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Guidelines for Amniocentesis and Chorionic Villus Sampling (CVS)

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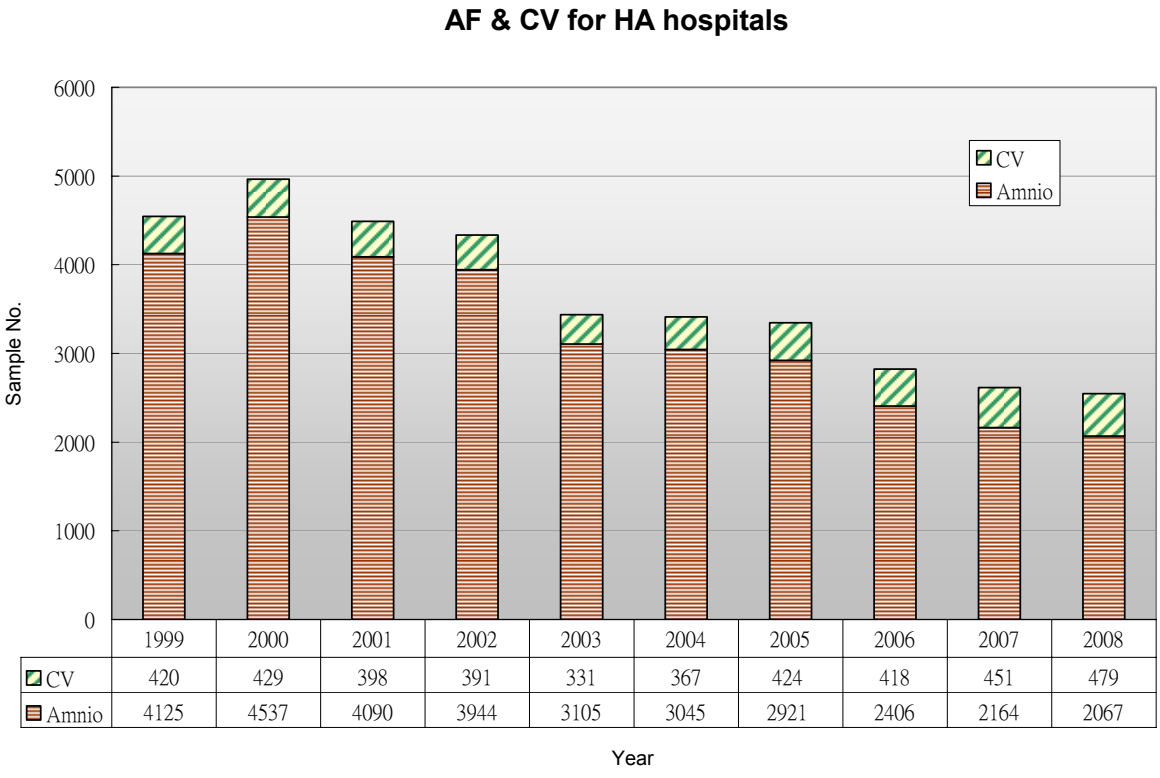
1 INTRODUCTION

Amniocentesis, followed by chorionic villus sampling (CVS), are the two most common invasive prenatal diagnostic procedures performed in Hong Kong.

The no. of amniocenteses & CVS performed annually from 1999 to 2008 in all Hospital Authority (HA) hospitals is shown in Figure 1 (data from Mrs. Wu Chung Prenatal Diagnostic Laboratory, Tsan Yuk Hospital). The no. of amniocentesis performed is decreasing in recent years while the no. of CVS remains stable.



	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
CV	420	429	398	391	331	367	424	418	451	479
Amnio	4125	4537	4090	3944	3105	3045	2921	2406	2164	2067

Figure 1

2 COMMON INDICATIONS

2.1 For detection of chromosomal abnormalities:

2.1.1 Positive Down screening test (1st trimester / 2nd trimester / integrated / sequential / contingent)

2.1.2 At risk of chromosomal abnormalities because of the presence of ultrasound markers or fetal structural abnormalities

2.1.3 Previous babies with chromosomal abnormalities, e.g. trisomy 21, trisomy 18 and trisomy 13

2.1.4 Couples known to be carriers of chromosomal translocations or other structural chromosomal abnormalities

2.1.5 Advanced maternal age - the commonly used cut-off is 35 years or above at expected date of confinement. This is becoming less commonly used as a sole indication for amniocentesis or CVS without Down screening test

2.2 For detection of non-chromosomal genetic diseases:

Couples known to be at risk of having fetuses with major genetic diseases, e.g. thalassaemias, haemophilias, inborn errors of metabolism, diagnosable by molecular or biochemical study of amniotic fluid cells or chorionic villi.

3 PROCEDURES

3.1 Timing

Genetic amniocentesis is usually performed at 15-20 weeks of gestation. Early amniocentesis before 14 weeks of gestation should preferably not be performed since it has been shown to be associated with a higher incidence of spontaneous abortion and neonatal talipes (4.4% and 1.8%) compared with CVS (2.3% and 0.2%)[1]. The Canadian Early and Midtrimester Amniocentesis trial (CEMAT) has shown a significantly greater loss in the early amniocentesis cases compared with the midtrimester ones (7.6% versus 5.9%) with a ten-fold increase in the incidence of fetal talipes in the early amniocentesis group[2].

Occasionally, amniocentesis is performed in the third trimester for late karyotyping usually in the presence of ultrasound fetal anomaly. Third-trimester amniocentesis does not appear to be associated with significant risk (0.7%) of emergency delivery[3]. Compared with mid-trimester procedures, complications including multiple attempts (5%) and bloodstained fluid (5-10%) are more common[3,4].

CVS is usually performed between 10 and 14 weeks of gestation and therefore provides an earlier diagnosis. It is recommended that CVS should not be performed before 10 completed weeks of gestation because of the risk of limb and other defects by transient fetal hypoperfusion and vasosplastic phenomena secondary to vascular disruption to the placental circulation[5].

3.2 Ultrasound Assistance

3.2.1 Types

Amniocentesis is usually performed under some form of ultrasound assistance transabdominally, either by "ultrasound guidance" or by "direct ultrasound control". In "ultrasound guidance", the operator determines the site of needle insertion using ultrasound, while the process of needle insertion is not under direct ultrasound visualization. This is seldom practised nowadays. In contrast, continuous visualization of the needle during the whole process of insertion is required in "direct ultrasound control". The

practice of amniocentesis without any form of ultrasound assistance should be abandoned.

CVS, both transabdominal and transcervical, should always be performed under "direct ultrasound control". In Hong Kong, CVS is usually performed transabdominally.

3.2.2 Advantages of ultrasound assistance

The use of ultrasound in amniocentesis significantly reduces the number of failed procedures (dry tap) and blood-stained samples, and enables the avoidance of the placenta and fetal parts[6]. "Direct ultrasound control" is preferred and should be the standard practice because when compared with "ultrasound guidance", there are fewer dry taps, blood-stained samples and multiple needle insertions[7,8]. "Direct ultrasound control" is also more likely to avoid bowel injury at needle insertion.

3.3 Needles

For amniocentesis, the needle diameter should not be wider than 20-gauge (0.9mm)[5]. The classic study of amniocentesis by Tabor *et al* [9] used a 20-gauge needle. One experimental model supported the use of a 22-gauge needle for routine amniocentesis: the defect created by 22-gauge needle and the subsequent flow rate is relatively small, yet the time to aspirate an adequate volume is short[10].

For CVS, the size of the needle (e.g. 18-gauge, 20-gauge, double-needles 17/19-gauge, 18/21-gauge) and method of aspiration (single-needle, double-needle) vary. As there are no published studies comparing clinical outcomes using different techniques, clinicians are advised to use the technique with which they are familiar[11].

3.4 Placental Passage in amniocentesis

The placenta should preferably be avoided during amniocentesis. Passage through the placenta may be acceptable if it provides the only easy access to a pool of amniotic fluid, as increased miscarriage following amniocentesis with placental passage has not been observed [12,13].

3.5 Local anaesthesia

Amniocentesis generates considerable anxiety but most women rate the pain as equivalent to that of venepuncture[14]. A randomised trial showed that injection of local anaesthetic did not reduce pain scores reported by women undergoing amniocentesis[14].

On the other hand, local anaesthetic (e.g. Lignocaine) is recommended for CVS because the procedure is more painful and a larger gauge of needle is used.

4 COMPLICATIONS

4.1 Fetal Loss

Fetal loss is the most important complication after amniocentesis and CVS. Spontaneous fetal loss must be taken into account for the estimation of procedure-related complications.

The largest randomized trial involving 4,606 women showed that amniocentesis was associated with a 1% excess of fetal loss (1.7% after amniocentesis vs. 0.7% without amniocentesis)[9]. A 20 gauge needle was used in the majority of cases in that study[15]. Roper et al reported a fetal loss of 1.2% within 8 weeks after amniocenteses performed beyond 15 weeks' gestation[16]. There was no control group for comparison. Since a smaller needle, usually 22-gauge, is used in most centers, the risk of amniocentesis-related fetal loss may be lower than 1%.

There are no studies that compare CVS with no testing. No significant difference was found in fetal loss rate between transabdominal CVS and mid-trimester amniocentesis from the latest Cochrane Review[17]. Local studies have also demonstrated that the procedure-related fetal loss rate of transabdominal CVS (0.74% in 1355 procedures)[18] was similar to that of mid-trimester amniocentesis (0.86% in 3468 procedures)[19].

Because there is a wide range in the reported incidence of procedure-related fetal loss after amniocentesis and CVS, local figures of individual centers should be used if available. However, a reliable estimation of this risk would require hundreds of procedures. In units where such a caseload is not met, the standard figure from the medical literature could be used for counselling.

4.2 Fetal Trauma

Fetal trauma attributed to amniocentesis had been described in case reports[20] but is rare. It is likely that the use of "direct ultrasound control" will minimize the occurrence of this complication.

4.3 Maternal Trauma

Maternal trauma is rare during amniocentesis and CVS with direct ultrasound control & adequate training.

5 ALTERNATIVE – FETAL BLOOD SAMPLING

Fetal blood sampling (FBS) is the alternative invasive sampling method for fetal karyotyping. FBS requires more skill than amniocentesis and CVS. FBS is performed under "direct ultrasound control", using a 20-gauge needle (size of needle depends on gestation) to aspirate fetal blood, usually from the umbilical vein at the placental cord insertion. The advantage of FBS is the ability to obtain a full cytogenetic study within 3-5 days and therefore is particularly useful if a high-risk woman presents beyond 21 or 22 weeks of gestation. However, FBS is associated with a fetal loss rate of 1.4% even in experienced hands among low risk pregnancies (i.e. all pregnancies where pathological conditions have been excluded)[21]. Therefore FBS should only be considered when there is a clear clinical benefit. FBS is less frequently required after the availability of rapid aneuploidy testing such as QF-PCR which can give results within 2 days after amniocentesis.

6 LABORATORY STUDIES

6.1 Karyotyping

This traditional method for prenatal diagnosis involves analysis of banded metaphase chromosomes from cultured amniotic fluid cells or chorionic villi. All 23 pairs of chromosomes are examined. Apart from the common aneuploidies - trisomy 21, trisomy 18, trisomy 13, 45,X (Turner's syndrome) and 47,XXY (Klinefelter's syndrome), a wide range of chromosomal abnormalities can also be identified by this technique, including rearrangements, such as translocations and inversions that may be balanced or unbalanced. Karyotyping is labour-intensive and it may take up to 14 days or more to have the results.

6.2 Rapid aneuploidy testing (RAT)

Advances in molecular diagnostics, using either fluorescence in situ hybridisation (FISH) with chromosome specific DNA probes or quantitative fluorescence–polymerase chain reaction (QF-PCR) with chromosome-specific small tandem repeat markers, can be applied to diagnose the common aneuploidies within 1 to 2 days. The sensitivity and specificity of FISH and QF-PCR, collectively described as rapid aneuploidy testing (RAT), have been demonstrated in large scale studies[22,23,24], and compare favourably with traditional karyotyping for the diagnosis of the common aneuploidies. A local study found 0.3% amniotic fluid and 0.8% chorionic villus samples showed discrepant findings between QF-PCR and karyotyping[25].

Unlike karyotyping, these technologies only allow the identification of the chromosomal abnormalities that are specifically sought (targeted testing). Currently, RAT (FISH or QF-PCR) is being used to give a rapid result for the common aneuploidies as an adjunct to karyotyping. Decision to terminate the pregnancy can be based on abnormal RAT result while normal RAT results can relieve the anxiety of the couples much earlier than karyotyping[26].

6.3 Microarray based comparative genomic hybridization (array CGH)[27]

In contrast to RAT, array CGH is a comprehensive, high-resolution, genomewide screening strategy for detecting gains (duplication) or losses (deletion) of DNA segments in a single test. Compared with traditional karyotyping, it is rapid, less labour-intensive, and readily amendable to automation. It enables the detection of genomic changes too small to be resolved by karyotyping, such as microdeletions & microduplications. On the other hand, array CGH does not detect balanced translocations and triploidy. Array CGH is not ready for routine use yet due to the costs, but it is likely to become increasingly important. There will be a higher demand for genetic counselling on the wide range of genetic or syndromal abnormalities detected by array CGH.

	Karyotyping	QF-PCR	Array CGH (Fetal DNA Chip*)
DNA	Not applicable	Minimum 10ng	Minimum 1ug
Cell culture	Required	Not required	Not required
Disease coverage	Common aneuploidies & visual structural abnormalities	Restricted to Trisomy 21, 18, 13 and Turner's syndrome	Common aneuploidies & over 100 genetic disorders
Turnaround time	14-21 days	3 days	7 days
Resolution	5Mb	Targeted aneuploidy	100kb
Limitation	Cannot precisely delineate the gain or loss region or single gene diseases	Difficult to scale up to a comprehensive, genome-wide screening	Cannot detect balanced chromosomal rearrangements (translocation and inversion), triploidies, or single gene abnormalities

*Fetal DNA Chip v1.0, Prenatal Genetics Diagnosis Centre, Department of Obstetrics & Gynaecology, The Chinese University of Hong Kong

Table 1

7 COUNSELLING

7.1 Principle

All women should be counselled carefully before amniocentesis and CVS. The indication (Section 2), details of the procedure (Section 3) and complications (Section 4) should be explained clearly. The results generated from the study of the amniotic fluid or chorionic villi sample (karyotyping, RAT, array CGH), and the limitations of the results, should be communicated clearly to the women (Section 6). If fetal blood sampling as an alternative invasive sampling (Section 5) is offered, the relative advantages and disadvantages must be explained clearly in terms that the

woman will understand. Written consent should be obtained before the procedure.

7.2 Multiple Pregnancies

The counselling of amniocentesis and CVS is more complicated in women with multiple pregnancies. Additional issues include the need for multiple sampling, the possibility of sampling error, and management options in case one of the fetuses is abnormal. Such counselling should preferably be provided by those with extensive experience in prenatal diagnosis.

Either two separate puncture sites or single-entry techniques can be used when performing amniocentesis or CVS in multiple pregnancies[28]. Miscarriage rate is likely to be somewhat higher than in singleton pregnancies[29]. Currently available evidence does not allow accurate estimates of excess risks.

The role of CVS in dichorionic placentae remains controversial because of relatively high risk of cross contamination of chorionic tissue leading to false positive or false negative[30]. Such procedures should be performed only in exceptional circumstances after detailed counseling[5].

A detailed section on invasive prenatal diagnosis in multiple pregnancies is available in HKCOG Guidelines No. 11 – Management of Multiple Pregnancies: Part I (November 2006).

8 RECOMMENDATIONS

8.1 Amniocentesis and CVS should only be offered to women who are at high-risk of carrying a fetus with chromosomal or genetic disease (Section 2). Amniocentesis and CVS should not be routinely offered to all pregnant women, or for fetal sexing without a medical indication.

8.2 The indications, risks and alternative options should be adequately explained to all women, preferably with their partners. All women should be given adequate time to decide whether to proceed with amniocentesis and CVS or not. Written consent should be obtained before the procedure.

8.3 The results generated from analysis of the amniotic fluid or chorionic villi sample should be communicated to the women clearly, together with the limitations, as soon as possible.

8.4 Amniocentesis and CVS should only be performed by competent operators, or trainees under direct supervision by competent operators.

8.5 Training in amniocentesis and CVS should preferably include ultrasound training to detect fetal structural abnormalities, patient counselling and management of pregnancies with abnormal test results[31].

8.6 Amniocentesis and CVS should be performed under direct ultrasound control. CVS should preferably be performed between 10 to 14 weeks and amniocentesis between 15 and 20 weeks of gestation respectively. Early amniocentesis before 14 weeks of gestation should not be performed.

8.7 The Rhesus status must be known and Rhesus-negative women should receive anti-D immunoglobulin after amniocentesis and CVS.

8.8 Complication rates and outcome of pregnancies after amniocentesis and CVS should be audited.

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This guideline was produced by the Hong Kong College of Obstetricians and Gynaecologists as an educational aid and reference for obstetricians and gynaecologists practicing in Hong Kong. The guideline does not define a standard of care, nor is it intended to dictate an exclusive course of management. It presents recognized clinical methods and techniques for consideration by practitioners for incorporation into their practice. It is acknowledged that clinical management may vary and must always be responsive to the need of individual patients, resources, and limitations unique to the institution or type of practice. Particular attention is drawn to areas of clinical uncertainty where further research may be indicated.

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