentesis. *Obstet Gynecol* 1986; **67**: 44–46.
40. Tabor A, Vestergaard CH, Lidegaard Ø. Fetal loss rate after chorionic villus sampling and amniocentesis: an 11-year national registry study. *Ultrasound Obstet Gynecol* 2009; **34**: 19–24.
41. Towner D, Currier RJ, Lorey FW, Cunningham GC, Greve LC. Miscarriage risk from amniocentesis performed for abnormal maternal serum screening. *Am J Obstet Gynecol* 2007; **196**: 608.e1–5.
42. Harper LM, Cahill AG, Smith K, Macones GA, Odibo AO. Effect of maternal obesity on the risk of fetal loss after amniocentesis and chorionic villus sampling. *Obstet Gynecol* 2012; **119**: 745–751.
43. Department of Obstetrics and Gynecology, Tietung Hospital, Anshan, China. Fetal sex prediction by sex chromatin of chorionic cells during early pregnancy. *Chin Med J (Engl)* 1975; **1**: 117–126.
44. Niazi M, Coleman DV, Loeffler FE. Trophoblast sampling in early pregnancy. Culture of rapidly dividing cells from immature placental villi. *Br J Obstet Gynaecol* 1981; **88**: 1081–1085.
45. Young C, von Dadelszen P, Alfirevic Z. Instruments for chorionic villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev* 2013; **1**: CD000114.
46. Jackson LG, Zachary JM, Fowler SE, Desnick RJ, Golbus MS, Ledbetter DH, Mahoney MJ, Pergament E, Simpson JL, Black S, et al. A randomized comparison of transcervical and transabdominal chorionic-villus sampling. The U.S. National Institute of Child Health and Human Development Chorionic-Villus Sampling and Amniocentesis Study Group. *N Engl J Med* 1992; **327**: 594–598.
47. Carlin AJ, Alfirevic Z. Techniques for chorionic villus sampling and amniocentesis: a survey of practice in specialist UK centres. *Prenat Diagn* 2008; **28**: 914–919.
48. Battagliarin G, Lanna M, Coviello D, Tassis B, Quarenghi A, Nicolini U. A randomized study to assess two different techniques of aspiration while performing transabdominal chorionic villus sampling. *Ultrasound Obstet Gynecol* 2009; **33**: 169–172.
49. von Dadelszen P, Sermer M, Hillier J, Allen LC, Fernandes BJ, Johnson JA, Shime J, Winsor EJ, Ryan G. A randomised controlled trial of biopsy forceps and cannula aspiration for transcervical chorionic villus sampling. *BJOG* 2005; **112**: 559–566.
50. Mastroiacovo P, Botto LD, Cavalcanti DP, Lalatta F, Selicorni A, Tozzi AE, Baronciani D, Cigolotti AC, Giordano S, Petroni F, *et al.* Limb anomalies following chorionic villus sampling: a registry based case–control study. *Am J Med Genet* 1992; **44**: 856–864.
51. Botto LD, Olney RS, Mastroiacovo P, Khoury MJ, MooreCA, Alo CJ, Costa P, Edmonds LD, Flood TJ, Harris JA, Howe HL, Olsen CL, Panny SR, Shaw GM. Chorionic villus sampling and transverse digital deficiencies: evidence for anatomic and gestational-age specificity of the digital deficiencies in two studies. *Am J Med Genet* 1996; **62**: 173–178.
52. Brambati B, Lanzani A, Tului L. Transabdominal and transcervical chorionic villus sampling: efficiency and risk evaluation of 2,411 cases. *Am J Med Genet* 1990; **35**: 160–164.
53. Papp C, Beke A, Mezei G, Tóth-Pál E, Papp Z. Chorionic villus sampling: a 15-year experience. *Fetal Diagn Ther* 2002; **17**: 218–227.
54. Odibo AO, Dicke JM, Gray DL, Oberle B, Stamilio DM, Macones GA, Crane JP. Evaluating the rate and risk factors for fetal loss after chorionic villus sampling. *Obstet Gynecol* 2008; **112**: 813–819.
55. Donner C, Simon P, Karioun A, Delneste D, Abramowicz M, Cochaux P, Rodesch F. Experience with 1251 transcervical chorionic villus samplings performed in the first trimester by a single team of operators. *Eur J Obstet Gynecol Reprod Biol* 1995; **60**: 45–51.
56. Smidt-Jensen S, Permin M, Philip J, Lundsteen C, Zachary J, Fowler S, Grüning K. Randomized comparison of amniocentesis and transabdominal and transcervical chorionic villus sampling. *Lancet* 1992; **340**: 1237–1244.
57. Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev* 2003; **3**: CD003252.
58. Basaran A, Basaran M, Topatan B. Chorionic villus sampling and the risk of preeclampsia: a systematic review and meta-analysis. *Arch Gynecol Obstet* 2011; **283**: 1175–1181.
59. Sotiriadis A, Eleftheriades M, Chatzinikolaou F, Chatzistamatiou K, Assimakopoulos E, Chasiakos D. Fetal growth impairment after first-trimester chorionic villus sampling: a case–control study. *J Matern Fetal Neonatal Med* 2015 **29**: 1–5.
60. Akolekar R1, Bower S, Flack N, Bilardo CM, Nicolaides KH. Prediction of miscarriage and stillbirth at 11–13 weeks and the contribution of chorionic villus sampling. *Prenat Diagn* 2011; **31**: 38–45.
61. Orlandi F, Damiani G, Jakil C, Rossi C, Maggio A, Scola B, Cittadini E, Quartararo P. Clinical results and fetal biochemical data in 140 early second trimester diagnostic cordocenteses. *Acta Eur Fertil* 1987; **18**: 329–333.
62. Society for Maternal-Fetal Medicine (SMFM), Berry SM, Stone J, Norton ME, Johnson D, Berghella V. Fetal blood sampling. *Am J Obstet Gynecol* 2013 Sep; **209**: 170–180.
63. Nicolaidis P, Nicolini U, Fisk NM, Tannirandorn Y, Nasrat H, Rodeck CH. Fetal blood sampling from the intrahepatic vein for rapid karyotyping in the second and third trimesters. *Br J Radiol* 1991; **64**: 505–509.
64. Tongsong T, Wanapirak C, Kunavikatikul C, Sirirchotiyakul S, Piyamongkol W, Chanprapaph P. Fetal loss rate associated with cordocentesis at midgestation. *Am J Obstet Gynecol* 2001; **184**: 719–723.
65. Maxwell DJ, Johnson P, Hurley P, Neales K, Allan L, Knott P. Fetal blood sampling and pregnancy loss in relation to indication. *Br J Obstet Gynaecol* 1991; **98**: 892–897.
66. Antsaklis A, Daskalakis G, Papantoniou N, Michalas S. Fetal blood sampling--indication-related losses. *Prenat Diagn* 1998; **18**: 934–940.
67. Liao C, Wei J, Li Q, Li L, Li J, Li D. Efficacy and safety of cordocentesis for prenatal diagnosis. *Int Gynecol Obstet* 2006; **93**: 13–17.
68. Royal College of Obstetricians and Gynaecologists. The use of Anti-D Immunoglobulin for Rhesus D Prophylaxis. Green-top Guideline No. 22. London: RCOG Press, March 2011. http://obgyan2015.org/wp-content/uploads/2015/11/Rh-negative-and-AntiD.pdf.
69. Tabor A, Jerne D, Bock JE. Incidence of rhesus immunisation after genetic amniocentesis. *Br Med J (Clin Res Ed)* 1986; **293**: 533–536.
70. Murray JC, Karp LE, Williamson RA, Cheng EY, Luthy DA. Rh isoimmunization related to amniocentesis. *Am J Med Genet* 1983; **16**: 527–534.
71. Henrion R, Papa F, Rouvillois JL, Henrion-Géant E. [Early amniocentesis, 1061 punctures and 1000 pregnancies]. *J Gynecol Obstet Biol Reprod (Paris)* 1979; **8**: 603–611.
72. Brandenburg H, Jahoda MG, Pijpers L, Wladimiroff JW. Rhesus sensitization after midtrimester genetic amniocentesis. *Am J Med Genet* 1989; **32**: 225–226.
73. Gagnon A, Davies G, Wilson RD; Genetics Committee, Wilson RD, Audibert F, Brock JA, Campagnolo C, Carroll J, Chitaya DT, Gagnon A, Johnson JA, MacDonald W, Murphy-Kaulbeck L, Okun N, Pastuck M; Executive and Council of the Society of Obstetricians and Gynecologists of Canada. Prenatal invasive procedures in women with hepatitis B, hepatitis C, and/or human immunodeficiency virus infections. *J Obstet Gynaecol Can* 2014; **36**: 648–655.
74. Giorlandino C, Cignini P, Cini M, Brizzi C, Carcioppolo O, Milite V, Coco C, Gentili P, Mangiafico L, Mesoraca A, Bizzoco D, Gabrielli I, Mobili L. Antibiotic prophylaxis before second-trimester genetic amniocentesis (APGA): a single-centre open randomised controlled trial. *Prenat Diagn* 2009; **29**: 606–612.
75. Alfirevic Z, Pilu G. Antibiotic prophylaxis for amniocentesis. *Prenat Diagn* 2009; **29**: 1094.
76. Ferrazzi E. Antibiotic prophylaxis before second-trimester genetic amniocentesis. *Prenat Diagn* 2010; **30**: 188.
77. Hobbins JC, Pilu G, Abuhumad A, Alfirevic Z, Bahado-Singh RO, Benacerraf BR, Berkowitz RL, Cetin I, Copel JA, Eik-Nes S, Frusca T, Galan HL, Guaschino S, Mahoney MJ, Marsal K, Malinger G, Marconi AM, Martinelli P, Moore TR, Papageorghiou AT, Platt LD, Rizzo N, Tabor A, Thilaganathan B, Timor-Tritsch IE, Todros T, Yagel S. Antibiotic prophylaxis before amniocentesis. *Prenat Diagn* 2011; **31**: 1213–1214.
78. Gramellini D, Fieni S, Casilla G, Raboni S, Nardelli GB. Mid-trimester amniocentesis and antibiotic prophylaxis. *Prenat Diagn* 2007; **27**: 956–959.
79. Mujezinovic F, Alfirevic Z. Technique modifications for reducing the risks from amniocentesis or chorionic villus sampling. *Cochrane Database Syst Rev* 2012; **8**: CD008678.
80. Mujezinovic F, Alfirevic Z. Analgesia for amniocentesis or chorionic villus sampling. *Cochrane Database Syst Rev* 2011; **11**: CD008580.
81. Technical and clinical assessment of fluorescence in situ hybridization: an ACMG/ASHG position statement. Technical considerations. American College of Medical Genetics. *Genet Med* 2000; **2**: 356–361.
82. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet al, Ledbetter DH, Shaffer LG, Jackson L. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med* 2012; **367**: 2175–2184.
83. Jansen FA, Blumenfeld YJ, Fisher A, Cobben JM, Odibo AO, Borrell A, Haak MC. Array comparative genomic hybridization and fetal congenital heart defects: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015; **45**: 27–35.
84. Grande M, Jansen FA, Blumenfeld YJ, Fisher A, Odibo AO, Haak MC, Borrell A. Genomic microarray in fetuses with increased nuchal translucency and normal karyotype: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015; **46**: 650–658.
85. Yi W, Pan CQ, Hao J, Hu Y, Liu M, Li L, Liang D. Risk of vertical transmission of hepatitis B after amniocentesis in HBs antigen-positive mothers. *J Hepatol* 2014; **60**: 523–529.
86. Towers CV, Asra T, Rumney P. The presence of hepatitis B surface antigen and deoxyribonucleic acid in amniotic fluid and cord blood. *Am J Obstet Gynecol* 1999; **184**: 1514–1518.
87. Grosheide PM, Quartero HW, Schalm SW, Heijtink RA, Christiaens GC. Early invasive prenatal diagnosis in HBsAg-positive women. *Prenat Diagn* 1994; **14**: 553–558.
88. Tess BH, Rodrigues LC, Newell ML, Dunn DT, Lago TD. Breastfeeding, genetic, obstetric and other risk factors associated with mother-to-child transmission of HIV-1 in Sao Paulo State, Brazil. Sao Paulo Collaborative Study for Vertical Transmission of HIV-1. *AIDS* 1998; **12**: 513–520.
89. Maiques V, García-Tejedor A, Perales A, Córdoba J, Esteban RJ. HIV detection in amniotic fluid samples. Amniocentesis can be performed in HIV pregnant women? *Eur J Obstet Gynecol Reprod Biol* 2003; **108**: 137–141.
90. Somigliana E, Bucceri AM, Tibaldi C, Alberico S, Ravizza M, Savasi V, Marini S, Matrone R, Pardi G; Italian Collaborative Study on HIV Infection in Pregnancy. Early invasive diagnostic techniques in pregnant women who are infected with the HIV: a multicenter case series. *Am J Obstet Gynecol* 2005; **193**: 437–442.
91. Ekoukou D, Khuong-Josses MA, Ghibaudo N, Mechali D, Rotten D. Amniocentesis in pregnant HIV-infected patients. Absence of mother-to-child viral transmission in a series of selected patients. *Eur J Obstet Gynecol Reprod Biol* 2008; **140**: 212–217.

92. Mandelbrot L, Jasseron C, Ekoukou D, Batallan A, Bongain A, Pannier E, Blanche S, Tubiana R, Rouzioux C, Warszawski J; ANRS French Perinatal Cohort (EPF). Amniocentesis and mother-to-child human immunodeficiency virus transmission in the Agence Nationale de Recherches sur le SIDA et les Hépatites Virales French Perinatal Cohort. *Am J Obstet Gynecol* 2009; **200**: 160.e1–9.
93. Shapiro DE, Sperling RS, Mandelbrot L, Britto P, Cunningham BE. Risk factors for perinatal human immunodeficiency virus transmission in patients receiving zidovudine prophylaxis. Pediatric AIDS Clinical Trials Group protocol 076 Study Group. *Obstet Gynecol* 1999; **94**: 897–908.
94. Millaire M, Bujold E, Morency AM, Gauthier RJ. Mid-trimester genetic amniocentesis in twin pregnancy and the risk of fetal loss. *J Obstet Gynaecol Can* 2006; **28**: 512–518.
95. Lenis-Cordoba N, Sánchez MÁ, Bello-Muñoz JC, Sagalá-Martinez J, Campos N, Carreras-Moratonas E, Cabero-Roura L. Amniocentesis and the risk of second trimester fetal loss in twin pregnancies: results from a prospective observational study. *J Matern Fetal Neonatal Med* 2013; **26**: 1537–1541.
96. Cahill AG, Macones GA, Stamilio DM, Dicke JM, Crane JP, Odibo AO. Pregnancy loss rate after mid-trimester amniocentesis in twin pregnancies. *Am J Obstet Gynecol* 2009; **200**: 257.e1–6.
97. Agarwal K, Alfirevic Z. Pregnancy loss after chorionic villus sampling and genetic amniocentesis in twin pregnancies: a systematic review. *Ultrasound Obstet Gynecol* 2012; **40**: 128–134.
98. Wapner RJ, Johnson A, Davis G, Urban A, Morgan P, Jackson L. Prenatal diagnosis in twin gestations: a comparison between second-trimester amniocentesis and first-trimester chorionic villus sampling. *Obstet Gynecol* 1993; **82**: 49–56.
99. Simonazzi G, Curti A, Farina A, Pilu G, Bovicelli L, Rizzo N. Amniocentesis and chorionic villus sampling in twin gestations: which is the best sampling technique? *Am J Obstet Gynecol* 2010; **202**: 365.e1–5.
100. Pergament E, Schulman JD, Copeland K, Fine B, Black SH, Ginsberg NA, Frederiksen MC, Carpenter RJ. The risk and efficacy of chorionic villus sampling in multiple gestations. *Prenat Diagn* 1992; **12**: 377–384.
101. Audibert F, Gagnon A; Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada; Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists. Prenatal screening for and diagnosis of aneuploidy in twin pregnancies. *J Obstet Gynaecol Can* 2011; **33**: 754–767.
102. Kidd SA, Lancaster PA, Anderson JC, Boogert A, Fisher CC, Robertson R, Wass DM. A cohort study of pregnancy outcome after amniocentesis in twin pregnancy. *Paediatr Perinat Epidemiol* 1997; **11**: 200–213.
103. McFadyen I. The dangers of intra-amniotic methylene blue. *Br J Obstet Gynaecol* 1992; **99**: 89–90.
104. Weisz B, Rodeck C. Invasive diagnostic procedures in twin pregnancies. *Prenat Diagn* 2005; **25**: 751–758.
105. Butwick AJ, Carvalho B. Anticoagulant and antithrombotic drugs in pregnancy: what are the anesthetic implications for labor and cesarean delivery? *J Perinatol* 2011; **31**: 73–84.
106. Patel IJ, Davidson JC, Nikolic B, Salazar GM, Schwartzberg MS, Walker TG, Saad WA; Standards of Practice Committee, with Cardiovascular and Interventional Radiological Society of Europe (CIRSE) Endorsement. Consensus guidelines for periprocedural management of coagulation status and hemostasis risk in percutaneous image-guided interventions. *J Vasc Interv Radiol* 2012; **23**: 727–736.

APPENDIX 1 Grades of recommendations and levels of evidence used in these guidelines

Classification of evidence levels	
1++	High-quality meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with very low risk of bias
1+	Well-conducted meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with low risk of bias
1−	Meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with high risk of bias
2++	High-quality systematic reviews of case–control or cohort studies or high-quality case–control or cohort studies with very low risk of confounding, bias or chance and high probability that the relationship is causal
2+	Well-conducted case–control or cohort studies with low risk of confounding, bias or chance and moderate probability that the relationship is causal
2−	Case–control or cohort studies with high risk of confounding, bias or chance and significant risk that the relationship is not causal
3	Non-analytical studies, e.g. case reports, case series
4	Expert opinion
Grades of recommendations	
A	At least one meta-analysis, systematic review or randomized controlled trial rated as 1++ and applicable directly to the target population; or a systematic review of randomized controlled trials or a body of evidence consisting principally of studies rated as 1+ applicable directly to the target population and demonstrating overall consistency of results
B	Body of evidence including studies rated as 2++ applicable directly to the target population and demonstrating overall consistency of results; or extrapolated evidence from studies rated as 1++ or 1+
C	Body of evidence including studies rated as 2+ applicable directly to the target population and demonstrating overall consistency of results; or extrapolated evidence from studies rated as 2++
D	Evidence level 3 or 4; or evidence extrapolated from studies rated as 2+
Good practice point	Recommended best practice based on the clinical experience of the guideline development group