Reducing risks in invasive genetic procedures

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Abstract

Objective. *Fetal loss evaluation after amniocentesis (intrauterine demise or miscarriage) 4 weeks after procedure or before 24 weeks' gestation in case of prophylactic protocol of medication and day monitoring associated to amniocentesis.* ***Materials and methods.*** *The study was conducted between 2008 and 2010 and included 143 singleton pregnancies, which underwent amniocentesis at 16-19 weeks in 24 hours hospitalization conditions with morphological evaluation of fetus, cervix and placental insertion. Antibiotics (cefuroxime axetile 2 g/day, orally) were administered 24 hours before starting the procedure and continued 4 days after. Tocolysis (indometacinum and, optionally, progestin), was maintained at least 24 hours after the complete procedure. Our results were compared with the ones suggested by literature.* ***Results and discussions.*** *There was no pregnancy failure in our group study.* ***Conclusions.*** *This approach has never been used or published. Preliminary results suggest improvement of prognostic in amniocentesis, under proposed protocol. A detailed analysis based on a large database is needed having in the view the cost price/benefit report.*

Keywords: *amniocentesis, risk, prophylaxis, protocol.*

Introduction

Although transabdominal amniocentesis is the oldest invasive procedure in prenatal diagnosis[1] it also represent the election method for late antenatal diagnosis - representing 90% of the used methods, despite the fact that it has not been standardized until now.

Generally, the specimen is obtained through discontinuous vacuum, created by a syringe, manually, without any pre-interventional preparation or post-interventional follow-up.

Available literature has noted superior abortion and preterm birth rates in pregnant women that underwent amniocentesis when compared to the rest of the pregnancies with an average of 1% fetal loss rate[1-5]. In many clinics is considered that the abortion rate directly caused by the procedure would be less than 1%, and a number of reports, mostly un-audited[6,7], have found even a slightly decreased risk in relation with amniocentesis.

Different protocols were proposed in studies aimed to investigate the effect of the antibioprophylaxy associated to amniocentesis, resulting conflicting views and no general decision concerning this matter[8-10]. Tocolytic prophylaxis was also investigated, with respect to the risk of spontaneous abortion within 4 weeks after the procedure, fetal loss rate within the 22nd week of gestation, also to the rate of preterm premature rupture of membranes. Use of progesterone prophylaxis did not revealed satisfactory results randomized controlled trial regarding the frequency of miscarriage, preterm delivery or neonatal outcome[11].

It seems to meet the terms regarding the impact of the ultrasound technique. The use of contemporary concurrent ultrasound guidance appears to reduce the number of punctures and the incidence of bloody fluid when compared to only pre-amniocentesis ultrasound evaluation. It is worth mentioning that it was associated with a reduced rate of loss when all studies were compared, but not among controlled studies[12].

The purpose of this paper is to evaluate pregnancy's fetal loss rate at less than 4 weeks after the invasive investigation, as well the miscarriage and intrauterine fetal death before 24 weeks of amenorrhea, in the group that have followed prophylactic protocol. The role of this study is to assess the potential benefit of the prophylactic protocol associated to the maneuver - oral antibiotic prophylaxis (cefuroxime/ampicilin) started with 24 hours before the maneuver, tocolysis with NSAIDs and hospital day monitoring following the maneuver.

Materials and Methods

The experimental study was realized between nov. 2008 - sep. 2010 in a group of 148 singleton pregnancies that were submitted to invasive genetic diagnosis using mid-trimester amniocentesis, at the gestational age of 16-19 weeks.

Recommendation for amniocentesis and subsequent genetic evaluation of the fetal karyotype was given to pregnant women that were included in the high-risk genetic group, taking into account the results of the following chromosomal screening methods: combined test (figure 3) for the first trimester pregnancies and triple-test or integrated-test (cut-off 1/250), in cases without first trimester evaluation, willingly-chosen by women that previously were offered genetic counseling for screening methods. Other accepted indications were considered fetal morphologic anomalies, parenteral or familial history of abnormal karyotype. The cases in which amniocentesis was performed in later gestational ages or comported other indications (eq. for dosing alpha-fetoprotein in amniotic fluid in case of an elevated risk for neural tube defects) were all excluded from our group. Also patients with history of reccurent abortion, preterm labour, gestational diabetes, bronchitis asthma, arterial hypertension pre-existing to pregnancy, patients with cervix length less than 24 mm, patients with other associated diseases that could not be subjected to protocol, were excluded from our group.

We were able to provide a complete monitoring in 96.6% of the cases (143 pregnancies), with a lost-to-follow-up of 3.4% (5 pregnancies).

Approval for the study was obtained from the hospital Ethics Committee. The procedure was performed after the counseling was completed and written consent of the patient was obtained. It consisted of hospitalization, complete ultrasound evaluation of the fetal morphology, placental and cord insertions, amniotic fluid amount; cervical length was evaluated transvaginally in all cases. Acquisition of ultrasound markers was realized using the ultrasound machine Voluson 730 Pro, GE Medical Systems Kretztechnik, OHG4871 ZIPF Austria, while evaluation of the genetic risk used Wallac LifeCycle 3, PerkinElmer Life and Analytical Sciences software equipment.

Amniocentesis was performed without local anesthesia, ultrasound-guided. It was used a standard 8.89 cm long spinal needle, with bright tip on ultrasound. The sample consisted of 10-20 ml amniotic fluid extracted in approximately 20-25 seconds, while trying to avoid as much as possible the placental puncture. As a precaution, we removed the first 0.5 ml of the amniotic fluid, in order to decrease the sample's risk of contamination with maternal cells. After the procedure, fetal heart-rate was monitored and recorded (figure 1).

Samples were personalized for transport and a standard amniocentesis form was filled in.

Antibiotherapy, consisting of second generation cephalosporin (cefuroxime axetile 2g/day, po), was already initiated 24h before the procedure in the study group; NSAID (indomethacin) was used for tocolysis and optionally 200 mg progestative recommended by her own obstetrician (mainly in relation to the pregnancy history), 24h before and after the procedure.

Patient was reexamined the following day, at 24 hours after the procedure: evaluation of fetal well-being, subjective estimation of the amniotic fluid amount, aspect of the placental insertion and transvaginal cervix length measurement were performed (figure 2).

We recommended in the study group continuation of antibiotic therapy for 4 days following procedure and only when necessary long-term tocolysis under different protocols (progestative/indomethacine/nifedipine or different associations) taking into account particularities of each case, especially to the cervix length of the patient, as it is known to be an important predictive marker of miscarriage or preterm labor.

In all cases the procedure was performed using the same technique, by the same physicians.

The groups were homogenous regarding gestational age in the moment of the procedure, quantity of amniotic fluid extracted, period of time used to take a sample, type of needle used, examination technique, ultrasound machine.

Figure 1. A) evaluation of fetal heart-rate at 1 minute, B) at 3 minutes and C) at 15 minutes after amniocentesis

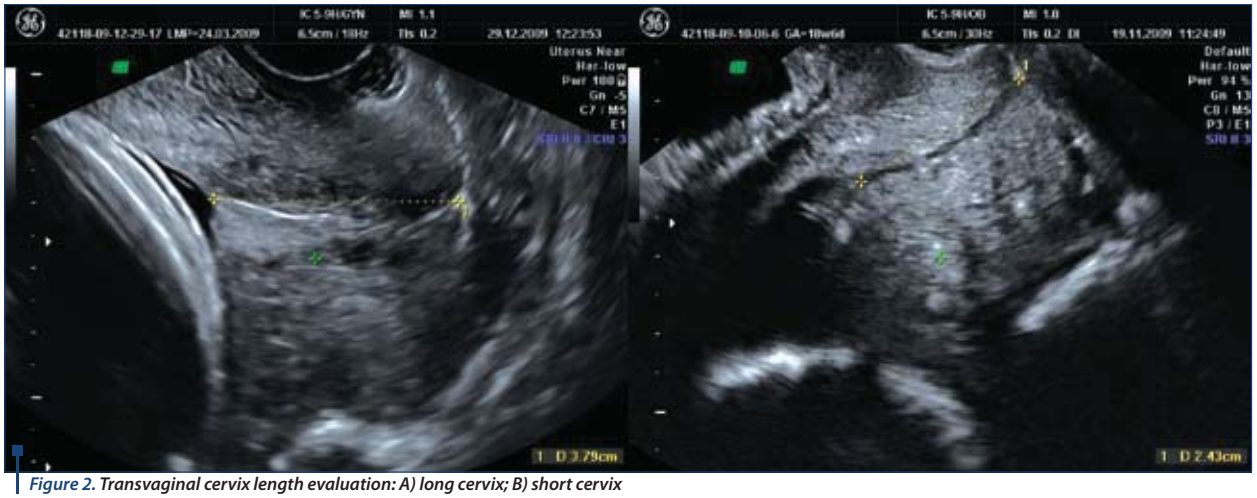

Figure 2. Transvaginal cervix length evaluation: A) long cervix; B) short cervix

Results and discussion

The results were obtained from medical charts of 148 patients treated in a one-day hospitalization period are presented in table 1 and chart 1.

In the study group there were no failures of the pregnancy regarding the 2 main parameters that we followed (intrauterine death of the fetus or spontaneous abortion at less than 4 weeks from the maneuver or before 24 weeks of gestation) (figures 3 and 4). Parameters undergoing analysis in the studied group are presented in table 2.

We further evaluated the variation of the cervical length measured at 24 hours after the procedure and compared the findings to a matched (gestational age, cervix length, needle type, number of punctures, placental penetration, quantity of amniotic fluid extracted, average time of the procedure) control group in which amniocentesis was performed without medical prophylaxis or day-hospitalization. A decrease in cervical length was recorded in both groups, but in the control group, without medical prophylaxis, the decrease was significantly more important (6.9mm compared to 3.2mm). However, in both groups, the mean cervical length after the procedure did not felled under the 30mm cut-off.

We also were interested in the variation of cervical length in procedure-related settings such as placental puncture, considering that post-procedure intra-amniotic hemorrhage was visualized by ultrasound in 30% of these cases and is considered to be associated with an increased adverse outcome (figure 4). We noted a decrease of the mean cervical length both in the study and control group, but the variation was smaller in the study group (3.5mm) and similar to the variation recorded in the cases without placental penetration during amniocentesis in the respective group (3.2mm). In the control group the decrease was more important (8.1mm) and perhaps these cases benefit the most when prophylactic protocol is applied.

Our search in international databases did not reveal studies investigating cervical length variation secondary to invasive procedures. Our results are based on small series, but suggest that cervical length may serve as a parameter for quantification of different tocolytic agents

Table 1 Follow-up of the patients that underwent amniocentesis

Studied lot	Outcome	Number	Percent	Completely analyzed	
				No.	%
148	Normal genetic testing	139	93.92	143	96.62
	Chromosome anomalies	4	2.7		
	Excluded (lost to follow-up)	5	3.38	5	3.38

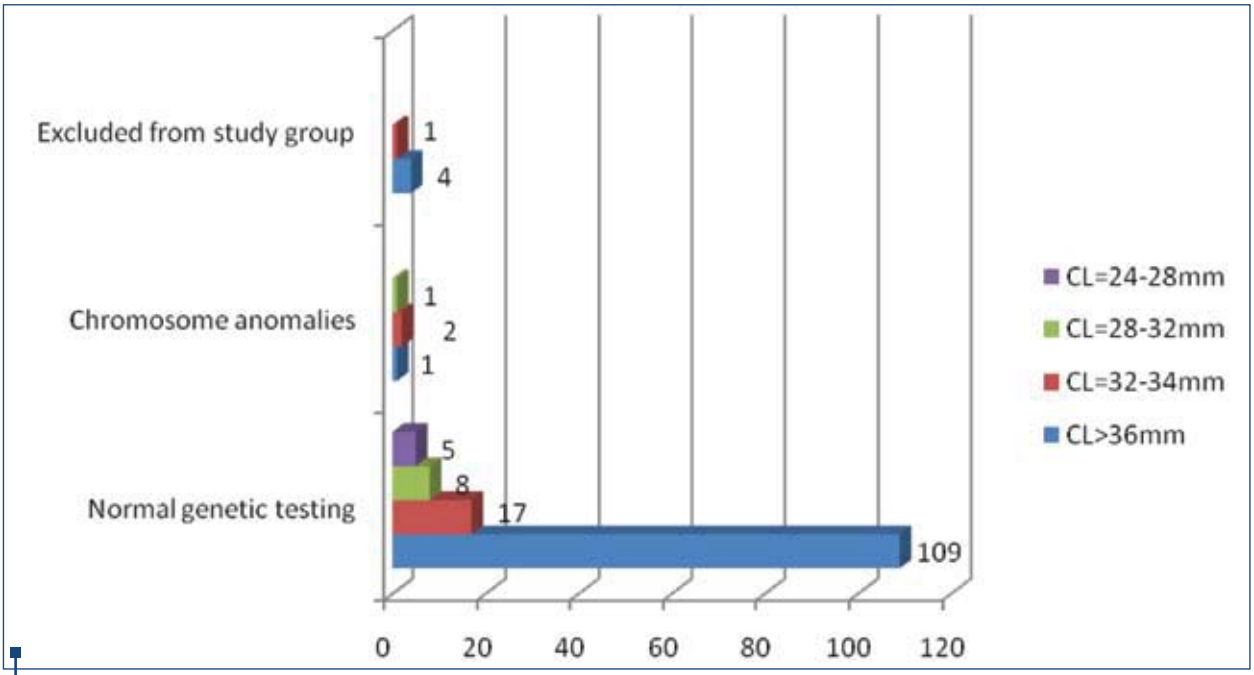

Chart 1. Structure of the studied lot according to genetic outcome and cervical length prior to the intervention. CL: cervical length

Table 2 Parameters undergoing analysis in the studied group

Evaluated parameter	Mean
Gestational age	17.4 gestational weeks (16-19 GW)
Cervix length	38 mm (24-56 mm)
Quantity of amniotic fluid extracted (ml)	16 ml (10-20 ml)
Number of punctures	1,027 (1 puncture performed in 145 procedures, 2 punctures necessary in 2 amniocentesis, 3 punctures performed in 1 case)
Macroscopically polluted samples (hemorrhagic amniotic fluid)	17 samples (11.48%)
Placental penetration	43 cases (29.05%)
Intra-amniotic hemorrhage ultrasound-visible	13 cases (8.78%)
Associated uterine fibroids	15 cases (10.14%)
Morphologic fetal anomalies	9 cases (6.08%)
Timpul mediu de desfăşurare	13 seconds (11-31 seconds)

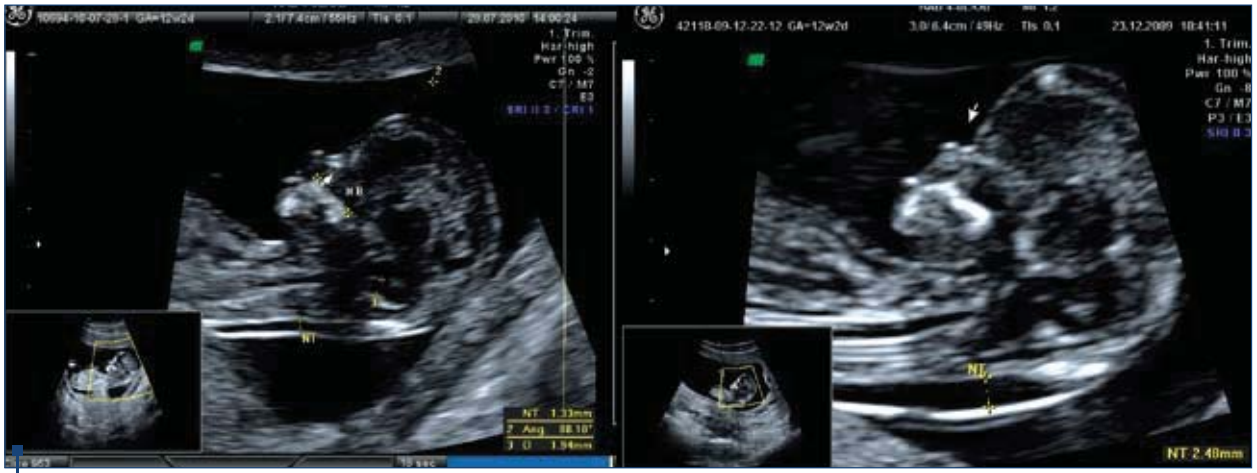

Figure 3. First trimester genetic ultrasound assessment according to Fetal Medicine Foundation criteria. Sagittal section of the fetal face showing: A) normal relations (nuchal translucency, frontal-maxillary-facial angle, nasal bone); B) abnormal ultrasound markers - absent nasal bone and increased nuchal translucency

efficiency in reducing the risk of miscarriage secondary to invasive genetic procedures. Also it would be interesting to assess the cervical length's dynamic in the event of amniocentesis performed in short cervix cases.

A very low risk of abortion associated to the procedure - 0.15% - was published in 2004 in the study "First and Second Trimester Evaluation of Risk" FASTER trial(13), conducted by the Society of Fetal-Maternal Medicine. There were presented information regarding patients that underwent amniocentesis in the second trimester (group of 1605 patients) which was compared with those that did not take the procedure (26187 patients), suggesting a post-procedural abortion rate at less than 24 GW of only 0.15%. However, recent comprehensive reviews show a total abortion rate of 1.9% in the case after

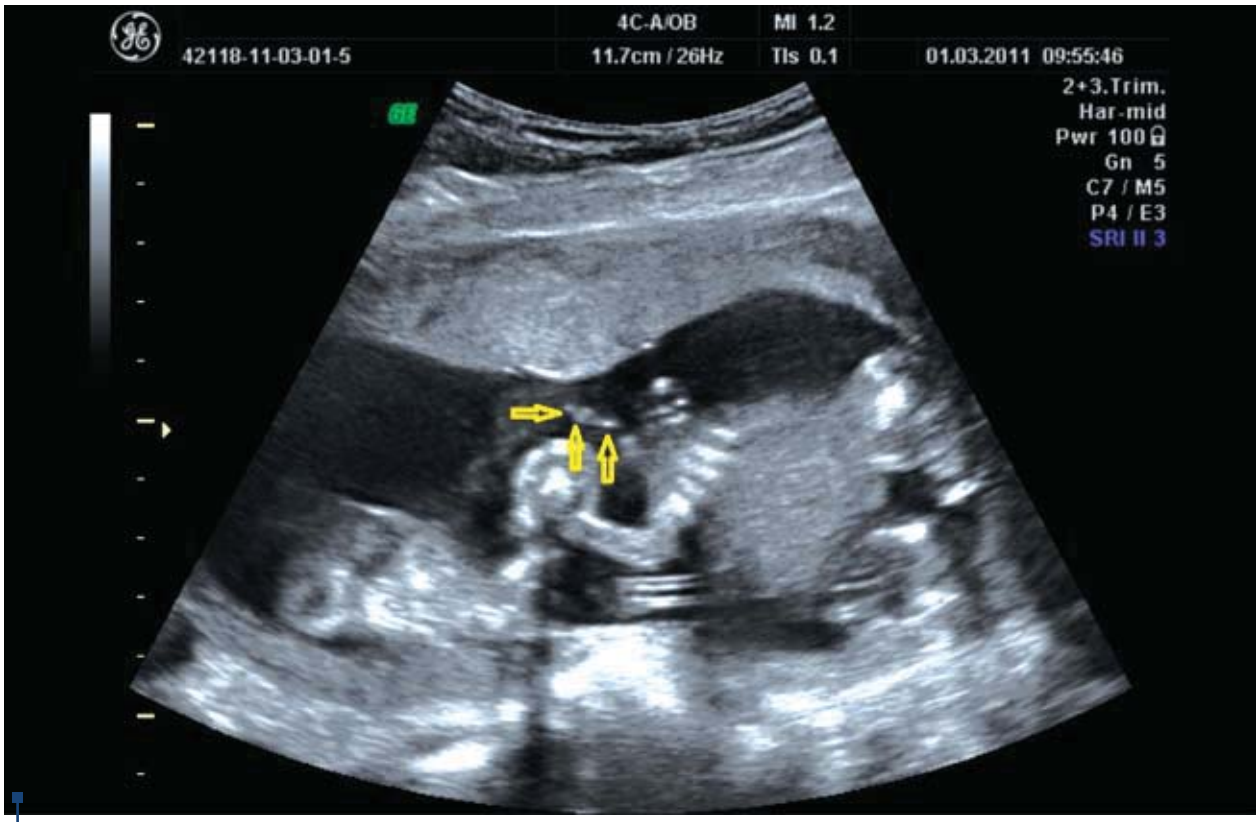

Figure 4. Intra-amniotic hemorrhage

amniocentesis and 2% after CVS(14). Muller et al.(15) studied a group of 3472 patients that underwent amniocentesis, with a control group of 47004 patients, finding an abortion rate of 0.7%. Tabor et al.(16) have analyzed a group of 32285 patients that underwent amniocentesis between 1996 and 2006 and defined "post-procedural abortion" the failure of a pregnancy through spontaneous abortion or intra-uterine demise before 24 weeks of gestation. The research group observed a spontaneous abortion rate of 1.4% (95% CI, 1.3-1.5) after amniocentesis and 1.9% (95 CI, 1.7-2.0) after CVS. Although results in studies made on large groups of patients have quite an important variation, it is universally recognized and accepted a 1% rate of miscarriage after undergoing amniocentesis(17). Following similar criteria we published our data in a previous research(18), remarking a 1.7% fetal loss rate after amniocentesis without any medical prophylaxis associated to the procedure, numbers that were positively correlated with previous studies. Spontaneous termination of pregnancies with chromosome defects may influence the fetal loss-rate of the pregnancies, taking into account the fact that these fetuses have a high risk of intra-uterine demise(19).

Large scale introduction of first trimester screening has determined a migration from amniocentesis to CVS as invasive first trimester screening test(20) and a decrease of the false positive rate. Currently, the sero-sonographic screening methods of selecting high-risk pregnancies for invasive genetic maneuvers have a detection rate of approximately 83-95% for the first trimester assessment, and 70-87% for the second trimester evaluation, for a 5% false-positive rate. Taking into account the real incidence of the most common diagnosed chromosomal abnormalities, it is easily recognizable that the vast majority of the invasive maneuvers are recommended and performed in pregnancies carrying perfectly normal fetuses.

Rates of fetal structural abnormalities requiring genetic analysis, along the rates of suspected neural tube defects in which it is recommended alpha-fetoprotein and acetyl-cholinesterase dosing from amniotic fluid, as well as other indications of late amniocentesis, show the fact that prevention of complications related to this maneuver start to have a demographic importance. Taking into consideration the prevalence of the suspected genetic, structural and metabolic anomalies, we can estimate an unacceptable high rate of normal pregnancies that are lost using this invasive maneuver.

Prophylaxis for reducing fetal loss rate in normal pregnancies can be done using two possibilities: reducing rate of amniocentesis (by increasing the specificity of screening techniques that may lead to invasive obstetrics) and reducing the complications related to the technique itself. Improving the specificity of genetic screening tests has experienced an impressive improvement during last decades; the policy of chromosomal screening performed nowadays almost halved the proportion of women performing invasive tests(16), but no further rise of specificity rate is possible without decreasing the sensibility. The concomitant improvement of the ultrasound systems and the increasing of the number

of well-trained examiners have significantly contributed to recommendations toward invasive genetic testing for structural anomalies, so the incidence of the maneuver was not reduced in time. Therefore, in these settings the only practical solution is to decrease the rates of adverse outcome related to the technique itself, by limitation the number of punctures, use of medical prophylaxis (antibiotherapy and/or tocolysis) and limitation of the procedure to experienced and audited centers/operators.

Using antibiotherapy associated to tocolysis in this small series we did not experienced any adverse outcome of pregnancy related to the procedure. In a previous research on a larger series(18) we obtained a similar outcome, using a different tocolytic agent (beta-mimetic). However, large prospective studies are required to confirm these findings. We consider the study of cervical length variation as a potential argument for the use of medical prophylaxis, because the use of prophylactic protocol seems to decrease the adverse effect secondary to the invasive procedure. Also, we noted the same benefic effect when using beta-mimetic in the study mentioned above.

Conclusions

Preliminary results suggest an improvement in invasive procedures outcome such as mid-trimester amniocentesis, when using combined medical prophylaxis (antibiotherapy and tocolysis). Results seem to be independent of the number of punctures, placental penetration, maternal age or the gestational age and cervix length, taking into account the limits we presented (16-19 weeks of gestation, cervix length >24mm). There is still needed detailed analysis based on randomized controlled trials with larger database to take into consideration the cost/benefit report. ■

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