Celocentesis for early prenatal diagnosis of hemoglobinopathies

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What are the novel findings of this work

1. The miscarriage rate following celocentesis is 2.3%.
2. In all cases of affected fetuses diagnosed by celocentesis where the parents chose termination, this was carried out at <10 weeks' gestation
3. Most women experienced none or only mild discomfort in association with celocentesis.

What are the clinical implications of this work

1. Celocentesis, as an alternative to CVS or amniocentesis for invasive prenatal diagnosis provides earlier diagnosis and potentially safer pregnancy termination for couples that choose this option.
2. The very early diagnosis following celocentesis offers the potential for in utero cell transplantation and intrauterine treatment by injection of donor hematopoietic stem cells into the celomic cavity.

ABSTRACT

Objective: Celocentesis, is an invasive technique that can provide prenatal diagnosis of single gene disorders, from as early as seven weeks' gestation. The objective of this study is to examine the safety of celocentesis.

Method: Celocentesis was performed for prenatal diagnosis of hemoglobinopathies in 402 singleton pregnancies, in which both parents were carriers of thalassaemia or sickle cell disease trait. We assessed procedure-related maternal discomfort or pain, success of sampling and obtaining of results, pregnancy outcome and postnatal follow up.

Results: First, celocentesis was carried out at a median gestational age of 8.6 (range 6.9-9.9) weeks and celomic fluid was successfully aspirated in 99.8% cases. Second, 67% of women had no or only mild discomfort, 18% moderate discomfort, 12% had mild to moderate pain and 3% had severe pain. Third, prenatal diagnosis from analysis of the celomic fluid was successful in 93.8% cases, but in the last 121 cases this was always successful..Fourth, in all cases of successful sampling and analysis of celomic fluid the diagnosis was concordant with results obtained from additional prenatal or postnatal testing. Fifth, in addition, to diagnosis of hemoglobinopathies, QF-PCR analysis which was performed to evaluate maternal contamination using several markers for chromosomes X, Y, 21, 18 and 13, led to the accurate diagnosis of chromosomal aneuploidies. Sixth, in all cases of affected fetuses diagnosed by celocentesis where the parents chose termination this was carried out <10 weeks' gestation. Seventh, in 97.1% (298/307) of the continuing pregnancies there was livebirth, in 7 (2.3%) there was miscarriage and in 2 there was loss to follow up. Eighth, fetal abnormalities were diagnosed in 3 (1%) cases, including unilateral transverse amputation of the forearm, unilateral moderate hydronephrosis and small bowel duplication. All neonates were examined by a pediatrician and were found to be phenotypically normal except the three cases with prenatally diagnosed defects.

Conclusions: Celocentesis can be used for early prenatal diagnosis of genetic abnormalities and the procedure-related risks of pregnancy complications appears to be low.

INTRODUCTION

Celocentesis, which involves the ultrasound guided aspiration of celomic fluid at 6-10 weeks' gestation, offers the potential for very early prenatal diagnosis.[1-10] The celomic fluid is derived from the extraembryonic mesoderm and contains embryo-fetal cells. Although culture of celomic cells for fetal karyotyping is not possible, molecular biology techniques can be applied for analysis of DNA extracted from these cells for diagnosis of hemoglobinopathies and other genetic disorders.[1-10] Consequently, with celocentesis prenatal diagnosis can be made at least four weeks earlier than the conventional methods of invasive testing; chorionic villus sampling (CVS) is used beyond 11 weeks' gestation, because if it is performed earlier it may be associated with the development of limp abnormalities,[11] while amniocentesis is used after 15 weeks, because if it is performed earlier it carries a higher risk for miscarriage and development of talipes.[12]

Celocentesis was initially described in the early 1990s[1] but it was not used widely for two reasons. First, high failure rate of obtaining a result due to maternal cell contamination and small number of fetal cells in the celomic fluid sample. These problems have now been overcome through optimization of the polymerase chain reaction (PCR) protocol and the use of specific antibodies for positive selection of fetal cells so reliable results can be obtained with a very small number of cells and with the use of a micromanipulator to hand-pick individual fetal cells when there is high maternal contamination or very limited number of fetal cells.[9,10,13,14] The second problem with celocentesis related to concerns on safety of the technique. A study of 20 women undergoing celocentesis prior to pregnancy termination, reported that 5 (25%) miscarried within a few days after the procedure.[15] In contrast, our study in 108 women undergoing celocentesis and 339 controls reported that within the subsequent 1-3 weeks and before elective termination of pregnancy, the rate of fetal loss in the celocentesis group was not significantly different from that in the controls.[16] Additionally, in a series of nine pregnancies undergoing diagnostic celocentesis we have reported that in two cases the fetus was affected and the pregnancies were terminated but all seven pregnancies with a normal result were uneventful and resulted in the livebirth of healthy babies.[7]

The objective of this study is to report the risks and complications associated with celocentesis used for prenatal diagnosis of hemoglobinopathies in a consecutive series of 402 singleton pregnancies.

PATIENTS AND METHODS

Study population

This was a prospective study in 402 consecutive singleton pregnancies undergoing celocentesis for prenatal diagnosis of hemoglobinopathies in the Department of Prenatal Diagnosis, Cervello Hospital, Palermo, Sicily, between April 2006 and October 2017. In all cases both parents were previously diagnosed as being heterozygous for β-thalassemia or sickle cell disease and transvaginal ultrasound examination diagnosed an intrauterine single live embryo with crown-rump length <18 mm. The parents were offered the options of celocentesis or the traditional techniques of CVS or amniocentesis. They were informed that celocentesis was a new technique that could provide earlier diagnosis and although there was some evidence that the associated risks of fetal loss were low the data were very limited.[7,16] In all cases choosing celocentesis a vaginal swab for microbiological investigations was carried out and for positive cases appropriate antibiotic therapy was given. The study was approved by the institutional review board (No 80, 26 January 2005) and all participating women gave written informed consent.

Celocentesis

The patient was placed in the lithotomy position and the vagina and external genitalia were carefully cleansed with an antiseptic solution. A transvaginal 5MHZ ultrasound transducer, covered with a sterile rubber, was then used for measurement of fetal crown-rump length and fetal heart rate, identification of the amniotic membrane, celomic space and yolk sac and diagnosis of any uterine anomalies.[5] A 20G needle was introduced transvaginally into the celomic cavity (Figure 1), through a guide attached to the transducer and three consecutive samples of 0.2, 0.2 and 0.6 mL were aspirated into three different syringes. To minimize the risk of contamination, the third sample was used for diagnostic testing. The fetal heart rate was measured again immediately after the procedure. No local or general anesthesia was used. In all cases ampicillin (1 g) was administrated intramuscularly, about one hour prior to celocentesis. Half an hour after the procedure the women were asked to score the degree of discomfort or pain from a scale of 0 for none 1-2 for mild discomfort, 3-4 for moderate discomfort, 5-6 for mild to moderate pain and 7-10 for severe pain.

Laboratory investigations

Laboratory processing of celomic fluid samples was as previously described.[13,14] Essentially, the sample was first assessed for maternal contamination. If contamination was absent or very low (<5%) DNA was extracted, amplified and then sequencing for the ß-globin gene was carried out; additionally quantitative fluorescent polymerase chain reaction (QFPCR) of short tandem repeat sequences of chromosomes 13, 18, 21, X and Y was performed.[10] If contamination was between 5% and 60% embryo-fetal erythroid precursors were selected by use of anti-CD71 MicroBeads and if contamination was >60% selection of embryo-fetal cells was achieved by micromanipulation; these cells were then used for extraction of DNA, ß-globin gene analysis and QFPCR for chromosomes 13, 18, 21, X and Y.

Pregnancy and pediatric follow up

In all pregnancies the women were offered the option of CVS or amniocentesis for confirmation of the result from celocentesis. In cases with an abnormal result from celocentesis where the women chose to have pregnancy termination without waiting for confirmation from further invasive testing the diagnosis was confirmed by examination of placental tissue obtained after termination.

Subsequent pregnancy care was carried out in the local hospital of each woman and these hospitals provided us with details on outcome, including livebirth, termination, miscarriage, stillbirth, preeclampsia, gestational hypertension, abruption, gestational age at delivery, birth weight and any abnormalities detected by pediatricians. In the cases without confirmation of the findings of celocentesis by CVS or amniocentesis neonatal blood was examined for hemoglobinopathies. Similarly, the hospitals provided data on the outcome of children to the age of 48 months.

RESULTS

Study population

During the study period celocentesis was offered to 496 women presenting for prenatal diagnosis of hemoglobinopathies with a single live fetus before 8 weeks' gestation; 426 accepted celocentesis and 70 chose CVS (Figure 2). Celocentesis was booked 5-10 days following the initial scan, but at the ultrasound scan prior to celocentesis, which was carried out at a median gestational age of 8.6 (range 6.9-9.9) weeks, a missed abortion was diagnosed in 24 (5.6%) cases. In the study population of 402 pregnancies all women were Caucasian, 196 (48.8%) were parous and 206 (51.2%) were nulliparous and the median maternal age was 31 (range18-45) years.

Celocentesis

Celomic fluid was successfully aspirated in 99.8% (401/402) cases; in 380 (94.8%) after the first needle insertion and in 21 (5.2%) after the second insertion. The median fetal heart rate was 171 (range 129-173) bpm before and 171 (range 131-169) bpm after celocentesis; the median change was 0 (range -38 to 38) bpm and this was unrelated to subsequent miscarriage. In the questionnaire for degree of discomfort or pain due to celocentesis, 72 (17.9%) had none, 198 (49.3%) had mild discomfort, 74 (18.4%) had moderate discomfort, 47 (11.7%) had mild to moderate pain and 11 (2.7%) reported severe pain. There were no differences in the difficulty of the procedure or the size of celomoc fluid sample with advancing gestation.

Laboratory investigations

Prenatal diagnosis from analysis of the celomic fluid was successful in 93.8% (376/401) cases and in 25 (6.2%) there was failure due to heavy maternal contamination; in the last 121 cases of celocentesis, after introduction of micromanipulation for selection of fetal cells,[13] prenatal diagnosis was always successful.

In 25 of the 26 cases with failed sampling or analysis the diagnosis was subsequently made by CVS; in one case there was a miscarriage before CVS. In the 376 cases of successful sampling and analysis of celomic fluid the diagnosis was confirmed by prenatal sampling (amniocentesis n=152, CVS n=27) placental tissue after pregnancy termination (n=83) or cord blood from live births (n=108); in 6 cases of miscarriage there was no confirmatory testing.

The results from celocentesis (n=376) or CVS (n=25) for hemoglobinopathies were homozygous in 92 (22.9%) cases, heterozygous in 207 (51.6%) and normal in 102 (25.4%). In four of the cases of heterozygous or normal result a chromosomal abnormality was diagnosed from analysis of the celomic fluid (trisomy 21, n=2; trisomy 13, n=1; triploidy, n=2) and in another case Cockayne syndrome, which is a fatal autosomal recessive neurodegenerative disorder, was diagnosed from analysis of celomic fluid in a family of known carriers. There were no differences in the percentage of failed sampling with advancing gestation.

Pregnancy outcome

In 89 of the 92 pregnancies with fetal hemoglobinopathy the pregnancy was terminated at the request of the parents and in 3 the pregnancies continued and resulted in livebirths (Figure 1). Pregnancy termination was also carried in the 2 cases of trisomy 21 and the 1 case of Cockayne syndrome; in the 2 cases of triploidy and the 1 of trisomy 13 there was missed miscarriage before planned pregnancy termination. In all cases of affected fetuses diagnosed by celocentesis where the parents chose termination this was carried out at <10 weeks' gestation.

In the 307 continuing pregnancies (303 with heterozygous or normal result for hemoglobinopathies, 3 with homozygous result and 1 with no result) there was livebirth in 298 (97.1%), in 7 (2.3%) there was miscarriage at a median of 16 (range 7-40) days from celocentesis, and in 2 (0.6%) there was loss to follow up, but in one of the latter ultrasound examination at 22 weeks showed a normally developing fetus. Therefore, the overall rate of miscarriage was 3.2% (10/310) of all pregnancies that did not have termination or 2.3% (7/307) of the continuing chromosomally normal pregnancies.

In the livebirths the median gestational age at delivery was 39 (range 26-42) weeks. Birth <37 weeks occurred in 12 (4.0%) cases including 2 of iatrogenic delivery for severe preeclampsia, 2 for antepartum hemorrhage in association with placenta previa and 8 with spontaneous onset of labor. The median birth weight percentile for gestational age was 48.2% (range 0-99.6%); in 10.7% (32/298) cases the birth weight was <10th percentile for gestational age.[17] There was preeclampsia in 2 (0.7%) cases, gestational hypertension in 14 (4.7%) and abruption in 1 (0.3%).

Fetal abnormalities were diagnosed by mid-trimester ultrasonography in 3 cases, all after uneventful celocentesis at 8 weeks' gestation: first, unilateral transverse amputation of the forearm, second, unilateral moderate hydronephrosis and third, small bowel duplication. All neonates were examined by a pediatrician and were found to be phenotypically normal except the three cases with prenatally diagnosed defects.

Postnatal follow up

We received data on detailed follow up at 6, 18, 24, 36 and 48 months of age on 275, 249, 226, 199 and 160 children, respectively. The child with amputation of the forearm at 24 months of age had normal cognitive and motor development. The child with hydronephrosis had surgery at 4 months of age and at 24 months function in the affected kidney was 55% and normal in the other. The child with bowel duplication had surgery at 4 months of age and at 48 months was developing normally. One other child had a stroke at 40 days of age and was found to be MTHFR homozygous; at the age of 48 months there was language and motor delay, impaired vision and epilepsy. Another child had surgery at 5 months of age for intestinal invagination and subsequent follow up to 48 months showed normal development. Two children that originally were developing normally at 48 months of age were diagnosed with autistic spectrum disorder. All other children that were normal at birth had normal subsequent development.

DISCUSSION

Main findings

This study of diagnostic celocentesis in 402 singleton pregnancies has demonstrated that: first, celomic fluid was successfully aspirated in 99.8% cases; second, 67% of women reported none or only mild discomfort in association with the procedure, 18% moderate discomfort, 12% mild to moderate pain and 3% severe pain; third, prenatal diagnosis from analysis of the celomic fluid was successful in 93.8% of cases; fourth, in all cases of successful sampling and analysis of celomic fluid the diagnosis was concordant with results obtained from additional prenatal or postnatal testing; fifth, in addition, to diagnosis of hemoglobinopathies, QF-PCR analysis which was performed to evaluate maternal contamination using several markers for chromosomes X, Y, 21, 18 and 13, led to the accurate diagnosis of chromosomal aneuploidies; sixth, in all cases of affected fetuses diagnosed by celocentesis where the parents chose termination this was carried out at <10 weeks' gestation; seventh, in 97.1% (298/307) of the continuing pregnancies there was livebirth, in 7 (2.3%) there was miscarriage and in 2 there was loss to follow up; and eighth, fetal abnormalities were diagnosed in 3 (1%) cases, including unilateral transverse amputation of the forearm, unilateral moderate hydronephrosis and small bowel duplication.

Comparison with results of previous studies

The high success rate of celocentesis in obtaining a sample is consistent with the results of previous studies investigating early fetal physiology and the feasibility of this technique for prenatal diagnosis.[1,6,16,18-20] The concordance of results of celocentesis with those of subsequent prenatal or postnatal testing for both the hemeoglobinopathies and fetal chromosomal abnormalities is reassuring. Early studies had highlighted the high-risk of maternal cell contamination of the sample and the high failure rate of obtaining a result because of the very low number of embryo-fetal cells in the celomic fluid.[9,21] These problems have to a great extent now been overcome with the use of specific antibodies for positive selection of fetal cells and in cases of very high maternal contamination use of a micromanipulator to pick up individual cells.[9,13] Although in our whole series there was failure to obtain a result in 6% of cases due to heavy maternal contamination, in the last 121 cases of celocentesis prenatal diagnosis was always successful.

Our finding that the fetal heart rate is not altered by the procedure is consistent with that of our early experience with this technique.[22] We found that following celocentesis the rate of fetal loss was 3.3% and this decreased to 2.3% after exclusion of three lethal chromosomal abnormalities. Ultimately, the true procedure-related risk of fetal loss can only be established through a randomized trial in which celocentesis is compared to either no invasive testing or CVS. In the absence of such a study it is reasonable to assume that the risk may be only 1-2%. In a previous study of 447 women undergoing pregnancy termination for social indications 108 agreed to have celocentesis and the rate of fetal death between recruitment and the planned termination 11 (range 6-21) days later was 4.7% in the celocentesis group and 2.7% in the 339 controls (odds ratio, 1.804; 95% confidence interval, 0.5912-

5.504).[16]

Our finding of a transverse forearm amputation in one of our 307 continuing pregnancies is of some concern. A recent screening study in 100,997 singleton pregnancies attending for a routine ultrasound examination for fetal anatomy at 11-13 weeks' gestation reported that the incidence of limb amputations was 1 in 4,200.[23] Previous studies in relation to CVS had highlighted the possible association with transverse limb defects and with oromandibular-limb hypogenesis syndromes in cases where the procedure is carried out at <10 weeks' gestation.[11,24]

Implications for clinical practic

Analysis of cell free DNA in maternal blood provides effective screening for trisomies 21, 18 and 13, but the technique is usually carried out after 10 weeks' gestation because in earlier pregnancy the fetal fraction is low and the failure rate of the test is high.[25] Cell free DNA testing has also been used for prenatal diagnosis of autosomal recessive conditions, such as the hemoglobinopathies, but only in cases where the parents carry different mutations; in cases where the parents carry the same mutation the only available option for prenatal diagnosis at present is CVS or amniocentesis.[2] The main advantage of celocentesis, as an alternative to CVS or amniocentesis for invasive prenatal diagnosis is that it provides earlier diagnosis and potentially safer pregnancy termination for couples that choose this option. This is particularly important in Sicily and many Mediterranean countries where hemoglobinopathies are the most common indication for pregnancy termination for a fetal defect.

Another potential use for celocentesis could be intrauterine corrective therapy. Extensive studies have demonstrated that the injection of human stem cells in the celomic cavity of the preimmune sheep induced a significant and prolonged level of chimerism[27-29]. In humans, the celomic fluid contains embryonic erythroid precursors, or megaloblasts and the presence of such cells in the CF suggests cellular trafficking between the embryo and /or the yolk sac and the celomic cavity[14,30,31]. Injection of donor hematopoietic stem cells in the celomic cavity following very early prenatal diagnosis, could represent an alternative method for in utero cell transplantation and intrauterine treatment of genetic diseases such as hemoglobinopathies.

Strength and limitations

The main strength of our study is that it reports the feasibility and outcome of a large number of pregnancies undergoing prenatal diagnosis by celocentesis. The main limitation is that the number of cases is too small to define the possible association with limb reduction abnormalities.

Conclusions

Celocentesis can be used for early prenatal diagnosis of genetic abnormalities and the procedure-related risks of fetal loss appears to be low. However, widespread clinical implementation of the procedure necessitates close scrutiny to define a possible risk of procedure-related transverse limbs defects.

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Conflict of interest
None

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Figure legends

Figure 1: Ultrasound picture of 8-week pregnancy demonstrating the needle in the celomic cavity.

Figure 2: Study flow chart.