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Feto-maternal hemorrhage after amniocentesis and cordocentesis – Implications on the prevention and treatment of Rh-alloimmunized pregnancy

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ABSTRACT

Objective: *i) To compare the frequency and the amount of feto-maternal hemorrhage (FMH) after amniocentesis and cordocentesis, ii) evaluate if placental penetration and prolonged procedure increase the risk for the appearance of FMH, and iii) to assess the significance of obtained results in the treatment of Rh-alloimmunized and Rh-negative-nonimmunized pregnancies.*
Design: *Prospective study*
Setting: *University Clinic of Gynaecology and Obstetrics*
Methods: *The study group consisted of 305 pregnancies that underwent prenatal karyotyping including 165 for amniocentesis and 140 for cordocentesis. Coomb's test was negative in all pregnancies. Fetal-maternal hemorrhage (FMH) was detected in maternal blood samples taken immediately before and one hour after the procedure. We used maternal serum alpha-fetoprotein levels (MSAFP) measurement and an increase in the second sample of 25% or more than the first sample as an indication for FMH. For the quantification of FMH original Kleihauer-Brown-Betke (KBB) test was used, and FMH was expressed in fetal erythrocyte concentration, and in percentage of total fetal-placental blood volume. After each intervention, the duration of the procedure, transplacental or extraplacental needle pathway, and number of stings used for the intervention were registered. We compared the incidence, the amount of FMH, incidence of placental penetration and procedural time.*
Results: *Gestational age in amniocentesis group was earlier when compared to the cordocentesis group (16.5 $\pm$1.3 vs 24.2 $\pm$2.4 gestational weeks). Silent FMH was registered in one case (0.3%). De novo FMH was registered in 8 pregnancies after amniocentesis and in 41 after cordocentesis using KBB test (4.8% vs 29.3%); while in 24 women after amniocentesis and 55 women after cordocentesis had elevated MSAFP levels (14.5% vs 39. 3%), showing statistical difference between the used methods. Following the cordocentesis greater mean FMH volume (0.80 $\pm$0.56 vs 4.10 $\pm$2.72), greater individual levels of the volume range, more frequent severe FMH (> 5ml of fetal erythrocytes), and greater percentage loss of total fetal-placental blood volume (0.90 $\pm$0.60 vs 4.49 $\pm$2.69) were registered. In the cordocentesis group there were more frequent placental penetration (11.52% vs 52.14%), and increased duration of the procedure, > 3 min (4.24% vs 22.86%). FMH was more frequent after using both procedures due to placental penetration.*
Conclusions: *We suggest that before and after each intrauterine intervention MSAFP levels should be determined in Rh-negative-nonimmunized pregnancies. If FMH is undetected, a minimal dose of 50 μg of anti-D-imunoglobulin (RhIg) should be applied. If MSAFP levels suggest the occurrence of FMH, KBB test should be performed for the quantification of FMH. If FMH values are less than 2.5ml of fetal erythrocytes, 100 μg of anti-D-imunoglobulin should be given, and for levels greater than 2.5ml, 300 μg (or more) of anti-D-imunoglobulin could be administered.*

Key words: *feto-maternal hemorrhage, amniocentesis, cordocentesis, Kleihauer-Brown-Betke test, alpha-fetoprotein measurement, Rh-alloimmunization*

INTRODUCTION

Amniocentesis and cordocentesis present irreplaceable invasive methods for prenatal diagnostics and therapy, especially in Rh-alloimmunized pregnancies. Indications for the type and frequency of fetal monitoring, time of pregnancy termination, and fetal intravascular transfusion (FIVT) are based on the amniotic fluid and fetal blood tests obtained by these procedures. The methods employed are reliable in prenatal management of Rh-alloimmunized pregnancies.

In fact in the 1960s, amniocenteses were performed predominantly for the purpose of predicting severity of erythroblastosis[1]. The procedures, often multiple, were carried out between 21 and 37 weeks' gestation, frequently in physicians' offices, as there was no method other than clinical acumen, of determining an amniocentesis site that would avoid needle trauma to the placenta.

Woo Wang et al[2], using a Kleihauer fetal cell screening technique[3] capable of demonstrating a fetal transplacental hemorrhage greater than 0.5 mL of fetal blood, detected fetal transplacental hemorrhages ranging from 0.58 to 58 mL in the blood of 10.8% of 74 Rh-immunized women examined after amniocentesis. The true prevalence of fetal transplacental hemorrhages in their series might have been greater than 10.8% because maternal blood samples were not taken until 30 minutes after the amniocenteses in nine of 15 patients in whom the procedure was difficult.

Peddle[4], reported data from the Winnipeg Rh laboratory in which a Kleihauer fetal cell screening method capable of detecting 0.1 mL of fetal red cells in the maternal circulation was used[5]. He studied 410 consecutive amniocenteses performed on 248 Rh negative-immunized pregnant women. Forty-six procedures (11.2%) were associated with fetomaternal transplacental hemorrhages of from 0.1 to 13 mL of red cells. Fifty-five percent of the women who experienced a fetal transplacental hemorrhage had a more than two-tube Rh antibody titer rise compared with a more than two-tube titer rise in only 20% of women who had not experienced a fetal transplacental hemorrhage at the time of amniocentesis. Therefore, one of the hazards of amniocentesis was placental trauma, fetal transplacental hemorrhage, rising antibody titers, and increasing severity of erythroblastosis if the mother was Rh immunized. If she was Rh negative but not immunized, fetal transplacental hemorrhage significantly increased the risk of Rh immunization. Rarer hazards from placental trauma at amniocentesis were fetal exsanguination and abruptio placentae.

One would expect that localization of the site of implantation of the placenta would markedly reduce, possibly even eradicate, placental trauma and fetal transplacental hemorrhages at the time of amniocentesis. Studies conducted by Pauls and Boutros[6], using Rh laboratory statistics, reported the use of a Cr^{51}-labeled red cell technique for localizing the placenta before amniocentesis. A total of 70 patients underwent amniocentesis after Cr^{51} placental localization. Kleihauer screening tests, revealed no detectable fetal cells in the circulations of the 70 women after amniocentesis.

This Cr^{51}-radiolabeled red cell placental localization is, however, cumbersome and expensive. Routine placental localization before amniocentesis have been rendered safe and efficient with the development of ultrasonography, which was rapid, accurate, convenient, and inexpensive. Such methods are available and are used in the authors' tertiary level fetal assessment units.

As a complication, both amniocentesis and cordocentesis may cause fetal-maternal hemorrhage (FMH), and worsening of the existing, or the appearance of de novo alloimmunization[7–11]. Bibliographic data of the frequency and the amount of these FMH and immunization risks are few and contradictory.

To determine the effectiveness of ultrasound placental localization in prevention of fetal transplacental haemorrhage, this investigation was carried out in which amniocentesis was always preceded by ultrasound placental localization. The objective of our study is to: 1) compare the frequency and the amount of fetal-maternal hemorrhage after amniocentesis and cordocentesis, 2) evaluate if placental penetration and prolonged procedure increase the risk for the appearance of FMH, and 3) indicate the significance of obtained results in the treatment of Rh-alloimmunized and Rh-negative nonimmunized pregnancies.

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MATERIALS AND METHODS

The study group consisted of 305 normal pregnant women subjected to amniocentesis or cordocentesis for fetal karyotyping. The method employed was based on gestational age – amniocentesis was done between 16 and 20 gestational weeks in 165 women while cordocentesis was used between 20 and 30 weeks in 140 cases. All these cases were singleton pregnancies, with negative indirect Coomb's test, and without any fetal anomalies, intrauterine growth restriction, infection, hypertension, and low-lying placenta. Any case of previous pregnancy bleeding (threatened abortion) and previous invasive procedures (amniocentesis or cordocentesis) was carefully evaluated in the two months preceding these invasive procedures. Amniocentesis and cordocentesis were carried out under continuous real time ultrasound guidance with 20-gauge needles, and "free hand" technique, using the color Doppler ultrasonic apparatus ALOKA SSD 1700. During the amniocentesis we avoided placental penetration, if possible. The optimal place for cordocentesis was considered to be placental insertion of the umbilical cord, and was approached, regardless of the placental localization. After each intervention we registered: the duration of the procedure, transplacental or extraplacental needle pathway, and if one or two stings were used. Procedure was considered to be prolonged if it lasted more than 3 minutes.

FMH was detected in maternal blood samples taken immediately before the intervention (for the detection of silent FMH or FMH caused by previous intervention or threatened abortion) and one hour after the intervention for the detection of FMH caused by the procedure itself. We used "acid elution tests", originally described by Kleihauer-Braun-Betke, and maternal serum alpha-fetoprotein levels (MSAFP) measurement.

MSAFP levels were used in the following way: if there was an increase in the sample taken after the procedure of 25% or more of the first value, we considered that FMH has occurred[12]. MSAFP levels were determined using the hemiluminiscency method and the original apparatus DPC, IMMULITE.

We used the original method of acid-elution test described by Kleihauer-Brown-Betke (KBB test). Fifty low-power microscope fields were observed and if fetal erythrocytes were seen, another 200 cells were examined for the determination of their percentage. The following formula was used: FMH volume (blood ml) = mother blood volume (ml) × no. of fetal erythrocytes over no. of maternal erythrocytes. When this value is divided by the fetal hematocrit, volume of FMH, expressed in ml of fetal erythrocytes is obtained. The fetal hematocrit is taken as 0.50. The minimum detection limit in our laboratory is 0.05 ml of fetal erythrocytes[13].

FMH was also expressed in percentage of total fetal-placental blood volume, which was calculated as 120 mg/kg of estimated fetal weight. Fetal weight was estimated before the intervention during routine ultrasonic examination, using the Haddlock and Shepard formula.

Severe FMH was defined as a hemorrhage of 5ml and more of fetal erythrocyte volume. KBB test was considered reliable for the detection of FMH. Our technologists are very skilled and experienced in performing these procedures. Commercial screening kits were not used, because they are rather complicated and expensive. The incidence of beta thalasemia and other hemoglobinopathies connected with the increase of maternal hemoglobin F are very small in our population, and therefore of no significance to the validity of this test[14–17].

Statistical analyses included the Pearson's, Chi square test, Fisher's exact test, Mann Whitney U test, Students' t-test and calculation of relative risk and odds ratios. Differences were considered statistically significant when $p < 0.05$.

RESULTS

The study group consisted of 305 women with uncomplicated pregnancies, the only risk factor being maternal age over 35 years. Considering the procedure type, gestational age in amniocentesis group was minor to cordocentesis group (16.5 ± 1.3 vs 24.2 ± 2.4 gestational weeks). Amniocentesis was performed in 165 women, while cordocentesis was conducted in 140 cases (Table 1). There were no significant differences between the groups with respect to maternal age, body weight and parity. The Coomb's test was negative in all these parameters examined.

Before the procedure, FMH was registered in only one case in which cordocentesis was done, presenting a frequency of 0.3%. In this particular case, the volume of FMH was 0.05ml of fetal erythrocytes which essentially remained the same following this procedure, implying that FMH was not the consequence of the procedure itself. This case could be interpreted as silent FMH, because it wasn't preceded by bleeding (threatened abortion) in 60 days period prior to the invasive procedure. During this 60-day period, bleeding was reported in 37 women, unsuccessful procedure occurred in 17 (14 amniocentesis and 3 cordocentesis).

D novo FMH was observed in 8 pregnancies after amniocentesis and in 41 pregnancies after cordocentesis using the KBB test (Table 1). The

frequency difference (4.8% vs 29.3%) was statistically significant. MSAFP test was positive in 24 pregnancies after amniocentesis and 55 after cordocentesis, which was also of statistical significance (14.5% vs 39.3%) (Table 1). Significant differences were also noted between the two laboratory investigations employed including the KBB test and MSAFP levels in these groups of patients.

After cordocentesis we observed a greater mean FMH volume, greater individual values of the volume range, significant increases in severe FMH (> 5 ml of fetal erythrocytes), and greater loss of total fetal-placental blood volume (estimated as 120 ml/kg of average fetal mass for gestational age) (Table 2).

Placental penetration occurred in 19 amniocentesis (11.52%) and 73 cordocentesis (52.14%). When analyzed together, FMH is more frequent after needle insertion into the placenta (Table 3) with the odds ratio (OR) = 12.92; confidence interval (CI = 6.20 – 26.95). The procedure was prolonged in 7 cases subjected to amniocentesis (4.24%) and in 32 women undergoing cordocentesis (22.86%). FMH was significantly associated with longer duration of the procedure (Table 3); with OR of 4.89 and CI between 2.35 and 10.20. No statistical differences were found in both variables in the group of women subjected to amniocentesis (needle pathway and the intervention duration).

The largest individual volume of FMH (9.75 ml) was registered after the cordocentesis where placenta was penetrated three times, with duration longer than 5 minutes.

TABLE 1
Characteristics and frequency of fetal-maternal hemorrhage after amniocentesis and cordocentesis using Kleihauer-Braun-Bettke test and increase of alpha-fetoprotein levels

	N (%)	FMH after the intervention (KBB positive test) N (%)	FMH after the intervention (MSAFP positive test) N (%)
Amniocentesis	165 (54.1)	8 (4.8)	24 (14.5)
Cordocentesis	140 (45.9)	41 * (29.3)	55 † (39.3)
Total	305 (100)	49 (16.1)	79 (25.9)

FMH – fetal-maternal hemorrhage
AFP – alpha-fetoprotein
KBB test – Kleihauer-Braun – Betke test
* – Pearson Chi – square test, Chi square = 33.54; p = 0.000 < 0.001
† – Pearson Chi – square test, Chi square = 24.15; p = 0.000 < 0.001

TABLE 2
Amount of fetal-maternal hemorrhage expressed as ml of blood containing fetal erythrocytes following amniocentesis and cordocentesis

	Mean value (ml)	Value range (ml)	> 5 ml n (%)	% of total fetal-placental blood volume
FMH Amniocentesis N = 8	0.80 ± 0.56	0.05 – 1.45	0	0.90 ± 0.60
FMH Cordocentesis N = 41	4.10 ± 2.72 *	0.05 – 9.75 *	14 (34.15) †	4.49 ± 2.69 ‡

FMH – fetal-maternal hemorrhage
* – Mann Whithey U test, z = 3.57; p = 0.000 < 0.001
† - Fisher's exact test; p = 0.001 < 0.01
‡ - Mann Whitney U test, z = 3.21; p = 0.001 < 0.01

TABLE 3
The frequency of fetal-maternal hemorrhage after amniocentesis and cordocentesis based on the needle pathway and duration of the intervention

Needle pathway and procedure duration	N – total (AC + CC) (%)	FMH – total N (%)	FMH - after AC N (%)	FMH – after CC N (%)
Transplacental	92 (19 + 73) (100)	38 (41.3)	5 (5.4)	33 (35.9)
Extraplacental	213 (146 + 67) (100)	11 * (5.2)	3 (1.4)	8 † (3.8)
< 3 min	266 (158 + 108) (100)	33 (12.5)	5 (1.9)	28 (10.6)
> 3 min	39 (7 + 32) (100)	16 ‡ (41.0)	3 (7.7)	13 § (33.3)

FMH – fetal-maternal hemorrhage
AC – amniocentesis
CC – cordocentesis

* – Student's proportion test, t = 6.75; p = 0.000 < 0.001
† – Student's t-test, t = 6.21; p = 0.000 < 0.01
‡ – Student's t-test, t = 3.51; p = 0.00051 < 0.001
§ – Student's t-test, t = 2.92; p = 0.003 < 0.01

DISCUSSION

In this study, we have considered the group of women with non-immunized pregnancies for investigations on frequency and amount of FMH after the amniocentesis and cordocentesis. If this study was performed in the alloimmunized pregnancies group, FMH values would be considerably lower due to influence of maternal antibodies on fetal erythrocytes.

Our results suggest that the FMH frequency after the cordocentesis procedure (29.3%) is 6.1 times greater than after the amniocentesis (4.8%). We have also observed that the frequency of severe FMH (> 5 ml) after the cordocentesis technique is significantly higher. This can be explained by longer duration and frequency of placental puncture and by greater mechanical trauma with this procedure.

Moreover, the shorter life-span of the fetal erythrocytes in maternal circulation is compounded by the fact that we didn't register FMH after 54 immunized events during pregnancy (threatened abortion with the bleeding, previous amniocentesis and cordocentesis), occurring in the time interval of 60 days before these procedures. It seems plausible to suggest that the shorter life-span of fetal erythrocytes could be due to accelerated erythrocyte destruction because of ABO incompatibility between mother and fetus, a larger cellular volume, or by some unknown mechanism.

The results of Bowman and associates[7] suggest even higher incidence of FMH after cordocentesis (57.5%), which is 15 times higher than after the amniocentesis (3.6%) and even around 150 times higher than incidence of spontaneous FMH (0.3%). These authors, in the Rh-alloimmunized group with FMH caused by invasive diagnostic procedures, registered significant growth of antibodies between 50 – 83% of all the cases.

In addition, Bowel and associates[8] found that amniocentesis has an increased risk of alloimmunization of about 30%, while the risk estimate for cordocentesis is 75%. The data of Wiener and associates[9] appeared to be contradictory in that cordocentesis has the same risk as the amniocentesis in inducing FMH and rarely leads to a detrimental alloimmunization. Chitrit and co-workers have reported an incidence of FMH after cordocentesis of 38.8%[10]. Sikovanyecz et al found the incidence of FMH after this procedure to be 36.7%[11].

MSAFP levels have been used in the detection of FMH in many studies. Fuhrman and associates used increase of 20% and more of MSAFP in the diagnosis of FMH after the chorionic villus sampling[6], while Nicolini and co-workers used a 50% increase in MSAFP levels as an indicator of FMH[12].

In our opinion, however, the KBB test, if well-performed, still represents a valid test for the detection of FMH, especially as a screening model[18]. This test should be performed by a team of skilled technologists to ensure reproducability of results using this laboratory investigation[16].

There was a significant difference between the two tests employed in the diagnosis of FMH. MSAFP levels were elevated in 25.9%, while KBB test was positive in 16.1% of all cases. The differences observed could be explained in two ways. As KBB test detects FMH equal to or higher than 0.05ml of fetal erythrocytes, MSAFP may detect even a small extent of hemorrhage in such cases. The other explanation could be attributed to the fact that MSAFP levels may give a certain number of false positive results as a consequence of AFP leakage from amniotic fluid to maternal circulation. On this basis it was suggested by Emery and co-workers that the KBB test should be performed in the cases of unexplained increase of MSAFP levels[15]. In this investigation, we observed a significant difference in FMH frequency which was dependent on the needle pathway – transplacental (41.3%) or extraplacental (5.2%), as well as on the duration of the intervention – 41% after prolonged intervention, and 12.5% after lasting up to 3 minutes. Nicolini and associates[18] reported a more frequent FMH after transplacental (65%) than extraplacental (17%) cordocentesis. Increased risk that follows placental penetration suggest that major risk for the occurrence of the FMH is not dependent on the type of procedure employed, but direct mechanical interruption of the placental membrane integrity. This risk could be decreased, in a certain number of cases with placental insertion on the anterior uterine wall, by puncturing the free umbilical loop via the extraplacental pathway. This could be due to the instability of the free loop when compared with the placental insertion of the umbilical cord, making this area a less favorable place for performing FIVT.

The number with two or more stings is small and doesn't allow statistical analysis. In our experience the worst cases of FMH occurred after the prolonged cordocentesis with placental penetration and duration of more than 3 minutes.

Severe FMH (> 5ml) after cordocentesis was usually associated with longer duration, placental penetration and two or more placental punctures. Similar results were reported by Chitrit et al[10] and Sikovanyecz et al[11], though they evaluated only cordocentesis.

In the treatment of Rh-alloimmunized pregnancy, if the benefit from amniocentesis and cordocentesis, as the method of choice in evaluating fetal anemia is equal (measuring bilirubin concentration in amniotic fluid and fetal hemoglobin and hematocrit), amniocentesis could be the method of choice, since it is associated with only a slightly increased risk of alloimmunization.

The clinical significance of FMH determination is in individualization of immunoprophylactic dose of anti-D-immunoglobulin (RhIg) after the immunizing effects during pregnancy. The current recommended dose of 250–300μg RhIg neutralizes 30ml of D-positive fetal blood (15ml of fetal erythrocytes). Our results shows that FMH after the cordocentesis procedure could overcome this critical amount.

We suggest that before and after each intrauterine intervention, MSAFP levels should be determined in Rh-negative-non-immunized pregnancies. If there is an increase of 25% and more of AFP values, we can conclude that FMH occurred during the intervention.

A minimal doze of 50μg of anti-D-immunoglobulin (RhIg) should be administered if FMH is not detected. However, if MSAFP levels suggest the occurrence of FMH, KBB test should be performed for the quantification of FMH. Based on our clinical experience, if FMH is less than 2.5ml of fetal erythrocytes, 100ug of anti-D-immunoglobulin may be given, and for FMH greater than 2.5ml, 300μg (or more) of anti-D-immunoglobulin is recommended.

In conclusion, amniocentesis in the postultrasound placental localization era carries with it a reduced but still significant risk of fetal transplacental hemorrhage. For this reason, alloimmunized women should have amniocenteses carried out only if they meet the criteria outlined. The nonimmunized Rh-negative woman should be given 300μg of Rh immunoglobulin after amniocentesis.

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