J. Perinat. Med.
15 (1987) 244

Chorionic villi sampling with an echogenic catheter: experiences of the first 500 cases

Wolfgang Holzgreve¹ and Peter Miny²

¹Department of Obstetrics and Gynecology and ²Institute of Human Genetics, Westf. Wilhelms-University Münster, West Germany

1 Introduction

Since chorionic villus sampling (CVS) was established as a first-trimester alternative to amniocentesis for the prenatal detection of genetic disorders four years ago [3, 15, 16], three major reports from different centers with about 1000 cases each have recently provided valuable informations on the safety of sampling and the reliability of diagnosis [4, 13, 17]. An editorial in the "Lancet" at the beginning of 1986 concluded from the discussion at the 2nd International Conference on CVS in Birmingham that "the best technique of CVS is not yet agreed" [1]. Most samples are at present obtained transcervically by a specific type of plastic catheter with a bendable aluminum obturator which was originally developed by H. WARD [15]. A disadvantage of this particular instrument can be an occasionally significant reduction in visibility on the ultrasound screen after removal of the metal obturator with resulting loss of control over the catheter tip during sampling. We therefore started in 1984 to develop a CVS catheter with improved echogenicity [5] and ultimately have used in our diagnostic series a transparent polyethylene catheter (figure 1 a) with a highly echogenic stripe in its wall (figure 1 b). Here our experience with more than 500 diagnostic cases using this technique and their complete follow-up according to an investigative protocol is reported.

Curriculum vitae

WOLFGANG HOLZGREVE, born October 15, 1955, started Medical School at the Westf. Wilhelms-University in Münster in 1973. In 1976 he received a Master of Science in Health and Medical Sciences degree from the University of California at Berkeley. He was a fellow of the Studienstiftung des Deutschen Volkes and of the International Rotary Foundation. After his residency in the Department of Obstetrics and Gynecology at the University of Münster he was appointed Oberarzt. From 1982 to 1984 he was a fellow of the German Research Foundation in the Department of Obstetrics, Gynecology and Reproductive Sciences at the University of California in San Francisco. In 1985 he was board certified in Obstetrics and Gynecology as well as Clinical Genetics. In 1986 he became Privatdozent at the Westf. Wilhelms-University in Münster and in the same year he was awarded a prize of the European Association of Perinatal Medicine.

2 Methodology

The diagnostic CVS program at the University of Münster, F. R. G., started after approval of our protocol by the Institutional Ethics Committee on Human Research at the beginning of 1985. All patients received detailed genetic

Figure 1. New echogenic catheter for CVS.

a) Polyethylene catheter (upper picture) and stainless steel obturator (lower picture).

b) Echo-reflecting stripe in the wall of the catheter.

counseling before the procedure and the statistical uncertainties of the risk evaluation due to the yet limited experience with CVS were specifically stressed [6]. Samplings were performed between 8 and 13 weeks from the last menstrual period with the use of our echogenic catheter (Angiomed, Karlsruhe, F. R. G.) which has a length from 20 to 26 cm and a diameter of 1.1 mm so that the dilatation of the cervix before the procedure is not required. After removal of the obturator there is no impairment in the visibility of the catheter due to its echogenic stripe (figure 2). In all cases we determined maternal serum alpha-fetoprotein values before and after sampling.

Figure 2. Ultrasound picture showing the catheter after removal of the obturator. The tip of the catheter is in the chorion frondosum of a pregnancy at 9 gestational weeks.

Maternal serum samples were drawn immediately before and shortly after CVS for AFP determinations by radioimmunoassay with commercially available kits with proper quality control (Professor FUHRMANN, Giessen).

In the last 403 cases we took swabs from the cervical os for bacteriological studies before the cervical disinfection with polyvinylpyrrolidone-iodine-complex and cultured the tip of the catheter after the procedure. In 124 consecutive cases estradiol, progesterone and β -HCG were measured with commercially available kits before the procedure, especially in those cases where ultrasound evaluation revealed, despite fetal heart action, findings suspicious of threatening abortion prompting us to wait with the sampling.

All patients were requested to fill out a questionnaire before the procedure with inquiries about their medical history and some socioeconomic factors. The procedures were done under continuous ultrasound visualization with a 3.5 MHz sector transducer (Sono-line SL, Siemens, Erlangen, F. R. G.).

Immediately after sampling the tissue was placed in Petri dishes with RPMI medium and evaluated under a dissecting microscope ($\times 60-100$). Chorionic villi were separated and transferred by forceps into clean medium,

washed and evaluated again microscopically in order to separate any decidual contamination. In cases with blood admixture heparin was added to the sample. The amount of tissue was evaluated by comparison with photographically documented reference standards [16]. In all cases long term cultures were established in Chang's medium (Hana Biologicals, Berkeley, California) and direct preparations were also performed routinely for the last 270 cases. In cases of female fetal karyotypes maternal cell contamination was excluded by comparison of fluorescence polymorphisms [14], when no metaphases from direct preparations were available.

The samplings took place as an outpatient procedure. All patients with ongoing pregnancies were seen again at 16 weeks after the last menstrual period for a detailed ultrasound follow-up and maternal serum AFP screening. All the relevant birth informations were mailed to us by a standardized questionnaire, and if necessary, follow-up was completed by telephone. All cases were documented on the data sheets of the International CVS Registry kindly provided by L. JACKSON, Philadelphia, USA, and all data were recorded on computer.

3 Results

The indications for CVS are in accordance with those for amniocentesis with the exception of neural tube defects (table I).

The one case for paternity testing using HLA antigen determination was investigated prior to a scheduled termination of pregnancy in the course of a legal investigation following rape (HLA-testing by Professor H. GROSSE-WILDE, Essen). The 7 cases at risk for sickle cell anemia or thalassemias were examined by DNA-studies [9] following an active screening program in Germany by our group. Of 8 patients at risk for Duchenne muscular dystrophy 3 had female fetuses and DNA-analysis was performed in the remaining 5 pregnancies at risk for hemophilia A. A female karyotype was found in two cases and a Turner syndrome in one. By DNA analy-

Table I. Indications for chorionic villi sampling.

Indications	Patients	
	n	%
Maternal age	377	76
Previous chromosome anomaly	41	8
Risk for X-linked disease	16	3
Hemoglobinopathies	7	2
Parental balanced translocation	4	1
Biochemical disease	3	1
Paternity testing (HLA)	1	—
Sexing because of risk for campomelia	1	—
Other indications	46	9
Total	496*	100

* 5 cases of twin pregnancies

sis in the latter case hemophilia A was excluded. Sexing was also performed in a pregnancy each at risk for X-linked hydrocephalus, Menke's and Wiskott-Aldrich syndrome as well as campomelia.

The bacteriologic studies on the cervical swabs revealed aerobic and/or anaerobic bacterial contamination in almost all cases, but a positive culture of the catheter tip was only encountered in 46 out of 403 cases (12%) including 23 cases with discordant bacteriologic findings in the cervix and catheter possibly suggesting secondary contamination [8].

Table II outlines the abnormal results. Terminations of pregnancy due to pathologic results were only performed in 13 cases (2.6%). In one case each of true trisomy 18 and 9 mosaicism and two cases with true marker chromosome mosaicism amniocentesis was performed which could not confirm the CVS findings, and the pregnancies are continuing uneventfully in two patients and resulted in a normal child in the other case with mosaic marker chromosomes.

Until December 1986 a total of 223 deliveries were documented.

So far we have complete records of pregnancy outcome from 259 cases; only two patients with intact pregnancy at 17 weeks were lost to follow-up due to move to the USA.

Table II. Abnormal results after chorionic villi sampling.

Unbalanced translocation	2
Trisomy 21	1
Trisomy 18	4
46,XY/47,XY, +19	1
46,XY/47,XY, +18	1* ¹
46,XY/47,XY, +9	1* ¹
46,XY/47,XY, +20	1
Trisomy 13	1
46/47, +mar	2* ¹
Fragile X	1
47,XYY	1
47,XXY	1
45,X/46,XX	1
46,XY/46,XX	2* ²
69,XXX	1
45,X	2
β-thalassemia	2
M. Hurler	1

*¹ normal karyotype at amniocentesis
*² maternal contamination as demonstrated by comparison of fluorescence polymorphisms

There was a more than 20% increase in maternal serum AFP levels in about 53% of the patients after CVS in our series [2]. The rises in maternal AFP levels showed a statistically significant positive correlation to the sample weights, but we did not observe a statistically significant correlation with the number of insertions so far.

At least 7% spontaneous abortions in the group of patients counseled before the sampling by sonography at the time of our first examination and about 3% of the intact pregnancies ended in spontaneous miscarriage between our first scan which had shown fetal heart action and the scheduled CVS date. In this latter group low levels of maternal estradiol, β-HCG and

especially progesterone were indicative of threatened abortion in 82% of the cases. Of interest is the observation of a fetal trisomy 18 with a significant drop of all three hormone levels prior to sampling and an ultrasonically still viable pregnancy 9 days after sampling immediately before spontaneous abortion occurred.

Of the 501 samplings 21 failed, but the rate of unsuccessful sampling decreased significantly with increasing experience and is now about 1%. The break-down of gestational ages is given in table III, sample weights and number of insertions in table IV and V, respectively. In the 486 pregnancies which were intended to continue 21 fetal losses occurred prior to 28 weeks of pregnancy. This abortion rate of 4.3% is not higher than the background rate seen in pregnancies without intervention matched for maternal age [20]. In WILSON's series [20] of follow-up investigations of women with normal ultrasound scans at 7 to 12 weeks 6 out of 133 pregnancies ended in spontaneous abortions already at 20 weeks gestation or less. The newborn parameters of the 223 babies delivered so far were within the expectation for our population and there was no indication for an increase in the rate of placental abnormalities or growth retardation. There were 2 still-births and 4 neonatal deaths, one in a prematurely delivered twin pregnancy. There is no indication for an increased rate of birth defects in our series.

Our questionnaire study [7] indicated that patients with higher socio-economic backgrounds were clearly overrepresented. The patients had received the primary information about CVS from their obstetrician in 74%, from other doctors in 6%, media in 7% and family members or other sources in 13%.

Table III. Weeks of pregnancy at time of sampling determined by ultrasound crown-rump length measurements.

Gestational age (weeks)	8	9	10	11	12	13	not recorded	total
n	14	81	179	158	60	5	4	501
%	3	16	36	32	12	1	—	100

Table IV. Weights of chorionic tissue.

Weight (mg)	n	%
No villi	21	4
< 10	52	10
10 to 20	124	25
20 to 30	157	31
30 to 40	76	15
> 40	68	14
Not recorded	3	1
Total	501	100

Table V. Number of insertions.

No. insertions	n	%
1	315	63
2	113	23
3	60	12
4	10	2
5	1*	—
Not recorded	2	—
Total	501	100

* two sampling sessions two weeks apart

4 Discussion and conclusion

Even though recent series [4, 13, 17] have documented that CVS can be a safe and reliable diagnostic procedure in experienced hands, single aspects of this new technique still have to be further elucidated.

The fetal loss rate (4.3%) in our series is in accordance with the reports from other centers [12] and does not differ significantly from the reported rates of spontaneous abortions in the first trimester [4]. The abortion rate may be a reflection of our practice of relatively low “aggressiveness in seeking in analyzable sample” [11] as indicated by our sampling failure rate of 4% which is in the middle of the range from 10% to 0% unsuccessful samplings reported from experienced centers. Our echogenic catheter, which is currently also used in the CVS program at Rotterdam (M. JAHODA, personal

communication), could be visualized well in all cases sampled which may explain the low rate of maternal cell contamination in the samples obtained. Only in three cases with male karyotype 1 to 3 cells with 46,XX karyotype were detected in the direct cytogenetic preparation.

No patient in our program was excluded from CVS because of e. g. previous bleeding or discharge during pregnancy or because of uterine or placental position. 7 (= 30%) of the 23 patients with spontaneous abortion had vaginal bleeding before sampling. This difference, however, was not significant. The only selection requirement was intact fetal heart action and a valid indication.

A further analysis of the abortion rates in the group of pregnancies intended to continue in our series shows a clear correlation to the gestational age at the time of sampling. Whereas the spontaneous abortion rates were 7,1% in the cases sampled before 9 and 7,8% after 11 weeks, it was only 3.3% in the group of patients between 9 and 11 weeks. This is in accordance with the findings of HOGGE et al. [4] and JACKSON [10]. Like others [1, 17] we also found a positive correlation between the risk of CVS and the number of insertions. The abortion rate was 4,1% (13/315) in the group of cases with one insertion, but rose to 6,2% (7/113) after two and 12,7% (9/71) after three or more insertions. Additionally we evaluated the abortion risk in relation to the sample weights. The highest abortion rate (23%) occurred in the patients where the sampling failed which might also be influenced by the increased number of insertions in these cases.

It is of interest that the abortion rate in our first 100 consecutive patients who have reached their due date was about 6% which in comparison to the overall results is not suggestive of a pronounced “learning curve” as observed in the beginning of other CVS programs [18, 19]. This may be explained by a proper training period of more than 50 cases in a pre-diagnostic series, careful ultrasound evaluation prior to CVS and the easy handling of our new echogenic catheter.

Summary

Using a newly developed CVS catheter with enhanced echogenicity we performed CVS in 501 consecutive cases. The abortion rate of 4.3% prior to 28 weeks of gestation in this series is within the background rate of controls matched for maternal age. The loss rate was clearly correlated to the number of insertions, time of sampling and sampling success. CVS is safest between 9 and 11 weeks of pregnancy. We recommend to restrict the number of insertions to a maximum of 3. The rate of failed samplings was 4, reflecting a low "aggressiveness in seeking a sample". Complete follow-up of 259

consecutive cases gave no indication of an increased rate of congenital anomalies following CVS in early pregnancy. In our study we also performed cervical swabs, pregnancy hormone testing, maternal serum AFP determination prior to and after CVS as well as maternal AFP screening at 16 weeks of pregnancy together with a detailed sonographic examination. We conclude that CVS can be considered now a safe and reliable diagnostic procedure, but requires further detailed documentation and close follow-up in controlled trials.

Keywords: Chorionic villi sampling, echogenic catheter, first trimester prenatal diagnosis.

Zusammenfassung

Chorionzotten-Biopsie mit einem echogenen Katheter: Erfahrungen mit den ersten 500 Fällen

Mit einem von uns neu entwickelten Katheter, dessen sonographische Visualisierbarkeit durch die Einlage eines echogenen Streifens gesteigert wurde, führten wir in 501 Fällen Chorionzotten-Aspirationen durch. Die Spontanabortrate bis zur 28. Schwangerschaftswoche betrug 4,3% und entspricht somit etwa der Hintergrundrate von Spontanaborten in diesem Schwangerschaftszeitraum. Die Abortrate zeigte bei näherer Analyse klare Korrelationen mit der Anzahl der Insertionen, dem Entnahmezeitpunkt und mit der gewonnenen Gewebsmenge. Wir empfehlen auf Grund der eigenen und publizierten Ergebnisse, nicht mehr als drei Insertionen

durchzuführen. In insgesamt 4% der Fälle konnte keine ausreichende Gewebsmenge gewonnen werden. Diese Rate, die im Verlaufe der Serie wesentlich gesenkt werden konnte, reflektiert eine „geringgradige Aggressivität bei der Gewebsgewinnung“. Ausführliche Nachuntersuchungen bei 259 aufeinanderfolgenden Fällen ergab keinen Anhalt für erhöhte Fehlbildungsraten nach Chorionbiopsien. In unserer Serie untersuchten wir auch bakteriologische Abstriche von der Portio vor Chorionbiopsie und der Katheterspitze nach dem Eingriff. Außerdem führten wir in allen Fällen Serum-AFP-Bestimmungen vor und nach Chorionbiopsien sowie in der 16. Schwangerschaftswoche durch im Zusammenhang mit sorgfältigen Nachuntersuchungen.

Schlüsselwörter: Chorionzotten-Entnahmen, echogener Katheter, pränatale Diagnostik im ersten Schwangerschafts-Trimenon.

Résumé

Le prélèvement de villosités chorales avec un cathéter échogénique: Des expériences après 500 examinations

Nous avons pratiqué la biopsie de villosités chorales dans 501 instants avec un cathéter plus échogénique, inauguré par nous-mêmes. Le risque de fausse-couche spontanée avant la 28ème. semaine était 4,3%, correspondant avec la fréquence de fausse-couche dans la population normale pendant la même période. Dans notre collectif nous avons trouvé une corrélation dis-

tincte entre le chiffre d'insertions, l'âge de la grossesse au temps de la prélèvement de tissu et la quantité de matériel. Dans 4% la biopsie ne donnait pas de tissu suffisant pour des examinations demandées. Jusqu'au présent, 259 enfants ont été nés et examinés. Nous n'avons pas trouvé une indication pour une élèvement de malformation dans ces cas. Dans toutes examinations nous avons pris des épreuves bactériologiques et de l'AFP avant et après la biopsie.

Mots-clés: Biopsie de villosités chorales, catheter plus échogénique, diagnostic prénatal.

Acknowledgements: We would like to thank S. BASARAN, U. EXLER, M. BUDDENDIEK, M. SANDKAMP and B. HOLZGREVE for excellent technical assistance and F. K. BELLER, J. HORST and I. H. PAWLOWITZKI for their continuous support of our program. This study was sponsored by grants from the German Research Foundation and the Bundesministerium für Forschung und Technologie, F. R. G.

References

- [1] Editorial: The potential of chorionic villus sampling. *Lancet* i (1986) 76
- [2] FUHRMANN W, K ALTLAND, IH PAWLOWITZKI, P MINY, W HOLZGREVE, V JOVANOVIC, R RAUSKOLB: Veränderungen der MS-AFP-Konzentration durch Chorionzottenbiopsie. 5. Tagung der Arbeitsgemeinschaft Klinische Genetik der Gesellschaft für Anthropologie und Humangenetik. Erlangen, May 8–10, 1986
- [3] GOSDEN JR, AR MITCHELL, CM GOSDEN, CH RODECK, JM MORSMAN: Direct vision chorion biopsy and chromosome-specific DNA probes for determination of fetal sex in first trimester prenatal diagnosis. *Lancet* ii (1982) 1416
- [4] HOGGE WA, SA SCHONBERG, MS GOLBUS: Chorionic villus sampling: Experience of the first 1000 cases. *Am J Obstet Gynecol* 154 (1986) 1249.
- [5] HOLZGREVE W, P MINY: Improved echogenicity of the catheter for chorionic villi sampling, In: FRACCARO M, G SIMONI, B BRAMBATI (eds): *First Trimester Fetal Diagnosis*, pp 63. Springer-Verlag, Berlin 1985
- [6] HOLZGREVE W, A REISCH, P MINY, FK BELLER: Sample size needed to assess risk of abortion after chorionic villus sampling. *Lancet* i (1985) 223
- [7] HOLZGREVE W, P MINY, B HOLZGREVE, U EXLER, FK BELLER: Socioeconomic characteristics of women selecting villi sampling for prenatal diagnosis. Third International Conference "Chorionic villi sampling and early prenatal diagnosis" Strasbourg, Dec. 17–18, 1986
- [8] HOLZGREVE W, P MINY, W RITZERFELD: Bacterial contamination of the catheter after transcervical CVS. Third International Conference "Chorionic villi sampling and early prenatal diagnosis". Strasbourg, Dec. 17–18, 1986
- [9] HOLZGREVE W, C AULEHLA-SCHOLZ, EU GRIESE, R OEHME, P MINY, J HORST: Erfahrungen mit der pränatalen Diagnostik von Sichelzellanämie und Thalassämien im ersten Schwangerschaftstrimenon. Aspekte für den Frauenarzt, Geburtshilfe Frauenheilkd., in press
- [10] JACKSON LG: Prenatal genetic diagnosis by chorionic villus sampling (CVS). *Semin Perinatol* 9 (1986) 209
- [11] JACKSON LG: Surveillance of risks in first trimester prenatal diagnosis, In: BRAMBATI B, G SIMONI, S FABRO (eds): *Chorionic Villus Sampling*, pp 213. Marcel Dekker, New York 1986
- [12] JACKSON LG: Chorionic Villus Sampling Newsletter. Dec. 1, 1986
- [13] JACKSON LG, RA WAPNER, MA BARR: Safety of chorionic villus biopsy. *Lancet* i (1986) 674
- [14] MINY P, W HOLZGREVE, S BASARAN, KH GERBAULET, FK BELLER, IH PAWLOWITZKI: Maternal cell contamination in chorionic villi cultures — Exclusion by chromosomal fluorescence polymorphisms. *Clin Genet* 28 (1985) 262
- [15] OLD JM, RHT WARD, F KARAGOZLU, M PETROU, B MODELL, DJ WEATHERALL: First trimester fetal diagnosis for hemoglobinopathies: three cases. *Lancet* iii, (1982) 1413
- [16] SIMONI G, B BRAMBATI, C DANESINO, F ROSELLA, GL TERZOLI, M FERRARI, M FRACCARO: Efficient direct chromosome analyses and enzyme determinations from chorionic villi samples in the first trimester of pregnancy. *Hum Genet* 115 (1983) 739
- [17] SIMONI G, G GIMELLI, L CUOCO, L ROMITTI, G TERZOLI, S GUERNERI, F ROSELLA, L PESCIOTTO, A PEZZOLO, S PORTA, B BRAMBATI, E PORRO, M FRACCARO: First trimester fetal karyotyping: one thousand diagnoses. *Hum Genet* 72 (1986) 203
- [18] WARD RHT, B MODELL, M PETROU, S KANAKONGSUKDI, R PENKETH: Chorionic villi sampling in a high-risk population using the Portex cannula. FRACCARO M, G SIMONI, B BRAMBATI (eds): *First Trimester Fetal Diagnosis*, pp 19. Springer Verlag, Berlin 1985
- [19] WARREN RC, L KEARNEY, C MCKENZIE, CH RODECK: Single-operator aspiration of chorionic villi using an ultrasound-guided silver cannula. FRACCARO M, G SIMONI, B BRAMBATI (eds): *First Trimester Fetal Diagnosis*, pp 32. Springer Verlag, Berlin 1985
- [20] WISON RD, V KENDRICK, BK WITTMANN, BC MCGILLIVRAY: Risk of spontaneous abortion in ultrasonically normal pregnancies. *Lancet* i (1984) 920

Received December 30, 1986. Revised March 22, 1987.
Accepted April 6, 1987.

PD OA Dr. Wolfgang Holzgreve
Zentrum f. Frauenheilkunde der
Westf. Wilhelms-Universität
Albert-Schweitzer-Str. 33
D-4400 Münster, West Germany