Royal College of Obstetricians and Gynaecologists

Setting standards to improve women's health

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AMNIOCENTESIS AND CHORIONIC VILLUS SAMPLING

This is the third edition of this guideline, which was previously published in October 1996 and February 2000.

1. Aim

It is estimated that around 5% of pregnant population (approximately 30 000 women per annum in the UK) could be offered a choice of invasive prenatal diagnostic tests (amniocentesis or chorionic villus sampling). The aim of this guideline is to ensure that the timing and techniques for these procedures do not vary significantly between practitioners and healthcare settings, thereby minimising associated risks.

The guideline will provide up-to-date information, based on clinical evidence, rates of miscarriage associated with the procedures, optimal techniques and timing, training and competence and clinical governance issues.

2. Introduction and background

Amniocentesis is the most common invasive prenatal diagnostic procedure undertaken in the UK. Most amniocenteses are performed to obtain amniotic fluid for karyotyping and the majority are undertaken from 15 completed weeks (15+0) onwards. Amniocentesis performed before 15 completed weeks of gestation is referred to as 'early.'

Chorionic villus sampling (CVS) is usually performed between 10 and 13 weeks of gestation and involves aspiration of placental tissue rather than amniotic fluid. CVS can be performed using either percutaneous transabdominal or the transcervical approach. Transabdominal CVS can be performed at gestations greater than 13 weeks.

3. Identification and assessment of evidence

The Cochrane Database of Systematic Reviews and the Cochrane Register of Controlled Trials were searched for relevant randomised controlled trials (RCTs), systematic reviews and meta-analyses. A search of Medline and PubMed from 1966 to 2003 was also carried out.

The databases were searched using the relevant MeSH terms, including all subheadings. This was combined with a keyword search using 'amniocentesis', 'chorionic villi sampling', 'standards' and 'adverse effects'.

The definitions of the types of evidence used in this guideline originate from the US Agency for Health Care Research and Quality. Where possible, recommendations are based on and explicitly linked to the evidence that supports them. Areas lacking evidence are highlighted and annotated as 'good practice points.'

4. Rates of miscarriage

The rate of miscarriage associated with amniocentesis is approximately 1%.

A

The best estimate of a miscarriage risk associated with amniocentesis comes from a randomised trial from Denmark reported in 1986.[1] This study randomised 4606 low-risk women aged 24–35 years to have or not to have an amniocentesis, which was carried out using a 20-gauge needle under real-time ultrasound guidance. Most procedures were performed at between 16 and 18 weeks of gestation. The amniocentesis group had a loss rate which exceeded the control group by 1%, a figure which is often quoted in counselling. More than 50% of the amniocenteses were performed by one operator and the rest by four who were somewhat less experienced. The placenta was avoided whenever possible but was perforated in 15% of cases. Bloody fluid was obtained in only 0.5% of cases overall.

Evidence level Ib

Several more recent large uncontrolled series suggested that procedure-related loss rates around 0.5% can be achieved.[2,3] There is debate, however, on what constitutes a procedure-related loss, particularly relative to the time interval after the procedure. Practitioners should be aware of these issues and, if quoting lower loss rates than 1%, should be aware of the adequacy of their own follow-up data.

The rate of miscarriage following CVS is higher than after second trimester amniocentesis.

A

Unfortunately, there are no studies that compare CVS with no testing. Randomised trials comparing CVS by any route with second trimester amniocentesis showed an excess pregnancy loss of 3%.[4] However, only one randomised trial compared transabdominal CVS with second-trimester amniocentesis and found similar total pregnancy loss (6.3% versus 7%).[5] As already mentioned above, recent uncontrolled series report significantly lower 'procedure-related losses'.[3,6] However, the potential for bias is considerable in all these studies and these data have limited value for counselling purposes in different settings.

Several randomised trial studies show almost identical miscarriage rates after transcervical CVS compared with the transabdominal approach.[7,8] Only the trial from Denmark[5] found the transabdominal approach to be significantly safer but operator experience in the two techniques differed. Interestingly, meta-analysis comparing transcervical CVS with second-trimester amniocentesis showed amniocentesis to be significantly safer (excess miscarriage rate of 3% associated with CVS).[4]

Evidence level Ia

5. Timing of amniocentesis and CVS

Early amniocentesis performed before 14 completed weeks of gestation (14+0) is not a safe alternative to second-trimester amniocentesis or CVS.

A

One randomised study compared transabdominal chorionic villus sampling to early amniocentesis and suggested that loss rates might be higher in the latter.[9] A large prospective randomised Canadian study has recently reported a significantly greater loss in the early amniocentesis cases compared with the 'late' ones (7.6% versus 5.9%).[10] An additional feature was a virtual ten-fold increase in the incidence of fetal talipes in the early amniocentesis group.

It is recommended that an early amniocentesis is undertaken only in exceptional circumstances after the mother has been made fully aware of the potential complications.

Evidence level Ia/Ib

It is recommended that CVS should not be performed before 10 completed weeks of gestation (10+0). **B**

The association between CVS, oromandibular limb hypoplasia and isolated limb disruption defects has been debated since the issue was first raised in 1991 when a cluster of five babies with limb reduction defects were reported among a series of 289 women undergoing transabdominal CVS between 8 and 9+3 weeks.[11] A subsequent analysis showed no difference in the rate of this abnormality compared with the population incidence, although the vast majority of procedures were performed after 10 weeks of gestation.[12] Although a few publications subsequently appeared to support this association, most found few, if any, cases of oromandibular-limb hypogenesis syndrome and an incidence of limb reduction defects no higher than the usual background difference of one in 2000. Despite reassuring reports, most units stopped performing CVS before 10 weeks of gestation and thus most subsequent analyses include later procedures. It remains possible, even probable, that early CVS before 9 weeks might cause limb and other defects by transient fetal hypoperfusion and vasospastic phenomena secondary to vascular disruption to the placental circulation.

Evidence level III

6. Consent

It is good clinical practice to obtain formal consent for amniocentesis or CVS before the procedure. Practice should conform to recommendations on consent from the General Medical Council and the RCOG. Use of the Department of Health consent form 3 is recommended.

Written or oral information should include: what results are possible from the procedure, how, when and by whom it is performed and how their practice is monitored. Information should also be given on:

- national and local risks of the procedures
- analysis (and subsequent storage) of the sample in the local cytogenetics laboratory
- accuracy of the particular laboratory test being performed
- culture failure rates
- reporting time
- method of communication of results
- indications for seeking medical advice following the test.

Where written material is not used, the counselling process, including verbal consent, needs to be clearly recorded in the patient's notes.

7. Method

Amniocentesis is associated with higher rates of successful taps and lower rates of 'bloody' taps when performed under direct ultrasound control with continuous needle tip visualisation. **B**

The method of amniocentesis used has been described variously in the literature. Normally, a 'blind' procedure involves palpating the outline of the uterus and inserting a needle into a selected spot. With 'ultrasound guidance', the contents of the uterus, particularly the position of the placenta, are visualised prior to amniocentesis and a suitable point on the mother's abdomen marked. The ultrasound probe is then removed from the abdomen and the needle inserted through the mark. Although this technique allows the operator to assess the feasibility of the amniocentesis, it does not ensure the safety of the fetus. In contrast, the use of real-time equipment allows the insertion of the needle under 'continuous ultrasound control.'

Evidence level III

Improvements were evident by undertaking amniocentesis under 'continuous ultrasound control' compared with 'ultrasound guidance'. Continuous visualisation of the amniocentesis needle by ultrasound reduced blood-staining from 2.4% to 0.8%.[13] Similar evidence is drawn from two

overseas studies.[14,15] Although the studies used historical controls, the trend of improved outcome, reduced blood-staining of the amniotic fluid and greater success in obtaining fluid, seems clear. There are also case reports documenting serious fetal trauma caused by an amniocentesis needle, although continuous ultrasound guidance undoubtedly minimises such a risk.[16] Continuous guidance is more likely to avoid bowel injury at needle insertion. Much larger studies would be needed to show clinically and statistically significant reduction in rare complications including fetal trauma but the opportunity for a randomised controlled trial using 'blind' or 'guided' techniques as a control has long passed. The current recommendation for 'continuous ultrasound control' rests on the need to avoid 'bloody taps,' because the presence of blood interferes with amniocyte culture.

Evidence level III

Best practice is that ultrasound scanning during the procedure should be performed by the person inserting the needle. An alternative technique involves ultrasound scanning being performed by a separate practitioner. Whatever the individual's views, there is no objective evidence favouring one technique over the other.

A transplacental approach may be appropriate if it provides easy access to a pool of amniotic fluid but care should be taken to avoid the cord insertion.

B

Although traditionally amniocentesis techniques have been employed that avoid the placenta, recent evidence suggests that penetration may not be associated with increased complications where continuous ultrasound guidance is used. Tabor *et al.*[1] suggested an increased miscarriage rate following placental puncture. However, three large studies[17–19] involving over 2000 cases have not demonstrated any increase in miscarriage rates where the transplacental approach has been used. Unfortunately, needle size is only mentioned in one of the studies.[17] In fact, if a clear pool of amniotic fluid can be reached **only** by passage through the placenta then this is the approach of choice. All authors have emphasised the need to place the needle through the thinnest available part of the placenta.

Evidence level IIb

The outer needle diameter should not be wider than 20-gauge (0.9 mm).

B

Needle thickness is likely to be important but there are few clinical data to guide the choice. The study of Tabor *et al.*[1] used a 20-gauge needle (note that in the original report the size of the needle was reported as 18-gauge by mistake). One experimental model comparing 18-, 20- and 22-gauge needles[20] suggested that there was less amniotic fluid flow from the puncture site with smaller gauge needles.

Evidence level IIb

Some experts recommend particular angles of access[21] but the data are not robust enough to guide practice.

Evidence level III

Amniocentesis generates considerable anxiety but most women rate the pain as equivalent to that of venepuncture.[22] A randomised trial by van Schonbrock *et al.* showed that injection of local anaesthetic did not reduce pain scores reported by women undergoing amniocentesis.[22]

Evidence level Ib

CVS should always be performed under direct ultrasound control.

B

There is a consensus that CVS, both transabdominal and transcervical, has to be performed under continuous ultrasound control. Techniques for transabdominal CVS vary significantly both in the size of the needle used (e.g. 18-gauge, 20-gauge, double needle 17/19-gauge, double needle 18/21-gauge) and method of aspiration (e.g. negative pressure by syringe, negative pressure by vacuum aspirator, biopsy forceps). As there are no published studies comparing clinical outcomes using different techniques, clinicians are advised to use the technique with which they are familiar. The

Evidence level IV

same applies to transcervical CVS: although there is some evidence to support use of small forceps as opposed to aspiration cannulae, the evidence is not strong enough to support change in practice for clinicians familiar with aspiration cannulae.[23]

Evidence level IV

8. Skill of the operator

Very experienced operators performing amniocentesis may have a higher success rate and a lower procedure-related loss rate. Occasional operators who perform amniocentesis less than ten times per annum may have increased rates of procedure-related loss.

B

Operator experience, as well as technique, may be important. In a study in which the majority of amniocenteses were undertaken by a single operator, results were compared with those of an occasional operator. With the former, success at the first attempt occurred in 94% of amniocenteses, with 3% of bloody taps, compared with the latter in which 69% were successful, with bloody taps rate of 16%.[24] Although other authors have commented on the significance of operator experience in terms of reduced needle insertions and fewer bloody taps,[14] these studies generally span many years and include change from 'ultrasound-guided' procedures to 'continuous ultrasound control'. Early studies using static ultrasound images suggested that practitioners carrying out more than 50 procedures per annum had a loss rate of 0.3% compared with those carrying out less than ten, with loss rates of 3.7%.[25] A recent report demonstrated that those doctors undertaking less than 50 procedures in the study period (36 months) had a single pass success rate of 82% compared with those with greater than 50 procedures of 93%.[26] However, the inexperienced practitioners used continuous ultrasound control less frequently and data analysis did not take this into account. Studies comparing very experienced practitioners (more than 100 procedures per annum) with much less experienced practitioners have shown substantial differences in outcome, with six- to eight-fold increase in loss rates by the less experienced doctors.[27,28] Other authors who compared outcomes where the difference in experience was less marked were unable to demonstrate differences in any parameter.[29]

Evidence level III

Interestingly, a large Medical Research Council (MRC) trial found no clear evidence that, over the course of the trial (4 years) increased operator experience improved safety of chorionic sampling.[30] However, each operator was required to perform at least 30 procedures before participation.

The practitioner carrying out ultrasound as a part of the amniocentesis or CVS procedure should be trained to the competencies of RCOG/RCR Diploma in Obstetric Ultrasound level or equivalent.

✓

Adequate training and maintenance of skills are of crucial importance. Ultrasound skills for performing invasive prenatal procedures are greater than those required for the completion of the RCOG specialist training logbook. Specific training should include ultrasound training beyond this level. Best practice would require ultrasound training at the level of the current RCR/RCOG Diploma in Obstetric Ultrasound or equivalent.

Independent performance of amniocentesis and CVS should only occur following adequate training, which should include the use of a clinical skills model, assessment of interaction with patients and supervised procedures.

B

Before undertaking procedures on women, consideration should be given to initial training using a clinical skills model. Several good models have been constructed and some of these validated.[31] Pittini *et al*. used a well-validated educational approach that included examination of patient interactive skills.[32] They demonstrated improved performance amongst all levels of trainees but particularly amongst those with least experience prior to the training, suggesting an ability to

Evidence level III

shorten the learning curve. Nizard *et al*. have concentrated on the technical aspects such as proportion of time that the needle is visualised during a procedure.[33] This group suggested that between 50 and 100 procedures under such conditions are required before there is no further improvement.

Evidence level III

Postgraduate training is moving to competence-based assessments rather than adherence to a particular numerical goal. No data exist on the number of supervised prenatal invasive procedures necessary before competence is gained. Both procedures are practical skills and trainees will gain competence at different rates. As there are no data to guide practice, individual centres should agree a training and assessment process that is open and transparent, and with a clearly responsible trainer. Local deaneries and NHS trust clinical governance systems should have a role in ensuring quality training. It is suggested that trainers should be performing at least 50 ultrasound-guided invasive procedures per annum.

At present, therefore, it not possible to make evidence based recommendations on the number of procedures that should be carried out annually to maintain competency. An arbitrary number of at least ten procedures per annum seems reasonable. However, further research is needed to clarify whether adequately-trained operators should be deemed unsafe just because they failed to reach a certain threshold in the number of performed procedures.

Audit of practice is the best way to assess competency. ✓

Competence is best assessed through continuous audit of complications such as 'need for second insertion' and 'miscarriage rate'. The 95% confidence intervals for complications from experienced operators[1,2] indicate that 'second insertion' is permissible in at most four of 50 consecutive amniocenteses or seven in 100 consecutive cases. Miscarriage rate should not exceed three in 50 or four in 100 amniocentesis. Higher number of complications may be an unfortunate 'cluster' or consequence of high background risk of miscarriage. Nevertheless, an independent review of the operator's skills should be carried out to provide reassurance to patients and the operator concerned.

Comparable numbers for CVS are higher, because of the higher background risk of miscarriage. Also, CVS is often performed in the presence of increased nuchal translucency, cystic hygroma or genetic conditions, most of which are associated with a higher miscarriage rate. The Cochrane review quotes CVS sampling failure between 2.5% and 4.8% and spontaneous miscarriage rate between 3% after transabdominal CVS in the Danish trial[5] and 9% in the MRC Trial[30] to nearly 20% in some studies.[4] If one accepts 3% sampling failure and 3% pregnancy loss as the 'gold standard', an external audit of practice should be carried out when either five sampling failures **or** five miscarriages occur in 50 consecutive cases (eight in 100 consecutive cases). Obviously, any external audit should take into account the background risk of miscarriage, which is likely to be significantly higher in the presence of fetal anomaly or abnormal karyotype. All organisations where prenatal invasive procedures are carried out should have robust mechanisms for collecting and monitoring these data. The mechanisms of review should be agreed locally, though national or regional guidance should be developed.

When difficulties with amniocentesis are anticipated, a further opinion should be sought from a more experienced operator. ✓

It is crucially important that amniocentesis is not performed until the operator is certain that the fetus and cord are clear of the intended pool of amniotic fluid. When difficulties are anticipated, consideration should be given to referring the patient to a more experienced operator.

For either procedure, a more experienced operator should be consulted if two attempts at uterine insertion have failed to produce an adequate sample for analysis.

9. Multiple pregnancies

Amniocentesis and CVS in multiple pregnancies should be performed only by a specialist who has the expertise to carry out selective termination of pregnancy. ✓

A high level of expertise in ultrasound scanning is essential for operators undertaking amniocentesis or CVS in multiple pregnancies because uterine contents have to be 'mapped' with great care. This is essential to ensure that separate samples are taken for each fetus and clearly labelled as such. Labelling is greatly assisted by the presence of obvious fetal abnormality (e.g. hydrocephalus, heart defect) or discordant fetal gender. However, to minimise the risk of chromosomal abnormality being assigned to the wrong twin, invasive procedures in multiple pregnancy should only be performed by a specialist who is able to proceed to selective termination of pregnancy.

Most clinicians tend to use two separate puncture sites when performing amniocentesis or CVS in multiple pregnancies,[34] although there are series using single-entry techniques with low rates of complications. Miscarriage rate is, therefore, likely to be somewhat higher than in singleton pregnancies.[35] However, currently available evidence does not allow accurate estimates of excess risks.

The role of CVS in dichorionic placentae remains controversial because of a relatively high risk of cross-contamination of chorionic tissue leading to false positive or false negative.[36] Such procedures should be performed only in exceptional circumstances after detailed counselling.

10. Third trimester amniocentesis

Third-trimester amniocentesis does not appear to be associated with significant risk of emergency delivery. Compared with mid-trimester procedures, complications including multiple attempts and bloodstained fluid are more common. B

Amniocentesis in the third trimester is carried out for a number of indications. These include late karyotyping, amniotic fluid optical density assessments for rhesus disease, amniotic fluid insulin measurements, lung maturity studies and detection of indices of infection in suspected preterm labour or rupture of the membranes. Much of the literature on the risks of late amniocentesis predates the use of continuous ultrasound-guided amniocentesis. More recent series report more than one attempt in over 5% of samplings[37,38] and bloodstained fluid in 5–10% of cases. When amniocentesis is carried out in the presence of preterm premature rupture of membranes, failure rates are higher.[39] Serious complications are rare. Two series with 194 and 562 procedures did not have any emergency deliveries as a result of amniocentesis,[37,40] whilst Stark *et al*.[38] suggested a rate of 0.7% for procedure-related delivery.

Evidence level III

11. Control of infection

Unless well-audited processes for probe decontamination and ultrasound gel microbiological surveillance are in place, best practice is to enclose the probe in a sterile bag during any invasive prenatal procedure and to use separate sterile gel. ✓

Severe sepsis, including maternal death, has been reported following invasive prenatal procedures. The level of risk cannot be quantified as case report literature on this does not provide denominator information but the risk of severe sepsis is likely to be less than one in 1000 procedures. Infection can be caused by inadvertent puncture of the bowel, skin contaminants or organisms present on the ultrasound probe or gel. The first two sources should be avoidable by standard practices. Decontamination of ultrasound probes between patients is variable[41] and there are practical difficulties in balancing the need for cleaning with

prevention of degradation of the probe. Some centres encase the probe in a sterile bag. Ultrasound gel has been shown to contain organisms and many departments have mechanisms to minimise the risks including the use of sterile ultrasound gel when performing invasive procedures. Standards for control of infection should conform to those for any invasive diagnostic radiological procedure.

Invasive prenatal testing in the first or second trimester can be carried out in women who carry hepatitis B or C. The limitations of the available data should be explained. Testing in women with HIV should be avoided, particularly in the third trimester. **B**

Blood-borne viruses constitute both an infection-control risk and a possible risk factor for maternal–fetal transmission. For hepatitis B, individual studies are small[42] but show no evidence of a transmission risk. A recent review[43] concluded that the risk of transmission was very low. It has been suggested that 'e' antigen status may be important. There are fewer data on transmission of hepatitis C[44] but, to date, there is no evidence that transmission is increased following amniocentesis.[43] Evidence level III

Most studies examining HIV suggest that invasive testing may be a risk factor in transmission[45,46] and recommend avoidance. The data are most robust for third-trimester procedures[46] where the relative risk is 4. Some have suggested that testing earlier in pregnancy is safe provided that retroviral therapy is being used and the maternal viral load is low.[47]

Rhesus status should be available or obtained in every case. Rhesus prophylaxis with anti-D immunoglobulin must be offered following each procedure in line with national recommendations.[48] Evidence level Ia

12. Organisation of care

The scope of this guideline is confined to technical aspects of the two procedures. For the woman and her family, good care in these circumstances encompasses more than the simple performance of a technique.

Trusts and organisations should ensure that the equipment, environment, staff training, arrangements for follow up, and links with related services carrying out pregnancy termination or support for women with diagnosed chromosomal or genetic disease are of sufficient standard and that these aspects of care are continuously reviewed.

13. Auditable standards

- Rate of pregnancy loss at any gestation after procedure.
- Proportion of procedures requiring multiple insertions.
- Proportion of procedures with failure to obtain an adequate sample.
- Complication rates ('bloody' tap, amniotic fluid leakage).
- It is suggested that each department should maintain a register of invasive diagnostic procedures to facilitate audit.

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APPENDIX

Clinical guidelines are: 'systematically developed statements which assist clinicians and patients in making decisions about appropriate treatment for specific conditions'. Each guideline is systematically developed using a standardised methodology. Exact details of this process can be found in Clinical Governance Advice No. 1: *Guidance for the Development of RCOG Green-top Guidelines* (available on the RCOG website at www.rcog.org.uk/clingov1). These recommendations are not intended to dictate an exclusive course of management or treatment. They must be evaluated with reference to individual patient needs, resources and limitations unique to the institution and variations in local populations. It is hoped that this process of local ownership will help to incorporate these guidelines into routine practice. Attention is drawn to areas of clinical uncertainty where further research may be indicated.

The evidence used in this guideline was graded using the scheme below and the recommendations formulated in a similar fashion with a standardised grading scheme.

Classification of evidence levels

Ia	Evidence obtained from meta-analysis of randomised controlled trials.
Ib	Evidence obtained from at least one randomised controlled trial.
IIa	Evidence obtained from at least one well-designed controlled study without randomisation.
IIb	Evidence obtained from at least one other type of well-designed quasi-experimental study.
III	Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case studies.
IV	Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities.

Grades of recommendations

A	Requires at least one randomised controlled trial as part of a body of literature of overall good quality and consistency addressing the specific recommendation. (Evidence levels Ia, Ib)
B	Requires the availability of well controlled clinical studies but no randomised clinical trials on the topic of recommendations. (Evidence levels IIa, IIb, III)
C	Requires evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality. (Evidence level IV)

Good practice point

✓	Recommended best practice based on the clinical experience of the guideline development group.

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The final version is the responsibility of the Guidelines and Audit Committee of the RCOG.

The guideline review process will commence in January 2008
unless evidence requires earlier review