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Consequences of Amniocentesis and Chorionic Villus Sampling for Prenatal Diagnosis

BY

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ABSTRACT

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Amniocentesis (AC) and chorionic villus sampling (CVS) are the principal methods for fetal karyotyping. The aim of this thesis was to evaluate psychological reactions and risks associated with the procedures.

A semi-randomised study was made on 321 women, where AC (147) and CVS (174) at 10-13 weeks' gestation were done trans-abdominally. Spontaneous fetal loss occurred in 6.8% and 1.7% of the women in the AC and CVS groups, respectively. Repeat testing was required more often in the AC (19.0%) than in the CVS (5.2%) group.

A subgroup of 94 women answered a questionnaire prior to the procedure. Anxiety was stated as reason for invasive testing in 38% of the women. Mean scores according to the Hospital Anxiety and Depression Scale for anxiety and depression were low. Likewise, mean scores for the Impact of Event Scale, evaluating the psychological distress evoked by the procedure, were low. Yet, a number of women had higher scores, indicating a risk of clinical anxiety and depression or psychological distress. The women worried most about miscarriage, fetal injury by the procedure and waiting for the result.

Fetal, infant and maternal outcomes were evaluated in a cohort of 71 586 women aged 35 to 49 years old, with single births in Sweden during 1991 to 1996. Altogether, 21 748 were exposed to AC and 1984 to CVS. Women exposed to AC and CVS were compared with non-exposed. Outcomes were extracted from the Swedish Medical Birth Register, the Swedish Hospital Discharge Register, and the Swedish Malformation Register. An increased risk of musculo-skeletal deformities, such as club foot (OR=1.45) and hip dislocation (OR=1.22), and respiratory disturbances such as neonatal pneumonia (OR=1.29), was found for infants born in the AC group. Risk increased with earlier gestation at the procedure. Fewer women in the AC group had a normal delivery and more had a Caesarean section. Complications related to the amniotic cavity and membranes (OR=1.15), hypotonic uterine dysfunction (OR=1.12) and instrumental vaginal deliveries (OR=1.11) were more common in the AC group. No significant differences were found for the CVS group.

CVS is the method of choice for prenatal karyotyping in the first trimester. AC should not be performed before 15 weeks' gestation. Further research to develop methods to better identify women at increased risk of chromosomal abnormal pregnancies and to develop non-invasive tests for prenatal diagnosis is needed. Thereby, the number of women exposed to invasive procedures and the adverse effects caused by these procedures can be minimised.

Key words: Prenatal diagnosis, malformations, lung, labor, amnion.

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To all women

PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Cederholm M., Axelsson O. A prospective comparative study on transabdominal chorionic villus sampling and amniocentesis performed at 10-13 week's gestation. *Prenatal diagnosis* 1997; 17(4): 311-317
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II. Cederholm M, Axelsson O, Sjödén P-O. Women's knowledge, concerns and psychological reactions before undergoing an invasive procedure for prenatal karyotyping. *Ultrasound Obstet Gynecol* 1999; 14: 267-272
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III. Cederholm M., Haglund B., Axelsson O. Infant morbidity following amniocentesis and chorionic villus sampling for prenatal karyotyping.
Submitted.

IV. Cederholm M., Haglund B., Axelsson O. Maternal complications following amniocentesis and chorionic villus sampling for prenatal karyotyping.
Submitted.

CONTENTS

ABBREVIATIONS AND DEFINITIONS

AC	Amniocentesis
BMI	Body mass index
CI	Confidence interval
CVS	Chorionic villus sampling
HADS	The Hospital Anxiety and Depression Scale
ICD	The International Classification of Diseases
IES	The Impact of Event Scale
PROM	Premature rupture of membranes
SGA	Small-for-Gestational-Age, defined as a birthweight more than two standard deviations below the mean for gestation and gender [56]
Late fetal death	Intrauterine fetal death occurring from gestational week 28 until birth
Neonatal death	Death occurring during the first 27 days of life
Postneonatal death	Death occurring from 28 days of life until the age of 12 months
Extremely preterm	Gestational length <196 days
Very preterm	Gestational length <224 days
Preterm	Gestational length <259 days

INTRODUCTION

Prenatal diagnosis

Invasive procedures for fetal diagnosis are performed mainly to obtain the fetal karyotype. AC and CVS have become the two principal methods for this purpose. By far the most common indication for AC and CVS is increased maternal age, which is associated with an elevated risk of fetal chromosomal abnormality [40, 85]. Other indications are a previous pregnancy or child with a chromosomal abnormality, a high anxiety level or perceived elevated risk [58, 72, 77, 81, 98]. In Sweden, about 6000 women a year choose to have AC or CVS and a majority of these women are more than 35 years of age.

Amniocentesis

Since the introduction of AC in the 1960s, AC has gained the most widespread acceptance and has become the 'golden standard' against which other methods are compared. AC is traditionally performed in the second trimester after 15 weeks' gestation. AC is performed trans-abdominally under ultrasound guidance with a needle size of 18 to 22 Gauge. From the sample of 15 to 20 ml of amniotic fluid, fetal cells are cultured to obtain the fetal karyotype. The process from procedure to result will take up to four weeks. Improvements in ultrasound technology have made it possible to perform AC earlier in pregnancy. In the late 1980s several publications reported on the possibility to successfully obtain amniotic fluid in the first and early second trimester with maintained cytogenetic results [34, 36, 37, 65, 70, 89, 95]. AC can be performed in clinics away from the genetic laboratory and the sample of amniotic fluid can be sent by post, which is regarded as a major advantage.

Chorionic villus sampling

With the introduction of CVS in the 1980s, first trimester diagnosis became reality and procedures were performed as early as six weeks' gestation [6]. Primarily, CVS was performed trans-cervically under ultrasound guidance and the

method was adopted in many centres [10, 63, 74]. Trans-abdominal CVS was introduced as an alternative technique with advantages such as a lower risk of infection and a sampling technique similar to trans-abdominal AC [82]. The cytogenetic result can be obtained within a few days after CVS since the trophoblastic cells in the chorionic villi divide rapidly. For a successful result, the trophoblastic tissue must be prepared at the genetic laboratory without too much delay. Thus, the use of CVS has been restricted by the necessity of performing the procedures not too far from the laboratories and AC has remained the method of choice in many centres in Sweden.

Fetal and maternal risks

Fetal loss

All pregnant women have a certain risk of miscarriage and fetal loss, although the extent of that background risk is not exactly known. Maternal age, gestational length and type of method used to document pregnancy, are factors with impact on the risk figures of fetal loss [16, 79, 101].

With the introduction of AC and CVS, the question arose if these procedures brought an additional risk. Reports from non-randomised studies found no increased fetal loss rate after the second trimester AC [18, 66], whereas others reported an increased risk [103]. The only randomised study, comparing AC at 16 weeks' gestation with non-exposed controls, found the spontaneous fetal loss rate to be one percent higher in the AC group [93]. Several of the studies on AC before 15 weeks' gestation reported no additional risk of fetal loss compared with traditional AC [34, 36, 37, 70, 89, 95].

Regarding CVS and risk of fetal loss, there is no randomised study comparing CVS with non-exposed controls. Two multicenter studies compared first-trimester trans-cervical CVS with AC after 15 weeks' gestation and found no significant difference in fetal loss [10, 74]. In a European multicenter study where trans-cervical and trans-abdominal CVS were compared with second trimester AC, the pregnancy loss rate was substantially greater after CVS [63]. In a randomised comparison of AC and trans-abdominal and trans-cervical CVS, the risk of fetal loss

was similar after AC and trans-abdominal CVS but increased after trans-cervical CVS [83].

Infant morbidity

The amniotic fluid has an important role for the development of fetal lungs and the posture of limbs [68, 71]. When AC is performed, the amniotic membranes are punctured and amniotic fluid is withdrawn. Concern was raised when an increased number of infants with postural deformities and unexplained respiratory difficulties at birth was found after second-trimester AC [103]. A few reports of respiratory problems in the new-born [93, 99], as well as musculo-skeletal deformities were presented [19], while other studies found no such associations [18, 26, 43]. Non-randomised studies of AC before 15 weeks' gestation found no increased rate of infant morbidity [34, 36, 37, 70, 89, 95]. However, a randomised English study comparing AC and CVS, found an increased spontaneous fetal loss rate after AC at 10-13 weeks' gestation and an increased but not significant rate of club feet [67]. A few studies performed on animal models also suggested an influence on lung function by AC [39, 61].

Safety of CVS was seriously questioned when Firth and co-workers reported that a cluster of infants with severe limb abnormalities was found after CVS before 10 weeks' gestation [29]. In a follow-up study, a correlation was found between the severity of the defects and the gestation for the CVS procedures [30]. These alarming reports were followed by some large population-based studies in which no such associations were found [31]. Concerning infant respiratory problems, a few studies evaluated CVS and reported an association [96, 104].

Maternal complications

The number of reports addressing maternal complications after AC and CVS have been few and the studies small. Some of the studies have not included non-exposed controls. An association has been reported between AC and antepartum bleeding and placental abruption [23, 103], as well as with post-procedural leakage of amniotic fluid [93]. The risk of leakage was found to be higher when AC was performed earlier in gestation [11]. An association between CVS and post-

procedural bleeding has also been reported [74, 83]. Other studies have not found an increased risk of pregnancy complications [18, 66, 97].

Psychological evaluations

Apart from the safety and technical aspects of AC and CVS, interest has also focused on the psychological implications of prenatal diagnosis. Women have been found to be concerned about fetal injury caused by the invasive procedure, spontaneous abortion and waiting for the test result [20, 28]. A few studies have found increased levels of anxiety before and decreased levels after AC [4, 25, 73]. Anxiety levels varying with gestation were found in women not having prenatal diagnosis and the anxiety correlated with their obstetric history [54]. Some studies have found differences between AC and CVS regarding levels of anxiety and fetal bonding [8, 75].

AIMS OF THE STUDY

The specific aims of the studies were:

- To compare AC with CVS performed at the same gestational age concerning the risk of fetal loss and diagnostic accuracy.

- To evaluate women's reasons for submitting to an invasive procedure, their knowledge of prenatal invasive procedures and how the information was obtained, for women making different choices at the same gestational age.

- To evaluate women's satisfaction with the given information, their concerns about complications due to the procedure and psychological reactions and distress evoked by the procedure.

- To study if AC and CVS in routine obstetric care performed for low-risk indications, increase the risk of musculo-skeletal postural deformities, limb reduction defects and respiratory problems in the newborn, and if the risk for fetal and infant mortality, prematurity, low birthweight and fetal distress increases. The aim was also to study if the gestational age at the procedures had any impact.

- To study if AC and CVS in routine obstetric care performed for low-risk indications, increase the risk of maternal complications like bleeding in pregnancy, placental abruption, complications related to the amniotic cavity and membranes, dysfunctional labor and operative deliveries in comparison with non-exposed women. The aim was also to study if the gestational age at the procedures had any impact.

MATERIALS AND METHODS

Papers I and II

Population and study design

Women with single, viable pregnancies requesting invasive prenatal diagnosis and who resided in the geographical region of Uppsala University Hospital, Sweden, were invited to participate from September 1992 to July 1994. The options were AC or CVS at 10+5 to 13+6 weeks' gestation. The women could choose or be randomised into AC or CVS. Entry criteria were fetal karyotyping for low-risk indications such as advanced maternal age (≥37 years), a family history of chromosomal abnormality in the absence of balanced parental translocation and parental anxiety. Exclusion criteria were multiple pregnancy, missed abortion, intrauterine contraceptive device *in situ*, multiple fibroids or a major fetal abnormality. Complications and pregnancy outcome were obtained in connection with an anomaly scan at 20 weeks' gestation and from the patient records after delivery and discharge from the hospital. Before coming to the Fetal Medicine Unit, the woman had received written and verbal information from an obstetrician at the antenatal care unit. The women were informed that there was an increased risk of miscarriage of approximately one percent for AC and CVS and that the diagnostic accuracy of AC was somewhat better than for CVS. The study was set up to see if earlier AC held the same risk and to estimate the diagnostic accuracy in comparison with that of CVS.

From October 1993 to July 1994, a subgroup of 100 women were invited to join a questionnaire study. Upon arrival at the Fetal Medicine Unit, the woman received the invitation to participate. On acceptance, she answered a questionnaire in three parts in the waiting room before the procedure. The questionnaire was collected before the invasive procedure.

Invasive procedures

AC and CVS were performed trans-abdominally with a 20 G needle using a needle guide under ultrasound guidance. All procedures were performed by one of three specialists experienced in invasive techniques. At AC, puncture of the placenta was avoided and the aim was to aspirate 10 ml of amniotic fluid. An aspirated volume less than 10 ml was defined as an insufficient sample. The cytogenetic results for AC were achieved by cell culturing using Eagle's medium with calf serum. For CVS, placental tissue was aspirated by applying a negative pressure through a 20 ml syringe attached to the CVS needle by a connecting tube. Care was taken not to puncture the amniotic membrane. Chorionic villi were identified under a dissecting microscope and clean villi were transferred to a culture medium (RPMI 1640+20 per cent fetal bovine serum) for overnight culture analysis.

The questionnaire

The questionnaire was answered before the procedure and consisted of three parts (see Appendix). In the first part the questions concerned maternal age, number of children and indication for fetal karyotyping. Four questions concerned knowledge of invasive procedures and if the woman had considered fetal karyotyping before her first visit to the antenatal care unit, how she had obtained knowledge of the procedures, and if the decision to have an invasive procedure was made by the woman herself or influenced by others, e. g. her partner, a doctor or the midwife. One question concerned the woman's satisfaction with the information given by the midwife and doctor at the antenatal care unit. Satisfaction was estimated using a scale ranging from 'very satisfactory' (=5) to 'very unsatisfactory' (=1). Five questions assessed the level of anxiety concerning complications and the waiting time before the result became known (Appendix). The women could choose four alternatives from 'very anxious' (=4) to 'not anxious at all' (=1). The second part was the HADS. The third part was the IES.

The Hospital Anxiety and Depression Scale (HADS)

The HADS is a self-assessment mood scale specifically designed for use with non-psychiatric hospital patients and consists of two subscales, assessing anxiety and depression and with seven items each [105]. The subscale scores range from 0 to 21. A score of 0-7 is indicative of a 'non case', 8-10 a 'doubtful case' and ≥11 is indicative of a 'case' of clinical anxiety or depression, respectively (Appendix).

The Impact of Event Scale (IES)

The aim of the IES is to evaluate current subjective distress occasioned by any life event, in this case reactions due to the invasive procedure [42]. Studies of psychological responses to stressful life events have found two major response sets, intrusion and avoidance. The IES contains 15 items, of which 7 measure intrusion characterised by unbidden thoughts and images of the event, and 8 items measure avoidance characterised by denial of meanings and consequences of the event. By using four alternatives (not at all, rarely, sometimes, often), the woman estimated the frequency of each item during the week before the invasive procedure. These alternatives are scored 0, 1, 3 and 5 in that order. The maximum score for intrusion is 35 and for avoidance 40. The subscale scores are divided into three categories: low (0-8), medium (9-19) and high (>20) levels of distress, respectively [41] (Appendix).

Papers III and IV

National Health Registers

The Swedish Medical Birth Register, held by the National Board of Health and Welfare, contains data on more than 99% of all births in Sweden and information is prospectively collected through copies of the standardised individual antenatal, obstetric and paediatric records that are forwarded to the Medical Birth Register [13]. Information collected includes demographic data, reproductive history and complications during pregnancy, delivery and the neonatal period. Infant death in

the first year of life is also recorded. The Medical Birth Register includes all births from pregnancy week 28 and live births before 28 weeks' gestation. Diseases and complications in pregnancy, delivery and the infant are classified according to the Swedish version of the International Classification of Diseases (ICD). The Ninth Revision (ICD-9) was used from 1987 to 1996 and the tenth (ICD-10) since 1997. The Swedish Malformation Register contains data on major malformations detected up to six months after birth.

The Swedish Hospital Discharge Register, held by the National Board of Health and Welfare, contains data on more than 99% of all in-patient care in Sweden, including obstetric. The information includes the number of days in hospital, day of discharge and up to six diagnoses classified according to the ICD currently used.

Women exposed to AC and CVS

Women exposed to AC or CVS were identified by records from the seven genetic laboratories in Sweden, where all chromosomal analyses are performed and registered. Data collected were the women's personal identification number, the date for the invasive procedure, type of procedure (AC or CVS) and the karyotype. In cases where data were incomplete, the laboratories were contacted and further information collected, if available. Information on the indication for the procedures was available from the laboratories for all but one of the included regions. Women with more than one procedure or both kinds of procedures were identified, as were women registered in more than one laboratory in the same pregnancy and women with multiple pregnancies. Identification numbers found to be incorrectly recorded in the registers of the laboratories were corrected, if possible, by reference to the original records at the genetic laboratories.

Study population

Women 35 to 49 years old with single births in Sweden during the period 1991 to 1996 were included (Table 1). The women were classified as exposed to AC or CVS or not exposed. With only small differences between regions in Sweden, the routine during the study period was to offer women of age 35 or more an invasive

procedure. Due to incomplete information regarding the indication for the procedures, and the fact that considerably more women under the age of 35 had their invasive procedure due to a high-risk indication, for example a fetal malformation, the study cohort was limited to women of age 35 or more. The registration regarding women's exposure to an invasive procedure and the dates for the procedures were incomplete in one of the regions and, therefore, all women giving birth in this region were excluded. Women registered with incorrect personal identification numbers in the Medical Birth Register were excluded. To minimise the risk of getting exposed women in the unexposed group due to incorrect identification numbers and a subsequent failure to make a correct matching, women with the same day of birth as the women with incorrect identification numbers and who gave birth within 280 days after the procedure to an infant with sex correlating to the karyotype were excluded.

The obtained karyotypes after AC or CVS and the registered diagnoses of chromosomal aberrations in the Medical Birth Register and the Swedish Malformation Register were compared and chromosomal abnormalities were found to be under-reported. Therefore, cases of trisomy 13, 18, 21, Turner syndrome (ICD-9 codes 758 A, B, C, G) and abnormalities included in the ICD-9 codes 758D, F, X, reported to the Medical Birth Register, the Swedish Malformation Register, or to the Swedish Hospital Discharge Register, were excluded from the study population in order to obtain comparable groups of exposed and not exposed.

Records in the Medical Birth Register, probably incorrectly registered, with unrealistic differences between infant birthweight and gestational age as well as cases with different sex according to the Medical Birth Register and the karyotype after AC or CVS, were excluded. More procedures late in gestation were performed due to high-risk indications, and therefore, all women with an invasive procedure after 20 weeks' gestation were excluded (13 AC, 0 CVS). Moreover, records with an invasive procedure before nine weeks of gestation (15 AC, 8 CVS) or missing data on gestational age (27 exposed women, 105 non-exposed) were also excluded. Finally, women exposed to both CVS and AC were excluded (Table 1). In Table 2 outcome variables according to ICD-9 is given.

Table 1 Study population and number of procedures according to gestation

Women 35-49 years, single births, 1991-1996			81 930
Exclusions			
Women from the region with incomplete registers and women with incorrect identification numbers			9563
	Non-exposed		**Exposed**
Left to follow-up	48 276		24 091
Exclusions			
Chromosomal abnormalities	274		30
Incorrectly registered data	43		102
Gestation missing or >20w or <9w	105		63
Both AC and CVS			164
Final study population	**47 854**		**23 732**
	Non-exposed	**AC**	**CVS**
Total	47 854	21 748	1984
Live births	47 616	21 654	1980
Procedures per gestational week		n (%)	n (%)
9		7 (0)	168 (8)
10		22 (0)	748 (38)
11		142 (1)	700 (35)
12		1108 (5)	254 (13)
13		5628 (26)	61 (3)
14		7421 (34)	19 (1)
15		4797 (22)	6 (0)
16		1937 (9)	3 (0)
17		497 (2)	8 (0)
18		125 (1)	7 (0)
19		48 (0)	7 (0)
20		16 (0)	3 (0)

Table 2 Diagnostic codes according to ICD-9 used for outcome analysis

Outcomes	**Definitions, ICD-9**
Maternal outcomes	
Normal delivery	650
Ante- and intrapartum bleeding	641 A-X
Abruptio placenta	641 C
Unspecified bleeding in late gestation	641 X
Complications related to the amniotic cavity and membranes	658 A-X
Oligohydramniosis	658 A
PROM	658 B
Delayed delivery after rupture of membranes	658 C
Chorioamnionitis	658 E
Fever or sepsis in labor	659 C-D
Hypotonic uterine dysfunction	661 A-C
Hypertonic uterine dysfunction	661 D-E
Infant outcomes	
Musculo-skeletal deformities	754 D-H, 755 W
Hip dislocation	754 D
Club foot	754 F-G
Limb reduction malformations	755 C-E
Respiratory disturbances	770 A-X
Neonatal pneumonia	770 A

Statistical analysis

Paper I

The results were analysed for the total (choice + randomised) AC and CVS groups. The follow-up was complete in all cases. Differences in spontaneous fetal loss and need for repeat testing were calculated with 95 per cent CI and the χ^2 test.

Paper II

The results were analysed with the χ^2 test and analysis of variance using Fisher's positively least significant difference test for pairwise post hoc comparisons. The results were analysed for each group of women choosing AC, CVS or randomisation, respectively. The results were presented for the study group as a whole when no difference between groups was found.

Papers III and IV

Crude and adjusted OR with 95 per cent CI were calculated with logistic regression using the SAS programme, version 8. Comparisons were made between the AC and CVS groups versus the non-exposed, respectively. Maternal age, parity, BMI, smoking and delivery hospital were regarded as possible confounders and controlled for in all calculations. A previous infant with low birth weight (<2500) was used in the model as a possible confounder for SGA and preterm delivery. Likewise, a Caesarean section in a previous pregnancy was used in the model for the risk calculation of a planned or emergency Caesarean section. Preterm rupture of membranes and gestational length were regarded as possible intermediate variables for respiratory disorders. Gestational length was used in the model in a third degree polynomial. Maternal outcomes and the risks of late fetal, neonatal and post-neonatal death were analysed for the whole study population. All other infant outcomes were analysed for the population of women giving birth to a live infant.

RESULTS

Paper I

Of 321 women participating in the study, 109 chose AC, 126 CVS and 86 randomisation (38 AC, 48 CVS). The AC and CVS groups differed regarding smoking and a previous stillbirth (Table 3).

The numbers of women leaving the hospital with a live infant were 133 (91%) in the AC and 167 (96%) in the CVS group. Spontaneous loss occurred in ten cases (6.8%) in the AC and in three (1.7%) in the CVS group, a difference of 4.1% (CI 0.6 – 9.6). Due to the semi-randomised design, the difference in spontaneous loss has also been calculated with logistic regression. The difference in spontaneous loss corresponds to a crude OR of 4.2. The OR adjusted for maternal age, weight, parity, smoking and previous miscarriages is 5.0 (CI 1.3-19.8). Amniotic leakage occurred in 11 women (7.5%) after AC, of which four had spontaneous fetal loss, whereas one of two women with amniotic leakage (1.1%) had a fetal loss in the CVS group.

A repeat test was required in 28 women (19.0%) in the AC and in nine (5.2%) in the CVS group, a significant difference of 13.8% (CI 6.7-21.0). The indications for repeat testing after AC were a failed sample and failed cytogenetic analysis, all cases occurring before 13 weeks' gestation. In the CVS group the indications were an ambiguous result and confirmation of an abnormal karyotype.

Table 3 Maternal characteristics for women in the study population (Paper I)

	AC		CVS	
	Choice	Randomised		Choice
	(N=109)	(N=38)	(N=48)	(N=126)
Age, years	n (%)	n (%)	n (%)	n (%)
22-34	20(18)	9(24)	5(10)	23(18)
35-39	62(57)	22(58)	24(50)	65(52)
≥40	27(25)	7(18)	19(40)	38(30)
Weight, kg				
<60	36(33)	12(32)	9(19)	41(32)
60-79	64(59)	22(58)	36(75)	79(63)
≥80	9(8)	4(10)	3(6)	6(5)
Smokers	10(9)	7(18)	10(21)	24(19)
Previous miscarriages				
1	35(32)	8(21)	15(31)	26(21)
≥2	10(9)	6(16)	9(19)	18(14)
Previous stillbirth	1(1)	0(0)	5(10)	5(4)

Paper II

Ninety-four women agreed to participate, of whom 38 chose AC, 31 CVS and 25 to be randomised. No differences were found between the groups except for two items (see below). The main reasons for having an invasive procedure for the whole group were advanced maternal age (75.5%) and anxiety (38.3%). Anxiety was the only reason for 10.6%. On the question about knowledge already before the visit to the antenatal care unit, a majority of women stated they had knowledge of different methods for fetal karyotyping (57.4%), how procedures are done (57.4%) and what the methods can detect (74.5%). A minority of women stated knowledge of possible risks and discomfort (34.0%) and the reliability of the methods (24.5%). Most women obtained their knowledge through their doctor and midwife (73.4%). The mean score for satisfaction with the information from the doctor and midwife was 3.74 for the whole group of women. Women in the randomised group were more satisfied (4.28) than women in the AC (3.50) and CVS (3.61) groups ($F(2.91)=5.4$; $p<0.01$), respectively.

The women's concerns in connection with the procedure are given as mean scores. The women worried most about miscarriage (2.55), fetal injury by the procedure (2.23) and waiting for the result (2.38). They were less concerned about problems like pain and discomfort (1.88) and an unreliable result (1.56), although the randomised group expressed more concern about an unreliable result (1.88) than did the other groups (1.45, respectively), ($F(2.91)=3.54$; $p<0.05$).

The mean HADS scores were 4.8 for anxiety and 2.8 for depression. Seventeen women (19%) scored as 'cases' (n=11) or 'doubtful cases' (n=6) for clinical anxiety. The corresponding figure for depression was ten women (12%) (3 'cases'; 7 'doubtful'). The mean IES scores were 8.4 for intrusion and 7.7 for avoidance. Thirty-six women (39%) expressed medium or high levels of intrusion and avoidance, respectively.

Paper III

The risk of musculo-skeletal deformities, including club foot and hip dislocation, was increased in the AC group compared with the non-exposed (Table 4). The highest risk was found for AC before 14 weeks' gestation with OR=2.63 at less than

13 weeks' and OR=1.34 at 13 weeks' gestation. No increased risk was found for the CVS group.

A diagnosis of respiratory disturbance was more frequent in the AC group compared with the non-exposed, with the highest risk at 14 (OR=1.21) to 15 (OR=1.24) weeks' gestation. For the subcategories of respiratory disturbances, an increased risk was found for neonatal pneumonia. For other subcategories, like meconium aspiration (OR=1.29) and unspecified respiratory symptoms and tachypnea (OR=1.11), the ORs were increased although not significant. In the CVS group, the OR was on the same level as for the AC group, although not significant.

No increased risks of limb reduction defects, low Apgar, neonatal convulsions, idiopathic respiratory distress syndrome, preterm birth, SGA and fetal or infant death were found in either group.

Paper IV

Fewer women in the AC group had a normal delivery compared with the non-exposed (Table 5). Complications related to the amniotic cavity and membranes were found more frequently in the AC group compared with the non-exposed, with the highest risk at 13 (OR=1.19) to 14 (OR=1.26) weeks' gestation. Regarding each subcategory, an increased risk of delayed delivery after rupture of membranes was found. For subcategories like oligohydramniosis (OR=1.09), premature rupture of membranes (OR=1.13) and chorioamnionitis (OR=1.30), the ORs were above one, although not significant. No increased risk was found in the CVS group. The OR for fever or sepsis in labor was not significant (OR=1.19 for AC and 0.92 for CVS).

More women in the AC group had a diagnosis of hypotonic uterine dysfunction in labor and instrumental vaginal deliveries more often than the non-exposed. The highest risk of hypotonic uterine dysfunction was found for AC at 14 weeks' gestation (OR=1.22). The women in the AC group were more often delivered by elective Caesarean section but less frequently by emergency Caesarean section. No difference was found for the CVS group.

No increased risks of bleeding late in gestation, abruptio placenta, postpartum bleeding, retained placenta, protracted labor or hypertonic uterine dysfunction were found in the AC or CVS group.

Table 4 Infant outcomes for the AC and CVS groups vs non-exposed with significant increased risks expressed as adjusted OR with 95% CI

Outcome	AC		CVS	
	OR	**CI**	**OR**	**CI**
Musculo-skeletal deformities	1.32	1.11-1.57	0.84	0.49-1.45
-Hip dislocation	1.22	0.99-1.50	0.65	0.32-1.32
-Club foot	1.45	1.06-1.99	1.36	0.58-3.19
Respiratory disturbances	1.12	1.02-1.24	1.17	0.91-1.50
-Neonatal pneumonia	1.29	1.02-1.65	1.29	0.64-2.57

Table 5 Maternal outcomes for the AC and CVS groups vs non-exposed with significant increased risks expressed as adjusted OR with 95% CI

Outcome	AC		CVS	
	OR	**CI**	**OR**	**CI**
Normal delivery	0.93	0.90-0.97	1.06	0.96-1.16
Amnion related complication	1.15	1.06-1.24	0.88	0.71-1.09
-Delayed delivery after rupture of membranes	1.14	1.03-1.26	1.10	0.84-1.45
Hypotonic uterine dysfunction	1.12	1.06-1.18	1.10	0.94-1.30
Instrumental vaginal delivery	1.11	1.03-1.19	1.11	0.91-1.36
Elective Caesarean section	1.09	1.02-1.16	1.02	0.86-1.21
Emergency Caesarean section	0.93	0.87-0.99	0.97	0.82-1.16

GENERAL DISCUSSION

Every pregnancy carries a risk of a fetal chromosomal abnormality. To a certain extent, the level of risk is related to maternal age and previous obstetric history as well as gestational length [40, 77, 85]. The experience of risk is individual. For a majority of pregnant women the risk is low. For some women with higher risk or a perceived risk of an abnormality, the possibility to have AC and CVS is a prerequisite to become pregnant. Before a decision to have AC or CVS, women must be informed about what can be obtained by the procedures and the associated risks. This study aimed to investigate more about consequences and risks associated with AC and CVS.

Fetal loss and diagnostic accuracy

AC after 15 weeks' gestation has been shown to increase the risk of fetal loss [93]. After the introduction of AC performed earlier in gestation, several non-randomised publications reported no additional risk of fetal loss and a very good diagnostic accuracy [34, 36, 37, 70, 89, 95]. When AC and CVS performed at 10 to 13 weeks' gestation were compared, increased spontaneous fetal loss rate and less diagnostic accuracy was found after AC (paper I). The results are comparable with those presented in two other reports [64, 67]. These studies have a similar design and are therefore well suited for comparison. The preferable design to study fetal loss rate is a randomised study with complete follow-up, in which women are included at the same gestation and one procedure is compared with no procedure or second-best, another procedure. As AC and CVS have become established methods in obstetric care, randomisation is less likely to be accepted. To let women have the possibility to choose procedure for prenatal diagnosis if they do not accept randomisation, probably increases the number of women participating but it introduces the risk of bias. In the present study, the results are likely to reflect a true difference in risk since the procedures were done for the same indication, at the same gestational age, by the same operators, by the trans-abdominal technique and the samples were analysed at the same laboratory. The semi-randomised design might, however, influence the results. The number of smokers and women with a

previous stillbirth differed between the AC and CVS groups. Yet, when age, weight, parity, smoking and previous miscarriages were controlled for, the fetal loss risk was of the same magnitude. In the CVS group, the spontaneous fetal loss rate was in accordance with other reports [67, 83]. Moreover, the increased risk of performing AC before 15 weeks' gestation has been confirmed in a large randomised study [11].

Interestingly, no increased risk of late fetal and infant death was found after AC or CVS (Paper III). Furthermore, the risk of late fetal death was lower in the CVS group compared with the non-exposed, and regarding preterm birth and SGA the risk was lower in both the AC and CVS groups (Paper III). A possible explanation would be that more vulnerable pregnancies exposed to AC or CVS more often end in early spontaneous losses whereas the non-exposed continue to preterm or SGA births or late fetal losses.

The number of failed samples and cytogenetic failures after AC was considerably higher in comparison with some reports [64, 67, 91], but in accordance with others [48]. No different procedure than the one allocated was performed, the procedures were never postponed without an attempt and a sample less than 10 ml was characterised as a failure, circumstances contributing to the number of failed samples. At AC, puncture of the placenta was avoided and thereby the risk of tenting the membranes might be higher [46, 94]. Fewer fetal cells are available in the amnion before 15 weeks' gestation and the cells need a longer time in culture [21, 22, 48]. To some extent, the inconsistent results from this and previous studies, may be due to varying gestations at sampling. In this study, no failed cultures occurred after 13 weeks' gestation, which supports such a view. Genetic laboratories use different methods for cell culturing; in the present study Eagle's medium was used. After the end of this study, the laboratory introduced Chang's medium which improved the results (Professor G Annerén, personal communication). For CVS the sampling and culture success was very good.

The increased risk of miscarriage associated with standard AC and trans-abdominal CVS is close to 1 % [83, 93], and from the present study it can be concluded that AC performed at 10 to 13 weeks' gestation carries a substantial increase in fetal loss risk. AC also implies an increased risk of repeat testing due to failed samples and cultures compared with CVS.

Psychological reactions

No differences, except for two items, were found between women choosing or being randomised to AC or CVS regarding their experiences and psychological reactions prior to an invasive procedure (Paper II). As these women were part of an on-going trial, a question is whether the results can be regarded to reflect how women experience the situation prior to an invasive procedure in a routine clinical situation. Women were offered prenatal diagnosis on the same indications as in routine care. At the antenatal care unit, women were informed by the same doctors and midwives and in the same manner as in routine care. As an effect of the study, women might have been more thoroughly informed, although doctors and midwives had no additional education before the start of the study. The possibility for women to choose procedure resembles the routine clinical situation. Since no major differences were found between women being randomised and choosing procedure, there is reason to believe that the results can be regarded as representative for women in general prior to an invasive procedure. Furthermore, the most likely uncertainties associated with a study situation would, in this study, have been related to the new method (earlier AC) for which no differences were found compared with CVS.

In this study, the questionnaire allowed women themselves to state one reason or more for having an invasive procedure, which could explain the rather high figure for anxiety, which is consistent with another Swedish study [81]. In other studies, the figures for anxiety as an indication is considerably lower [35, 65]. Although women submitted voluntarily to an invasive procedure due to anxiety, they also worried about possible adverse consequences of the procedures. The women worried most about miscarriage and fetal injury due to the procedure, as well as waiting for the result, a finding also reported from other studies [4, 20, 28, 53, 59, 80].

Well-informed consent and knowledge are regarded as important aspects of prenatal diagnosis [57]. An objective assessment of women's knowledge was not performed in this study. According to the women's own statements, a majority of the women stated no knowledge about reliability of the methods and procedure-related risks before their visit to the antenatal care unit. This information is important to doctors and midwives, who are the women's main source of

information. The women were satisfied with the information they obtained from the doctor and midwife, even if women being randomised were more satisfied. A higher satisfaction with the information on both AC and CVS might have improved the women's confidence in medical professionals and might have increased the number of women accepting randomisation.

The impact of invasive procedures on anxiety has been investigated in several studies. The results are difficult to evaluate, due to non-randomised recruitment, different gestational lengths for the procedures, and different scales for the psychological evaluation [8, 25, 73, 92]. Irrespective of prenatal invasive testing, pregnancy itself may cause variations in mood [54]. Women's concerns and reactions did not differ according to the method chosen, and the invasive procedures were performed at the same gestational length. Despite many women stating anxiety as a reason for prenatal diagnosis, expressing worry about miscarriage and fetal injury due to the procedure, and also expressing limited knowledge about possible risks and reliability of the methods, it appears as if most women can handle the situation, according to the low mean scores on the HADS and IES. However, a certain number of women experience more distress regardless of which method they submit to. If these women would benefit from more support has to be investigated further.

The HADS is short and was developed as a screening tool for identifying individuals in somatic care at risk of the two most common forms of psychological disturbances, anxiety and depression [105]. The HADS has been widely used with proven reliability and validity [3, 38, 45, 76, 88]. To a certain degree, the two sub-scales correlate. In the HADS, items relating to both emotional and physical illness are excluded and, thereby, a depressive state might be under-estimated. The IES was developed to evaluate current subjective distress related to a specific event [41, 42]. It is often used for assessing post-traumatic stress, but several reports have also presented the results from obstetric settings [3, 49, 78]. A correlation between the HADS and IES was not assessed in this study, but can not be excluded. The results of the HADS and IES are consistent and indicate that women in general under-going prenatal invasive testing are at low risk of developing major anxiety, but some

women are at a higher risk. The use of both scales gives different aspects of women's psychological reactions before undergoing AC or CVS.

Maternal and infant complications and morbidity

Concerns raised in previous studies regarding an increased risk of infants being born with musculo-skeletal deformities and respiratory problems after AC, have been confirmed in this study (Paper III). Moreover, an increased risk of complications related to the amniotic cavity and membranes, uterine dysfunction in labor and a lower chance of a normal delivery was found for women after AC (Paper IV).

For this type of epidemiological studies, the definition of study population is crucial. The size of this study population makes the risk minimal that the findings are by chance. The cohort was limited to women from 35 years of age. This group of women were those offered invasive testing according to the routines in Sweden during this period. The indications for the procedures were recorded in the laboratories for all but one of the included regions. Most women had their invasive procedures for low-risk indications. More procedures after 20 weeks' gestation were performed due to high-risk indications, such as a fetal malformation, and therefore excluded. To avoid the risk of getting exposed women in the non-exposed group, extensive exclusions were made (Table 1).

The CVS group was smaller than expected at start of the study, which reduced the statistical power. Due to the possibility to perform AC in clinics far from the genetic laboratory, and the genetic laboratories being accustomed to one procedure, AC is the method of choice in most clinics.

Maternal and infant outcomes were collected from the Swedish Medical Birth Register, the Swedish Hospital Discharge Register and the Swedish Malformation Register. In this way, information was found to be more complete and there is no reason to believe that under-reporting to the registers differed between the exposed and non-exposed groups.

Before the analysis, factors that may introduce bias must be identified. For this study maternal age, parity, smoking, BMI and hospital were chosen. These are factors with a possible impact on women's uptake of invasive testing and also on pregnancy outcome [1, 12, 14, 15, 27, 62, 69, 86]. Routines for offering invasive

testing and the use of diagnostic codes at different delivery hospitals were also controlled for possible differences [13].

Several studies have reported on the association between AC and a risk of musculo-skeletal deformities [32, 67, 90, 103], of which one study reported an association between club foot and leakage of amniotic fluid [24]. A possible association between the amniotic cavity and membranes and the occurrence of postural deformities is indicated, since the membranes are punctured at AC but not at CVS, and the risk figure for CVS was not increased. The volume of amniotic fluid increases with each week of gestation, and a relatively larger amount of fluid is withdrawn when AC is performed earlier than 15 weeks' gestation [84]. After puncture of the membranes, leakage of amniotic fluid for shorter or longer time can occur. Before the membranes are fused to the cavity wall, a leakage may occur into the extra-amniotic space and not be visible outwardly. Accordingly, the fetus may be prevented from moving freely and, thereby, contract a deformity.

Previous studies have reported an effect on infant lung function after AC, such as respiratory distress and pneumonia [93], unexplained respiratory difficulties [103], increased respiratory morbidity [33, 104] and findings indicating an effect on lung growth and development [60]. This study confirms an association with the most evident impact when AC is performed at 14 to 15 weeks' gestation. The results were controlled for differences in gestational age at birth. Fetal lung growth seems to be influenced by factors such as amniotic fluid and fetal breathing movements [100, 102]. Fetal breathing movements were found to be reduced for two days after AC [55]. The lung growth in guinea pig was related both to the duration and onset of oligohydramniosis, with the greatest effect in early pregnancy [61]. An effect on lung function was seen after AC before 16 weeks' gestation, which corresponds to the pseudoglandular stage of fetal lung development, at which the tracheobronchial tree is formed [52].

This line of argument seems inconsistent with the finding of an OR on the same level for respiratory disturbances after CVS. However, a number of studies have reported associations between CVS and neonatal respiratory distress, high airway resistance and an increased respiratory morbidity the first year of life [33, 96, 104]. Moreover, AC in the monkey affected fetal lungs regardless of the amount of fluid

removed and even if no fluid was removed, which might support an association between any type of puncture and impaired lung function [39]. Whether the increased risk of respiratory disturbances is related to the amniotic cavity and membranes remains to be established, as well as the under-lying mechanisms. Regarding other complications related to the infant lung, the risk of idiopathic respiratory distress was not increased. However, the number of preterm births was decreased among exposed women.

The small CVS group reduced the statistical power. Concerning the risk of limb reduction defects, this was even more obvious. No increased risk was found in either the AC or the CVS group, but according to the power calculations, an OR of at least 1.8 was needed in the CVS group to reach statistical power. Still, this study gives no evidence of limb reduction defects occurring more often after CVS from 9 weeks' gestation, which corresponds to other reports [31, 50], and to the reported background incidence [2, 9, 51].

A further indication that the amniotic cavity, membranes and fluid could have a role for the increase in risk of musculo-skeletal deformities and respiratory disturbances, is the finding of an increased risk of amnion-related complications after AC (Paper IV). The risk of amnion-related complications was found to be highest for AC at 13 to 14 weeks' gestation, and for respiratory disturbances at 14 to 15 weeks' gestation. The study population included only women giving birth from 28 weeks' gestation and live births before 28 weeks. Spontaneous abortions and intrauterine fetal deaths before that gestation were not included, which might explain the rather small increase in the risk of amnion-related complications after AC, of which the risk of a delayed delivery after rupture of membranes was the only subcategory found to be significantly increased, although the ORs for the other subcategories were on the same level. Under-reporting as well as under–diagnosing might have reduced the number of cases in each subcategory. Even with a large study population like this, outcomes of low incidence and minor differences may be difficult to find.

Regarding amnion-related complications and an association with AC, post-procedural leakage of amniotic fluid has been extensively studied [7, 64, 90, 93], and a higher risk for AC before 15 weeks' gestation has been identified [11].

Although other studies have found contradicting results, i.e. a lower risk of PROM after early AC [17], the results from this and other studies indicate an effect on amnion-related complications after AC.

The risk of neonatal pneumonia was increased in the AC group, as were the ORs for chorioamnionitis and fever and sepsis in labor. A association between these outcomes was not found but can not be excluded. Therefore, discussion of mechanisms that cause complications later in gestation after an AC puncture before 16 weeks' gestation remains speculative. The puncture might start an inflammatory reaction progressing more or less slowly, or might introduce infectious agents, leading to complications like PROM, chorioamnionitis or a spontaneous fetal loss. Some reports have indicated an inter-individual difference in anti-bacterial activity in amniotic fluid [5].

The finding of an increased risk of abnormal labor and hypotonic uterine dysfunction after AC is difficult to explain. The corresponding OR for the CVS group was similarly elevated. At the same time, the number of instrumental vaginal deliveries was increased in the AC group, with a similar increase for CVS. No such association has been presented previously, except in a British study reporting an excess of dysfunctional uterine action after AC [103]. A few smaller studies have reported no association between invasive procedures and instrumental deliveries [44, 93]. However, in studies on pregnancies in older women, an association between age and instrumental vaginal deliveries and Caesarean section has been reported [47, 87]. Whether these women were exposed to invasive procedures or not, was not stated.

Mechanisms starting and regulating uterine action in labor, are not fully known. Whether the fetus has a role for uterine action remains a subject of speculation. No association was found in this study between hypotonic uterine dysfunction and adverse infant outcomes like respiratory disturbances or postural deformities. Nevertheless, the results indicate that, after AC, women have a slightly lower chance of normal deliveries and an increased risk of instrumental vaginal deliveries due to hypotonic uterine dysfunction. Women in the AC group were also delivered more often by an elective Caesarean section whereas the numbers of emergency Caesarean sections were decreased, compared with the non-exposed. Women's

uptake of prenatal invasive procedures may differ with regard to perceived risk, which may in turn explain differences in preferences for elective Caesarean sections and a subsequent reduction of emergency Caesarean sections.

This study does not suggest an association between AC and an increased risk of placental abruption and bleeding in late gestation. The women were not randomised to AC or CVS, which might introduce bias. For women with symptoms like vaginal bleeding, the procedure could have been postponed or cancelled after counselling and examination by the obstetrician. Thus, the result can probably be applied to women without risk factors for bleeding and placental abruption.

CONCLUSIONS

AC performed at 10 to 13 weeks' gestation carries a higher risk of unintended fetal loss and repeat testing compared with CVS performed at the same gestational age.

No increased risk of major psychological reactions is found for a majority of women prior to an invasive procedure.

A substantial minority of the women experience distress and are at risk of clinical anxiety and depression.

Further studies are needed to evaluate if these women would benefit from more support.

Women are concerned about spontaneous abortion, fetal injury by the invasive procedure and waiting for the result.

The obstetricians and midwives are the women's major source of knowledge.

AC is found to be associated with an increased risk of musculo-skeletal deformities in the infant, especially, when performed before 14 weeks' gestation.

AC is found to be associated with an increased risk of respiratory disturbances in the infant, especially, when performed at 14 to 15 weeks' gestation.

For CVS, a possible association with respiratory disturbances can not be excluded.

CVS performed from nine weeks' gestation, was not found to be associated with limb reduction malformations.

AC is associated with a slightly lower chance of normal deliveries and a somewhat increased risk of hypotonic uterine dysfunction and operative vaginal deliveries.

AC is associated with more complications in the third trimester related to the amniotic cavity and membranes.

An association between CVS and hypotonic uterine dysfunction and operative vaginal deliveries is suggested.

AC and CVS are not found to be associated with bleeding late in gestation and complications related to the placenta.

CVS is the method of choice for prenatal karyotyping in the first trimester.

AC should not be performed before 14, or even 16 weeks' gestation.

Further research to develop methods to better identify women at increased risk of chromosomal abnormal pregnancies and to develop non-invasive tests for prenatal diagnosis is needed, thereby minimising the number of women exposed to invasive procedures and the adverse effects caused by these procedures.

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