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A randomized study to assess two different techniques of aspiration while performing transabdominal chorionic villus sampling

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ABSTRACT

Objective *The technique used to perform transabdominal chorionic villus sampling (CVS) is not standardized, but aspiration of villi is generally obtained by discontinuous vacuum created in a syringe, manually or by a hand-grip device. We evaluated the feasibility of a new method of performing CVS which employs a 4-mL Vacutainer® connected to the needle, producing a continuous negative pressure.*

Methods *Two hundred pregnant women, whose gestational age ranged from 10 + 2 to 16 + 2 (mean, 12 + 1) weeks, entered the randomized study, which was powered to detect with 90% probability the absence of any difference in the size of chorionic samples obtained by using a 20-mL syringe with the vacuum obtained by a hand-grip device (Group 1) or by a vacutainer (Group 2). Four operators with different levels of experience performed all the procedures, which were done transabdominally using a freehand technique with a 20-gauge needle under ultrasound guidance.*

Results *Maternal age, body mass index, gestational age and the way the needle was inserted within the chorion were similar in the two groups. The median amount of villi sampled was 20 mg, with no differences between the two groups. The rate of fetal loss was 1.7%. All losses occurred in women of Group 1 who had only one needle insertion. A second needle insertion was required more frequently while using the vacutainer.*

Conclusion *This new technique for performing transabdominal CVS uses a readily available device and is as effective as traditional sampling systems to aspirate villi. It has the advantage of being a one-operator procedure.*

INTRODUCTION

Although transabdominal chorionic villus sampling (CVS) is the method of choice for early invasive prenatal diagnosis, the technique is not standardized[1]. One survey of 32 delegates attending the 2005 Fetoscopy meeting in Rodos (unpublished data) found that there were variations in the size of needle and the volume of the syringe used, the aspiration technique (by a hand grip – 'pistol' – or by applying a manual vacuum to the syringe) and the number of passes within the chorion. Almost all centers used a single rather than a two-needle system, and all but two required the assistance of one operator holding the ultrasound probe. We have devised a new method of performing transabdominal CVS which employs a Vacutainer® (BD Vacutainer Systems, Plymouth, UK) to obtain a continuous vacuum while advancing and withdrawing the needle within the chorion, in contrast to the standard technique of obtaining discontinuous negative pressures by 'pistol' or manual aspiration. The aim of this study was to evaluate the feasibility of this modified aspiration system compared to the technique most commonly used worldwide.

METHODS

This randomized study was powered to detect with 90% probability the absence of any difference in the size of chorionic samples obtained by using a vacutainer or a 20-mL 'pistol' syringe. Two hundred pregnant women referred during the period September 2005 to May 2006 entered the study. Inclusion criteria were: a singleton

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viable pregnancy and gestational age ranging from 10 to 16 weeks. During the study period 10 twin pregnancies were excluded from it. The randomization was done using sequentially numbered sealed envelopes. All the women involved had genetic counseling prior to the procedure and gave informed consent to take part. The indications for performing CVS were: advanced maternal age ($n = 173$), positive first-trimester screening for fetal anomaly ($n = 13$), suspicious ultrasound findings ($n = 11$), previous trisomic fetus ($n = 1$), maternal anxiety ($n = 1$) and risk of thalassemia ($n = 1$). Mean maternal age was 38 (range, 27–44) years and mean maternal body mass index (BMI) was 22.7 (range, 18–32). Gestational age, based on the last menstrual period and confirmed by sonography, ranged from 10 + 2 to 16 + 2 (mean, 12 + 1) weeks. Four operators with different levels of experience performed all the procedures. The CVS was done by Operator 1 in 74 cases, by Operator 2 in 35, by Operator 3 in 26, and by Operator 4 in 65. Operators 1 and 2 had each performed > 500 procedures prior to the study and Operators 3 and 4 between 200 and 300 each. Therefore, 109 CVSs were performed by a very experienced operator and 91 by one with intermediate experience.

The procedures were done transabdominally using a freehand technique with a 20-gauge needle. The skin was sterilized with topical application of povidone-iodine and the transducer was covered with a latex condom. The needle was introduced under ultrasonic guidance until the placenta was reached. In all instances, even when using the 'pistol' aspiration technique, the operator held the transducer with one hand and the needle with the other, hence dispensing with the need for an assistant. The needle was flushed with 0.5 mL heparin before the procedure. The chorion was mainly anterior in 97 cases and posterior in 103. Once the needle had entered the main body of the chorion, the maximum depth reached by the tip of the needle from the decidua and the angle between the needle and the chorionic plate were measured on still frames (Figure 1). The number of passes (advance and withdrawal movements of the needle along the measured angle of insertion and within the depth of the chorion thus calculated) was also recorded for each procedure. Ninety-nine aspirations of chorionic villi were performed with a 20-mL syringe containing 5 mL culture medium inserted in a syringe holder (the 'pistol'), and the aspiration was obtained by moving the plunger of the syringe upwards in order to create the discontinuous negative pressure necessary, while the needle was advancing through the chorion (Group 1). The number of times per CVS procedure the vacuum was obtained via this technique ranged from 3 to 8 and was left to the discretion of the operator. The remaining 101 samples were done using a 4-mL vacutainer connected to the needle, which produced a continuous negative pressure (Group 2). The presence of villi in the sample was verified by the naked eye in a Petri dish, and the weight of the sample was estimated using a visual analog scale (VAS) set to photographic standard[2]. The technician assessing the size of the CVS sample was blinded to the technique used.

Immediately after CVS, all the women were tested using a VAS to evaluate the perceived degree of pain experienced during the procedure on a scale of 1–10. For statistical analysis, block comparisons were made using the chi-square test, and continuous variables were compared using the Student's *t* or Mann–Whitney test, as appropriate, after confirmation that values were normally distributed or otherwise.

RESULTS

Maternal age, BMI, and gestational age at sampling did not differ between the two groups (Table 1). The way the needle was inserted within the chorion was also similar: the chorion was mainly anterior in 49 of the 99 women in Group 1 and in 48 of the 101 in Group 2, and the maximum depth reached by the tip of the needle within the chorion and the angle between the needle and the chorionic plate did not differ significantly (Table 1). The

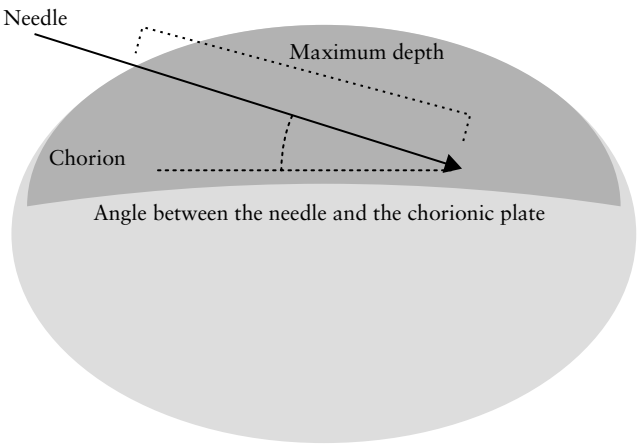

Figure 1 Diagrammatic representation of the placenta showing variables measured at chorionic villus sampling.

Table 1 Characteristics of the study groups

Parameter	*Group 1* (n = *99*)	*Group 2* (n = *101*)
Maternal age (years)	37 ± 3.4 (27–43)	38 ± 2.8 (30–44)
Body mass index (kg/m^2)	23 ± 7.1 (18–32)	22 ± 4.1 (18–30)
Gestational age (weeks)	12 + 1 ± 0.87 (10 + 5 to 16 + 2)	12 + 0 ± 0.6 (10 + 2 to 14 + 1)
Depth of needle insertion (mm)	31.6 ± 11.8 (4–65)	29.5 ± 12.1 (3–67)
Angle of needle (°)	29 ± 24 (0–90)	29 ± 23 (0–90)

Data are given as mean ± SD (range). Group 1, the standard technique group; Group 2, the Vacutainer® group.

number of passes of the needle within the chorion was also similar in the two groups (median number in each group, 4; 95% CI, 4–5).

The amount of villi yielded at the first sample was 5 mg or less in 16 procedures in both Group 1 and Group 2, and greater than 20 mg in 27 procedures in Group 1 and in 26 of Group 2. A second needle insertion was required in 10 cases (5%): 1 in Group 1 and 9 in Group 2 ($P = 0.006$). The median amount of villi sampled was 20 mg for both groups. The need for a second needle insertion and the amount of villi finally yielded did not correlate with maternal BMI, the experience of the operator, the chorion location, the angle of insertion of the needle, the depth of the needle within the chorion or the number of passes of the needle.

There was no difference in the VAS score of the pain experienced during CVS between women of Group 1 (mean, 2.4; 95% CI, 2.2–2.7) and those of Group 2 (mean, 2.4; 95% CI, 2.1–2.8). Only 12% of the women experienced pain expressed by a score of > 4.0, and no correlation was found with any of the analyzed variables.

An abnormal fetal karyotype was diagnosed in 17 women (six cases of trisomy 21, four of trisomy 18, two high-grade mosaicisms, one case of trisomy 13, one Turner syndrome, one tetraploidy, one inversion of chromosome 17, and one unidentified marker). Twelve of these women elected to undergo termination of pregnancy, and intrauterine death of a trisomy-21 fetus occurred in one 2 weeks after CVS. Of the remaining 187 women, eight (4.3%) were lost to follow-up (four of Group 1 and four of Group 2) and two additional women terminated their pregnancy following diagnosis of exomphalos and renal agenesis later in the second trimester. Three women with a normal fetal karyotype were diagnosed as having fetal demise at follow-up 1 month after CVS. No further perinatal losses occurred in the series. Therefore, the rate of fetal losses was 1.7% (3 of 177) (or 2.2% (4 of 178) if the case of trisomy 21 is included). All the cases of fetal loss occurred in women who had only one needle insertion, and all were in Group 1 ($P = 0.05$). We have tested *in vitro* the maximum negative pressures which are obtained with 2.5, 5, 10 and 20 mL syringes and with a 4 mL vacutainer: these are 40, 60, 70, 90 and 50 kPa, respectively. Therefore, the negative pressure exerted with the vacutainer is less than that obtained with the syringe used in this study (20 mL), but it is continuous rather than intermittent.

DISCUSSION

Since the introduction of the transabdominal approach to CVS, the technique has undergone few modifications[3,4]. Most centers and operators use a single- or double-needle system, and once the needle is within the chorion a syringe is attached to the needle and discontinuous negative pressure is applied, either manually or with the help of a hand grip ('pistol'), to achieve adequate aspiration of the villi. Although this technique is undoubtedly effective, some degree of experience is necessary to be confident that sufficient villi have been aspirated before withdrawing the needle, which means that it is not an easily standardized procedure. In addition, the plunger of the syringe is difficult to move upwards while avoiding lateral dislodgement of the needle, unless the operator holds the needle with one hand and the plunger (or the hand grip within which it is lodged) with the other. Based on the experience of most centers, this requires the assistance of a sonographer or a physician to hold the ultrasound probe. An alternative solution, which employs vacuum pump suction, has not found widespread favor, probably because it is cumbersome and expensive[5].

The method we have described is the application to CVS of a technique accepted worldwide to sample blood from a peripheral vein. It uses a readily available device and allows a continuous and stable negative pressure to be maintained while the needle is within the chorion.

Clearly, variables other than the negative pressure exerted may determine the amount of villi yielded by CVS. These include the maximum depth reached by the tip of the needle from the decidua, the angle between the needle and the chorionic plate and the number of passes within the chorion. These variables were found not to be significant, which contrasts with the common belief that there is an optimal way of performing transabdominal CVS. Indeed, there was no effect of the degree of experience of the operators on the amount of villi aspirated. However, all the operators had performed more than 200 CVSs prior to starting the study, hence all had done the initial training in this procedure. Although we flushed the needles with heparin, the negative pressure, however obtained, may be nullified by the formation of blood clots within the needle, but we could not standardize, nor document consistently, this occurrence in either group of women.

The study was powered to demonstrate the absence of any difference in the size of chorion samples obtained by using a vacutainer or a 20-mL 'pistol' syringe. A second needle insertion was required more frequently while using the vacutainer, which might be explained by the learning curve with a new technique. The median amount of villi finally aspirated with the two methods did not differ between the two groups and was similar to that reported in other series[6,7]. The advantage of using a test tube rather than a syringe lies in the fact that the operator holds and moves the needle with one hand without the need to use the other to create the negative pressure by repeatedly withdrawing the plunger of the syringe within the barrel. Hence, transabdominal CVS is a one- rather than a two-operator procedure. In times of careful consideration of costs, this might be an advantage.

The physics of the lever might be recalled to highlight an additional potential advantage of using the vacutainer rather than a syringe. The needle and the syringe or the vacutainer attached may be considered as a lever, with the fulcrum as the point of entry of the needle at the maternal skin. With the vacutainer, the hand of the operator can hold the luer of the needle closer to the maternal abdomen than when holding the plunger of a syringe. According

to the principle of the lever, the amount of work done is given by force times distance, i.e. the further from the fulcrum a force is applied, the greater the force resulting at the other arm. Therefore, it would be sensible to hold the needle, hence to apply a force to it, as close to the maternal skin as possible, in order to minimize the effect on the tip of the needle within the chorion. In other words, the vacutainer may allow the operator maximal control of the needle within the chorion, reducing the risks of untoward movements in the lateral and anteroposterior planes. Although we did not set out to compare fetal loss rates, it is noteworthy that there were no losses in the group randomized to the vacutainer. We are continuing to use this technique, in order to evaluate the rate of pregnancy loss: larger studies are needed to confirm that this is indeed an effect of a less traumatic sampling technique. For the time being, one can reasonably conclude that this technique is as safe as reported in most series. It is our impression that this new technique is easier to use than the conventional syringe system but we have not verified this with a formal assessment. This study shows that transabdominal CVS is a relatively pain-free procedure, with no differences between the two aspiration methods.

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