Amniocentesis-related adverse outcomes according to placental location and risk factors for fetal loss after mid-trimester amniocentesis

I. Kalogiannidis, S. Prapa, T. Dagklis, A. Karkanaki, S. Petousis, Y. Prapas, N. Prapas

Iakentro, Advanced Medical Center, Thessaloniki (Greece)

Summary

Purpose of investigation: Amniocentesis-related adverse outcomes in singleton pregnancies and possible risk factors for fetal loss after mid-trimester amniocentesis performed in a single institution were investigated. *Methods:* Amniocentesis-related adverse outcomes such as insufficient aspiration of amniotic fluid (AF), repeated puncture, and aspiration of hemorrhagic AF after mid-trimester amniocentesis were reviewed, while special consideration was given according to the placental location. Fetal loss rate up to 24 weeks of gestation and risk factors related to fetal losses were also investigated. *Results:* 5,948 cases with the inclusion criteria were analyzed. Advanced maternal age was the most common indication (53%) for amniocentesis. A need for repeated puncture was overall 2.1% (n = 128) and was associated with a fundal placental location. Aspiration of hemorrhagic amniotic fluid was observed in 3.7% (n = 222) and was significantly associated with an anterior or fundal placental position. Fetal loss rate was 0.3% and there was no relationship with advanced maternal age (≥ 35 years), gestational age at amniocentesis > 18 weeks, repeated procedure, aspiration of hemorrhagic AF or placental location. *Conclusion:* Anterior or fundal placental position is a risk factor for amniocentesis-related adverse outcomes, however without significant contribution to the fetal losses. Placental location, advanced maternal age, amniocentesis gestational age > 18 weeks, and the procedure's adverse outcomes seem to have no impact on fetal loss rate.

Key words: Amniocentesis; Mid-trimester; Fetal loss.

Introduction

Second trimester amniocentesis is the most common invasive procedure for prenatal diagnosis of chromosomal abnormalities [1]. The main indication for second trimester amniocentesis is advanced maternal age (≥ 35 years), with trisomy 21 being the most common chromosomal anomaly diagnosed after cytogenetic analysis [2, 3].

Today, enough data support the safety and efficacy of the procedure [4-6]. The potential risk for procedure-related fetal loss should be included in the counseling of women who opt for invasive testing. In current practice women are informed of a risk of about 0.5% for procedure-related pregnancy loss after mid-trimester amniocentesis [7], although recent studies suggest that this risk may be less [6].

The aim of the present study was to evaluate amniocentesis-related adverse outcomes, fetal loss rate and potential risk factors for fetal loss after mid-trimester amniocentesis. Special consideration was given to the evaluation of amniocentesis adverse outcomes according to placental location.

Methods

Mid-trimester amniocenteses for cytogenetic analysis performed in the period between September 1993 and February 2008 were reviewed. Data were collected from the Medical Center records and from the records of the Cytogenetic Laboratory of the same Institution. Multiple gestations were excluded, as well as cases with incomplete data or insufficient follow-up information. The Institutional Review Board approved the study. Informed consent had been taken from all women before the invasive procedure.

Indications for second trimester amniocentesis such as advanced maternal age (≥ 35 years), abnormal serum markers of first and second trimester screening for chromosomal anomalies, abnormal fetal ultrasound findings, previous pregnancy or a child with chromosomal or congenital defect, increased risk for a neural tube defect and family history were reviewed. Chromosomal abnormalities or congenital malformations affecting either the parents or close family were classified under the indication of family history. The association between abnormal karyotyping and indication of amniocentesis was calculated, as well as the distribution of the abnormal karyotypes of the study.

Aspiration of insufficient volume of amniotic fluid (AF), repeated puncture procedure and aspiration of hemorrhagic AF were considered as amniocentesis (procedure)-related adverse outcomes. The incidence of the previous outcomes after mid-trimester amniocentesis was calculated and a possible association between these and placental location was investigated.

Fetal loss rate of the population of the study was estimated and included all the unintended fetal losses up to 24 weeks of gestation. The registration desk of our institution performs the follow-up in all our cases by personal, phone, or postal communication with the women who perform the amniocenteses or with the referral physicians. Cases where parents opted for termination of pregnancy because of cytogenetic anomaly were not included. Potential risk factors for fetal losses such as advanced maternal age (≥ 35 years), gestational week at amniocentesis > 18 weeks, repeated puncture, aspiration of hemorrhagic AF and placental location were investigated.

Revised manuscript accepted for publication September 30, 2010

Amniocentesis procedure

All amniocenteses were performed by the same operator, under constant sonographic guidance. A "free-hand" technique with a 22G needle was used for amniotic fluid aspiration without local anesthesia. A local antiseptic was used before the insertion of the needle. A wide space of amniotic cavity free from umbilical cord or fetal segments was chosen for the aspiration of 20 ml of amniotic fluid for cytogenetic analysis. A transplacental puncture was avoided whenever possible. The first 1-2 ml of amniotic fluid was discharged to avoid maternal contamination.

Statistical analysis

The Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago, IL) was used for statistical analysis. The Student's t-test and Fisher exact test were used to calculate continuous variables, while chi-square was used to estimate cross-tabulated data. A p value of less than 0.05 was used to define statistical significance. All p values were two-sided.

Results

Out of 6364 amniocenteses performed in the period of the study, 5948 cases met the inclusion criteria. The mean maternal age was 34 ± 5 years. The mean gestational age at amniocentesis was 18 ± 1.5 weeks. Advanced maternal age was the most common indication (53%) for second trimester amniocentesis (Table 1). Overall, 129 cases (2.2%) with an abnormal karyotype were found. The distribution of the abnormal karyotypes by indication is presented in Table 1. Trisomy 21 was the most common cytogenetic anomaly of the study (50/129, 39%), while 47,XXY the most common sex chromosomal abnormality (Table 2).

Table 1. — *Indications for amniocentesis and abnormal karyotype*.

	Total n (%)	Abnormal karyotype n (%)
Maternal age (≥ 35 years)	3146 (53.0)	63 (2.0)
Family history*	333 (5.6)	10 (3)
Previous history**	186 (3.2)	2 (1.1)
Ultrasound abnormalities	654 (11.0)	17 (2.6)
NTD	51 (1.0)	0 (0.0)
Abnormal serum markers	1408 (23.6)	36 (2.6)
Others	170 (2.6)	1 (0.5)
Total	5948	129 (2.2)

*chromosomal defects and congenital malformations of parents or close family, **chromosomal or congenital anomalies in previous pregnancy or child, NTD: neural tube defect.

Insufficient aspiration of amniotic fluid (< 20 ml) occurred in 12 cases (0.2%). Overall, the procedure was repeated in 128 cases (2.1%), while in 222 cases (3.7%) the aspirated amniotic fluid was hemorrhagic. Analysis according to placental location showed that insufficient aspiration was more common in case of fundal placental position (2.1%, $p = 0.001$). The same was true concerning the repeated procedure, however did not reach any significant difference. Aspiration of hemorrhagic amniotic fluid was significantly more common in a case of anterior (4.5%) and fundal (4.5%) position of the placenta ($p = 0.01$) (Table 3).

Table 2. — *Distribution of the abnormal karyotypes of the study*.

Abnormal karyotypes	n	(%)
Autosomal chromosomal anomalies (n = 69)		
T 21	50	39
T 18	9	7
Others	10	7.8
Sex chromosomal anomalies (n = 17)		
45,X	5	4
47,XXX	4	3
47,XXY	7	5.4
48,XXXX	1	0.8
Unbalanced DNA abnormalities (n = 43)	43	33
Total	129	100

T: trisomy.

Table 3. — *Amniocentesis-related adverse outcomes, as well as according to placental position*.

Placental position	n (%)	Insufficient VAF n (%)	RP n (%)	HAF n (%)
Anterior	2,966 (49.7)	7 (0.2)	70 (2.4)	134 (4.5)*
Posterior	2,707 (45.5)	0	49 (1.8)	77 (2.8)
Fundal	179 (3.2)	2 (2.1)*	6 (3.4)	8 (4.5)*
Lateral	96 (1.6)	3 (0.1)	3 (3.1)	3 (3.1)
Total	5,948	12 (0.2)	128 (2.1)	222 (3.7)

VAF: volume of amniotic fluid, RP: repeated puncture procedure, HAF: hemorrhagic amniotic fluid.
* $p < 0.05$ (chi square was used to estimate p value).

Table 4. —*Estimated risk factors for fetal loss up to 24 weeks of gestation*.

Placental position	Fetal loss rate n (%)	p value
Maternal age		
≥ 35 years	7/3578 (0.2)	NS
< 35 years	8/2370 (0.3)	
Gestational week		
> 18 weeks	6/2384 (0.25)	NS
≤ 18 weeks	9/3564 (0.25)	
Repeated puncture procedure		
Repeated puncture	0/128 (0)	NS
Single puncture	15/5820 (0.26)	
Aspiration of hemorrhagic AF		
Hemorrhagic AF	0/222 (0)	NS
Clear AF	15/5726 (0.26)	
Placental location		
Anterior	7/2966 (0.2)	NS
Posterior	7/2707 (0.3)	
Fundal	0/179 (0)	
Lateral	1/96 (1)	

NS: non significant, AF: amniotic fluid.

Fetal loss rate in the present study was 0.3% (n = 15). In two out of 15 cases there was a chromosomal abnormality and spontaneous abortion occurred within two weeks after amniocentesis (12 and 14 days, respectively). Analysis of potential risk factors for fetal loss showed that there was no increased risk for fetal loss when amniocentesis was performed after 18 weeks of gestation (> 18 weeks, n = 9 vs ≤ 18 weeks, n = 6; p = NS). Similarly, advanced maternal age (≥ 35 years) was not a risk factor for fetal loss (≥ 35 years; n = 7 vs < 35 years; n = 8, p =

NS). Procedure-related adverse outcomes and placental location did not significantly influence the fetal loss rate in the present study (Table 4).

Discussion

A single institution large series of second trimester amniocentesis was analyzed in the present study. Amniocentesis-related adverse outcomes were rare after mid-trimester amniocentesis and were associated with an anterior and fundal placental location. There was no correlation of fetal loss rate up to 24 weeks of gestation with advance maternal age, amniocentesis gestational age > 18 weeks, the procedure's adverse outcomes and location of the placenta.

Advanced maternal age was the leading indication for mid-trimester amniocentesis, which remained stable during the period of the study. This is consistent with previous reports [3, 8-10]. In a recent report on a large series of 120,000 amniocenteses, 72% of these were performed because of advanced maternal age and this rate also remained stable during the period of the study [2]. An abnormal karyotype was found in 2.2% of our cases, which is similar to the rate of 2% to 5% reported in the literature [2, 3, 8-10]. Trisomy 21 was the most common chromosomal anomaly in the present study as in previous reports [2, 3, 10].

Fetal loss rate is a critical point in invasive prenatal diagnosis and special consideration must be given to offering careful counseling to the parents. The different methodologies that have been used in previous studies makes a review of the literature difficult and the outcomes controversial. Additionally, the studies related to pregnancy loss after mid-trimester amniocentesis are mainly retrospective, with lack of control groups and different follow-up periods, running from two weeks after amniocentesis up to the end of pregnancy. In these studies the fetal loss rate ranged from 0.2% to 3% [3, 8, 9, 11-15], which is in accordance with our 0.3% fetal loss rate. The amniocentesis fetal loss rate that was reported by the Center for Disease Control and Prevention in 1995 was 0.5% [7]. A similar rate was reported in a meta-analysis by Seeds in 2004, where 29 studies with more than 68,000 mid-trimester amniocenteses (at least 1,000 procedures for each study) were included in the final analysis [12]. The author concluded that procedure-related fetal loss rate up to 28 weeks, when only case-controlled studies were analyzed was 0.6%. The fetal loss rate of the amniocentesis group (1.68%) compared to the control group (1.08%), was not significantly different [12]. Evidence from randomized studies related to pregnancy loss is weak. Tabor *et al*., in 1986 in a randomized controlled trial of 4,606 women reported a 1% difference of spontaneous abortions between the amniocentesis (1.7%) and the control group (0.7%) [4]. Similar results were shown by the Canadian Early and Mid-Trimester Amniocentesis Trial Group (CEMAT), in the same setting of patients, while the total post-amniocentesis abortion rate up to 20 weeks including spontaneous and therapeutic abortion in the Canadian study was 2.9% [5]. However, a recent publication working on pregnancy loss after second trimester amniocentesis dramatically changed the current evidence related to amniocentesis fetal loss rate. In the FASTER Trial, 15 centers from the United States showed an extremely low procedure-related fetal loss rate of 0.06% (1/1.600) in a follow-up period of less than 24 weeks of gestation [6]. Challenging the current recommendation related to fetal losses after mid-trimester amniocentesis, the FASTER Trial demonstrated no significant difference in pregnancy loss between the amniocentesis and control group (1% vs 0.94%, $p = 0.74$) [6].

An analysis of potential risk factors related to fetal losses of our database showed no association with advanced maternal age (≥ 35 years), which is in accordance with previous reports [11]. However, others demonstrated significantly higher risk of fetal losses in selective analyses of women > 40 years (5.1%) compared with women aged 20-34 years (2.54%) [16]. Repeated puncture procedures did not influence the rate of fetal losses according to the present data. Kong *et al*., in a similar analysis, showed no significant association between fetal losses and number of punctures [11]. Concerning the gestational week at amniocentesis, previous studies reported that mid-trimester amniocentesis performed later than 18 weeks of gestation was associated with an increased risk for fetal losses [11, 17], which was not confirmed by our data. Placental location had on impact on the fetal loss according to the results of the present study. Consolidating the former prospect, another study showed that the transplacental puncture procedure during mid-trimester amniocentesis was insignificantly associated with pregnancy loss [11]. Different methodology and operator experience may give a possible explanation in the variability of the results between the different studies.

The present study has certain limitations, such as the retrospective nature and lack of control analysis, although the majority of the current evidence was based on observational cohorts and with lack of control groups. Moreover, this was one of the largest series derived from a single institution for prenatal diagnosis and a single laboratory for cytogenetic analysis. The follow-up period related to fetal loss rate was constricted up to 24 weeks of gestation and may considered by some as too short. However, it is already known that almost 60% of pregnancy losses are observed in the first two weeks and 90% up to four weeks after an amniocentesis procedure [6].

Conclusion

The present study showed that procedure-related adverse outcomes such as repeated puncture, aspiration of hemorrhagic or insufficient AF had a low incidence and were associated with an anterior or fundal placental position. Fetal loss rate was 0.3% and according to the present data no correlation was found with advance maternal age, amniocentesis gestational age > 18 weeks, procedure's adverse outcomes and location of the placenta.

References

[1] Brambati B., Tului L.: "Chorionic villous sampling and amniocentesis". *Curr. Opin. Obstet. Gynecol.,* 2005, *17,* 197.

[2] Nagel H.T.C., Knegt A.C., Kloosterman M.D., Wildschut H.I.J., Leschot N.J., Vanderbussche F.P.H.A.: "Prenatal diagnosis in the netherlands, 1991-2000: Number of invasive procedures, indications, abnormal results and terminations of pregnancy". *Prenat. Diagn.,* 2007, *27,* 251.

[3] Horger E.O., Finch H., Vincent V.A.: "A single physician's experience with four thousand six hundred genetic amniocenteses". *Am. J. Obstet. Gynecol.*, 2001, *185,* 279.

[4] Tabor A., Madsen M., Obel E., Philip J., Bang J., Gaard-Pedersen B.: "Randomized controlled trial of genetic amniocentesis in 4606 low-risk women". *The Lancet,* 1986, *327,* 1287.

[5] The Canadian Early and Mid-Trimester Amniocentesis Trial (CEMAT) Group: "Randomized trial to assess safety and fetal outcome of early and mid-trimester amniocentesis". *The Lancet,* 1998, *351,* 242.

[6] Eddleman K.A., Malone F.D., Sullivan L., Dukes K., Berkowitz R.L., Kharbutli Y. *et al*. for the First and Second Trimester Evaluation Risk (FASTER) Trial Research Consortium: "Pregnancy loss rates after mid-trimester amniocentesis". *Obstet., Gynecol.,* 2006, *108,* 1067.

[7] Centers for Disease Control and Prevention: "Chorionic villous sampling and amniocentesis: recommendations for prenatal counselling". *MMWR Recomm Rep.,* 1995, *44,* 1.

[8] Chaabouni H., Chaabouni M., Maazoul F., M'Rad R., Jemaa L.B., Smaoui N. *et al*.: "Prenatal diagnosis of chromosomal disorders in Tunisian population". *Ann. Genet.,* 2001, *44,* 99.

[9] Sirivatanapa P., Wanapirak C., Chanprapaph P., Sirichotiyakul S,. Chanprapaph P., Yampochai A. *et al*.: "Prenatal diagnosis: 10-years experience". *J. Med. Assoc. Thai*., 2000, *83,* 1502.

[10] Caron L., Tihy F., Dallaire L.: "Frequencies of chromosomal abnormalities at amniocentesis: Over 20 years of cytogenetic analyses in one laboratory". *Am. J. Med. Genet*., 1999, *82,* 149.

[11] Kong C.W., Leung T.N., Leung T.Y., Chan L.W., Sahota D.S., Fung T.Y. *et al*.: "Risk factors for procedure-related fetal losses after mid-trimester genetic amniocentesis". *Prenat. Diagn.,* 2006, *26,* 925.

[12] Seeds J.W.: "Diagnostic mid trimester amniocentesis: How safe?". *Am. J. Obstet. Gynecol*., 2004, *191,* 608.

[13] Crandall B.F., Howard J., Lebher T.B., Rubinstein L., Sample W.F., Sarti D.: "Follow-up of 2000 second-trimester amniocenteses". *Obstet. Gynecol.,* 1980, *56,* 625.

[14] Sant-Cassia L.J., MacPherson M.B., Tyack A.J.: "Midtrimester amniocentesis: is it safe? A single centre controlled prospective study of 517 consecutive amniocenteses". *BJOG,* 1984, *91*, 736.

[15] Blackwell S.C., Abundis M.G., Nehra P.C.: "Five-year experience with midtrimester amniocenetsis performed by a single group of obstetricians-gynecologists at a community hospital". *Am. J. Obstet. Gynecol*., 2002, *186*, 1130.

[16] Papantoniou N.E., Daskalakis G.J., Tziotis J.G., Kitmirides S.J., Mesogitis S.A., Antsaklis A.J.: "Risk factors predisposing to fetal loss following a second trimester amniocentesis". *Br. J. Obstet. Gynecol*., 2001, *108,* 1053.

[17] Roper E.C., Konje J.C., De Chazal R., Duckett D.P., Oppenheimer C.A., Taylor D.J.: "Genetic amniocentesis: gestation-specific pregnancy outcomes and comparison of outcome following early and traditional amniocentesis". *Prenat. Diagn.,* 1999, *19,* 803.

Address reprint requests to:
I.A. KALOGIANNIDIS, M.D.
4 A. Svolou str.
54622 Thessaloniki (Greece)
e-mail: kalogiannidis@mailbox.gr