An evaluation of cytogenetic diagnosis by chorionic villus sampling

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CHAPTER 1: LIST OF ABBREVIATIONS AND SUMMARY

1.1 LIST OF ABBREVIATIONS

Ac = Amniocentesis
AFP = Alpha-fetoprotein
CI = Confidence interval
CPM = Confined placental mosaicism
CS = Chromosomal studies
CV = Chorionic villi
CVB = Chorionic villi biopsy
CVS = Chorionic villi sampling
EDD = Expected date of delivery
FD = Fetal death
FT = Fetal tissues
HCG = Human choriogonadotrophin
IUFD = Intrauterine fetal death
IUGR = Intrauterine growth retardation
LMP = Last menstrual period
LTC = Long-term culture
MCC = Maternal cell contamination
MSAFP = Maternal serum alpha-fetoprotein
MSHCG = Maternal serum human choriogonadotrophin
NLB = Normal live born
NTD = Neural tube defect
PP = Post partum
RDR = Regression derived rate
TA CVS = Transabdominal chorionic villi sampling
TC CVS = Transcervical chorionic villi sampling
TOP = Termination of pregnancy

1.2 SUMMARY

The main indication for prenatal diagnosis by amniocentesis or chorionic villi sampling is the risk of a fetal chromosomal aberration. The risk of delivering a child with such an aberration increases with advancing maternal age. In The Netherlands, all women aged 36 years or older in the 18th week of pregnancy, are offered prenatal diagnosis. This advanced maternal age indication makes by far the largest contribution (about 80%) to the number of prenatal diagnoses.

The fetal chromosomal constitution can be investigated either in cells obtained by

amniocentesis in the second trimester of pregnancy or in cells, obtained by chorionic villi sampling (CVS) *i.e.* the aspiration of a very small portion of the future placenta, usually performed ten weeks after the last menstrual period (LMP). However, in a number of cases, the chromosomal constitution of these chorionic villi differs from the chromosomal pattern in the fetus. In this thesis, the reliability and the implications of CVS as a routine diagnostic prenatal test were investigated. The questions to be answered were:

To what extent do the cytogenetic diagnostic results, obtained from chorionic tissue, provide adequate and accurate information on the chromosomal constitution of the fetus?

Which biological mechanisms can be held responsible for a genetic difference between the extra-embryonic tissues and the fetus proper?

Which practical guidelines can be put forward?

Do chromosomal aberrations confined to the placenta have any clinical significance?

How many pregnant women had to undergo a second invasive procedure to establish the fetal chromosomal constitution?

How many of the women who had their pregnancies actively terminated by first trimester suction curettage after a chromosomal aberrant CVS diagnosis, would otherwise have had a spontaneous abortion before the 16th week of pregnancy?

How many induced abortions in the second trimester were replaced by a first trimester suction curettage by the application of CVS instead of amniocentesis in our group of patients?

To answer these questions, an evaluation was made of all the cytogenetic aberrations in our study group of 3277 consecutive CVS cases. In 2607 cases (79.6%), the indication for prenatal diagnosis was the advanced age of the mother.

In all cases, the cytogenetic diagnosis in the CV samples was made by the investigation of so-called direct preparations. These direct preparations reveal the chromosomal constitution of the cytotrophoblast cells in the chorionic villi. In a number of cases, an additional examination was performed on the chromosomal pattern of the mesenchymal core cells of the chorionic villi.

In 140 CV samples (4.3%), a chromosomal aberration was found (Table 5.3). The results of a cytogenetic follow-up study were available in 123 cases. In 65 cases (53%), the diagnosis was confirmed and in 58 follow-up studies (47%), a difference was demonstrated between the chromosomal pattern of the fetus (amniocytes, n=46; post-partum blood, n=2; fetal skin, n=10) and that in the chorionic villi. In 52 cases, the fetal chromosomal pattern turned out to be normal (Table 5.11).

In 48 patients (1.5% of all patients, 34% of all aberrations), a second trimester amniocentesis was indicated, because of ambiguous CVS results. In three of these, termination of pregnancy (TOP) followed because aberrant cells were traced in the amniotic fluid (a trisomy 9, a trisomy 18 and an additional marker chromosome). In

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In 86 women, TOP followed the CVS procedure based on an aberrant cytogenetic result. In 72 out of these 86 cases, a cytogenetic follow-up result was available (Table 5.7). The CVS diagnosis was confirmed in 62 cases. In 4 chromosomally aberrant fetuses, a discrepancy could be demonstrated between the chromosomal constitution of the chorionic villi and the fetus and in 6 cases, all the cells investigated in the products of conception showed a normal karyotype (including one case of intrauterine fetal death (IUFD) before the scheduled termination and one case with possible maternal cell contamination in the follow-up study (Table 5.8)).

Additional information can be obtained on the cytogenetic constitution of the mesenchymal core cells of the chorionic villi by a long-term culture (LTC) preparation technique. These LTC's are set up if chorionic villi sample size exceeds 25–30 mgrs. In case of chromosomal aberrations in the direct preparations, the LTC preparations are harvested and analyzed as well. Even when using this additional information provided by the LTC preparations, the cytogenetic diagnosis obtained can differ from that established by investigation of fetal cells. In 23 cases with a cytogenetic aberration in the direct preparations, enough chorionic villi for an LTC were available (Table 5.12). In 16 cases, the fetal chromosomal constitution was correctly predicted by the LTC preparations. In 4 cases, the fetal chromosomes could not be investigated (spontaneous abortion in two cases and termination of pregnancy without subsequent tissue culture in two cases) but in 3 cases the LTC karyotype differed from the fetal karyotype.

The predictive values for the various categories of chromosomal aberrations in the direct preparations of the CVS specimen were calculated (Table 5.21). The probability that the fetal chromosomal constitution will be correctly represented differs per diagnosis. The highest reliability could be assigned to a normal chromosomal complement diagnosed by CVS. Pregnancy outcome is known in 99.4% of our patients and no false negative diagnoses were noticed in our series. The predictive values for the various non-mosaic CVS aberrations were: 100% for trisomy 13, 97.7% for trisomy 21, 81.8% for trisomy 18, 75.0% for triploidy, 25.0% for an additional marker chromosome and 0.0% for 45,X. The relevance, the 95% confidence interval, of these predictive values varies between the different aberrations according to their frequency in our material.

Differential selection could be held responsible for many of the differences between the chromosomal make-up of the extra-embryonic tissues and that of the fetus. These selection mechanisms also underlie the differences in predictive values for the various chromosomal aberrations found in CVS. The higher the selection pressure, the lower the predictive value for a certain cytogenetic abnormality. This concept can serve as a practical guideline in the clinical application of CVS.

Out of the 140 chromosomal aberrations, 120 were found among the patients with a maternal age indication for the CVS procedure (n=2607) (Table 5.22). We calculated the number of aberrations that would have been found if this group of patients had chosen amniocentesis in the second trimester of pregnancy (Figure 5.4). In 45

patients, a normal cytogenetic diagnosis would have been made, 31 patients would have experienced a spontaneous abortion before the usual time of amiocentesis and 44 aberrations would have been found in the amniotic fluid cell cultures, leading to 41 second trimester terminations of pregnancy and three live borns with a chromosomal anomaly (47,XXY in two cases and 47,XXX in one case), assuming these parents had taken the same decision as they took after the CVS procedure.

There were 37 pregnancies which showed a chromosomal aberration in the extra-embryonic tissues and a normal fetal chromosomal pattern in a subsequently performed amniotic fluid cell culture. Among these, five fetal/perinatal deaths occurred (Table 5.29). This 13.5% is significantly higher than the fetal/perinatal loss rate of 3.5% (χ^2=8.96; p<.01) in our population and strongly supports that there is a causal relationship between confined chromosomal aberrations in the placenta and adverse pregnancy outcome.

The greatest advantage of CVS undoubtedly is its application in the first trimester of pregnancy, giving in more than 95 per cent of all cases a relieving early information on the normal chromosomal constitution of the fetus. Serious drawbacks result from its varying diagnostic reliability, specifically related to the different forthcoming cytogenetic abnormalities and expressed by specifically varying predictive values with regard to the fetal chromosomal constitution. The disadvantages of the test can be partly compensated by additional follow-up studies *i.e.* amniocentesis, involving of course the serious consequences of delays, increased risk of miscarriage, and second trimester induced abortion in the case of a fetal chromosomal aberration.

CHAPTER 2: INTROD

2.1 INTRODUCTION

Prenatal diagnosis

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