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Middle cerebral artery Doppler in prediction degree of fetal anemia and the best timing for the second intrauterine intravascular transfusion in red cell alloimmune disease

I. Babović[1,2], S. Plešinac[1,2], Z. Radojičić[4], O. Antonović[1,2], R. Sparić[2], D. Plećaš[1,2], N. Radunović[1,2,3]

[1] *School of Medicine, University of Belgrade, Belgrade*
[2] *Department of Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade*
[3] *Serbian Academy of Sciences and Arts, Belgrade*
[4] *Faculty of Organizational Sciences, University of Belgrade, Institute for Statistics, Belgrade (Serbia)*

Summary

Aim: To determine the role of fetal multiples of the median of middle cerebral artery peak systolic velocity (MoM MCA-PSV), predicts the rate of decline in fetal hematocrit (Hct) for determination of the best timing for the second intrauterine intravascular transfusion (IUIVT) in fetuses with Rh alloimmunisation. *Materials and Method*: Retrospective study of 59-monofetal alloimmunized pregnancies from 2005 to 2012 that underwent first and second IUIVT were assessed in Department of Gynecology and Obstetrics, Belgrade, Serbia. *Result:* There was an inverse statistically significant correlation between measurements MCA MoM-1 and fetal Hct-1 before the first IUIVT r = - 0.622; $p = 0.001$ and MCA-MoM-3 and Hct-3 before the second IUIVT r = - 0.381; $p = 0.001$, also as the significant correlation between the interval between both procedures (expressed in day) and measurement MCA-MoM-3, before the second IUIVT r = - 0.284; $p = 0.029$. *Conclusion:* The measurements MoM-MCA before every IUIVT can be useful for prediction of the best timing for the next IUIVT.

Key words: Doppler; Hemolytic disease; Hydrops fetalis; Intrauterine intravascular transfusion; RhD antibodies.

Introduction

The introduction in the late 60s and 70s of prophylactic anti-D immunoglobulin (IG) for RhD negative women has changed the landscape of hemolytic disease of fetus and newborn (HDFN) and counts as one of the great of success stories in modern medicine. However, is a still remains a relevant pregnancy complication in some countries, mainly because of failure in prophylaxis [1].

Hemolytic anemia due to Rh D antibodies can be of different intensity**.** Therefore, diagnosing fetal anemia in a non-invasive and accurate way is fundamental [2-4]. Doppler assessment of the fetal middle cerebral artery (MCA) peak systolic velocity (PSV) has emerged as the best tool for predicting fetal anemia in at-risk pregnancies. It is based on the principle that the anemic fetus preserves oxygen delivery to the brain by increasing cerebral flow of low viscosity blood [5].

Standarized fetal hematocrit (z-Ht) was defined as the number of the standard deviations (SDs) from the normal mean for gestational age. Severe fetal anemia was defined as zHct ≤ -5 SDs. Mary *et al.* have proposed the cut off value for multiples of the median of middle cerebral artery peak systolic velocity (MoM MCA-PSV) of 1.29 for mild and one of 1.50 MoM for moderate, as finally MoM-MCA of 1.55 for severe anemia. These cutoff values results in 100% sensitivity, based on their retrospective analysis of 111 fetuses. The sensitivity of increased MCA-PSV above 1.5 MoMs for the prediction of moderate or severe anemia was 100 percent (95% CI 86-100), either in the presence or absence of hydrops [6].

The first fetal blood sampling was indicated in pregnancies with an antibody titer 1:64 Coombs (critical titer in the present laboratory), when Δ OD 450 reached Liley zone 3 or the upper zone 2, or MCA-PSV values of >1.50 multiples of the median (MoM). IUT is an effective treatment for severe fetal anemia. Perinatal loss occurs in about 1.6% of the procedures. Additional complications include thrombosis v. umbilicalis (includes emergency cesarean section), infection, and rupture of the membranes. [7, 8] Fetal medicine teams aim at optimizing the number of intrauterine intravascular transfusions (IUIVTs) and avoiding unnecessary procedures.

After the first IUIVT, most centers time subsequent procedures based on the expected rate of decline in fetal hemoglobin (Hb) or hematocrit (Hct) levels or MCA PSV measurements. Indeed, Scheier *et al.* showed that MCA PSV is useful in the prediction of fetal anemia in the second transfusion but less accurate for the third transfusion [3]. Possibly, this is due to hemodynamic changes induced

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by the presence of transfused adult cells in the fetal circulation. Recent studies show an estimated fetal Hct drop of 0.7% to 1% per day. This parameter depends on the presence of fetal hydrops, because of the association between fetal hydrops and higher fetal Hct decline [9].

Since 1987, Department for Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade has been the national referral center for the management and intrauterine treatment of fetal anemia. The first IUIVT was performed on November 1987. In over 22 years, 498 IUIVTs were performed in 149 fetuses. The incidence of HBFN due to red cell Rh alloimunization in Serbia is 1.5-2%.

The aim of the present study was to determine standardized MCA peak velocity MoM-MCA as a predictor fetal Hct decrease between first and second IUIVT, which indicated the best time for the second IUIVT for fetal anemia due to red-cell alloimunization.

Materials and Methods

Fifty-nine monofetal Rh D alloimunized pregnancies were retrospectively studied at the Department of Gynecology and Obstetrics, Clinical Center of Serbia, from January 2005 to January 2012.

A computer database search was performed to identify all pregnancies with maternal Rh D alloimmunization that underwent first and second IUIVTs during the study period.

The ultrasound and Doppler examination was performed using an ultrasound scanner with 3.75 MHz curvilinear probe. Axial section of brain, including thalami, cavita septi pellucidi was obtained and the Circle of Willis was identified. All Doppler measurements were performed with the angle between the ultrasound beam and the direction of the blood flow as close to 0° as possible and never exceeding 30°. If the angle was > 0°, an angle correction was applied. MCA-PSV measurements were performed before IUIVT (24 hours) and the day after (12-24 hours).The highest point of the flow velocity waveform (peak systolic velocity) was measured Mari's normograms in MoM established for various gestational ages used standardized MCA peak velocity (MoM-MCA). The maximum velocity was measured when a uniform Doppler signal of at least three seconds was obtained. Ideally, MoM-MCA is measured in the resting state.

The volume of blood to be transfused was calculated using the formula described by Plećaš *et al*. [10] at each procedure was calculated to achieve a post transfusion fetal hematocrit level equivalent to 40-50%.

$$\text{Volume (ml)} = 169.43\text{-}13.29\ (\text{GA}) + 0.274\ (\text{GA}^2) - 4.17\ (\text{Hct increase}) + 0.209\ (\text{GA x Hct increase}),\ r^2 = 0.85$$

Standarized fetal hematocrit (z-Ht) was defined as the number of the SDs from the normal mean for gestational age. The fetal IUIVT was immediately carried out when fetal Hct was 20-25% or the cut off value for MoM MCA of 1.29 for moderate anemia or the cut off value for MoM-MCA of 1.50 for severe anemia.

The rate of fetal Hct fall after the first IUIVT was calculated by dividing the difference between the post-transfusion (post-2 Hct) and the pre-transfusion Hct (pre-Hct-3) at the second IUIVT and interval in days between both transfusions.

$$\text{Hct decline (\% / day)} = \text{post-transfusion (post-Hct-2)} - \text{pre-transfusion (pre-Hct-3) / time interval between IUIVT-1 and IUIVT-2}$$

Table 1. — *First and second intrauterine intravascular transfusions data from 59 alloimunized pregnancies.*

	Intrauterine intravascular transfusion	
	First	Second
Gestational age (weeks)	26.46 ± 3.96	28.37 ± 4.18
Middle cerebral artery*		
PSV (cm/sec)	53.66 ± 17.07	60.32 ± 16.67
MoM	1.58 ± 0.20	1.54 ± 0.18
Hct (%)		
Before	0.24 ± 0.09	0.27 ± 0.07
After	0.41 ± 0.08	0.42 ± 0.07

*Doppler assessment before transfusion; Hct = hematocrit;
PSV = peak systolic velocity; MoM = multiples of the median;
SD = standard deviation. Values expressed as mean ± standard deviation.

From the study the authors excluded: pregnancies submitted to only one IUIVT and cases in which post-transfusion or pre-transfusion blood samples were not obtained, multifetal pregnancies, intrauterine fetal demise and suspected fetal congenital malformations and fetal anemia due to other antibodies (C, Kell).

Results are described as mean, SD, and relative frequencies. Hct mean decline rates were compared between medical procedures with paired t test. Pearson's correlation test was used to determine variables that correlate significantly with rate of fetal Hct decline. Statistical analysis included: gestational age at each procedure, standardized middle cerebral artery peak systolic velocity just before and after the IUIVT, fetal Hct levels before and after the first transfusion, and volume of blood transfused. Continuous variables were presented as mean (SD as 95% confidence interval, CI) assessed for normality.

Results

During the study period, 59 singleton pregnancies underwent first and second IUIVTs to treat fetal anemia because of Rh alloimmune disease. Thirty-one women (52.54%) had previous history: 19 (32.2%) moderate titer (1:64) and 12 (4.83%) were with high (1:128) and of anti-D antibodies. Hydrops was present at the first IUIVT in 28/59 (47.45%) fetuses.

First IUIVT was performed at a mean gestation 26.46 (SD 3.9), and mean volume of blood transfused was 38.3 ml (SD16.8). The mean pre-transfusion Hct-1 was 24% (SD 9%), the mean post-transfusion Hct-2 was 41% (SD 8%). The mean pre-transfusion Hct-3 before second IUIVT was 27% (SD 7%) Mean interval to the second procedure was 12.19 (SD 6.04) days and average Hct decline rate 1.13%/day. Table 1 summarizes data on first and second transfusions.

The PSV-MCA decreased immediately after transfusion in 58 cases but no changed in one case. This study also showed that MCA MoM-1 before the first IUIVT exhibits inverse statistically significant correlate with pre-transfusion Hct-1 r = - 0.622; $p = 0.001$ and MCA-MoM-2 with post-transfusion Hct-2 r = - 0.512; $p = 0.001$. The study also exhibited a significant correlation MCA-MoM-3 with

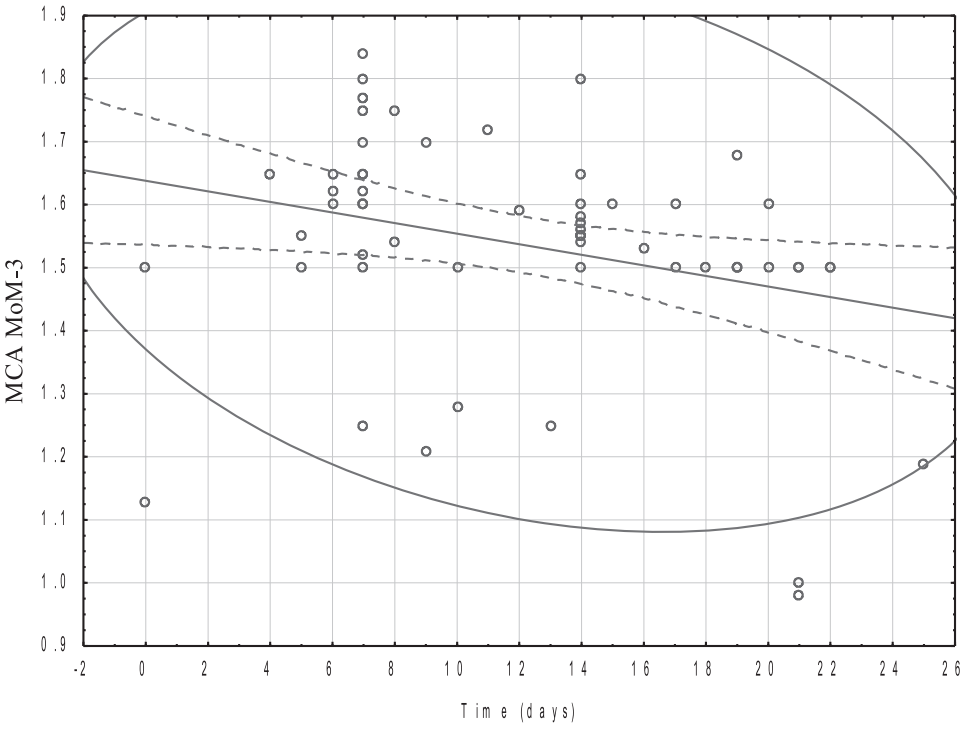

Figure 1. — There is insignificant negative correlation between the interval between both procedures (expressed in day) (T) and measurement MCA MoM-3 before the second IUIVT r = - 0.284; p = 0.029. *T-interval between both procedures (expressed in day). **PSV-MCA MoM-3 before the second IUIVT/ (multiples of the median of middle cerebral artery peak systolic velocity).

pre-transfusion Hct-3 before the second IUIVT. r = - 0.381; p = 0.001.The sensitivity of pre-transfusion MCA-MoM-1 for severe anemia before the first IUIVT was 100% (95% CI for difference 78.12 - 100%) and the sensitivity of pre-transfusion MCA-MoM-3 before the second IUIVT was 88.89% (95% CI for difference 63.93 - 98.05%). The specificity MCA-MoM-3 before the second IUIVT was 51.22% (95% CI for difference 35.37 - 66.85), slightly higher than the specificity MCA-MoM-1 before the first IUIVT was 24.39% (95% CI for difference 12.91 -40.64).

Pearson's test demonstrated that mean decline rate in fetal Hct levels between first and second IUIVT (expressed in percentage/day) insignificant correlate with post-transfusion Hct level (Hct-2) r = - 0.047; p = 0.755 and pre-transfusion Hct-3 r = - 0.012; p = 0.939.There were also insignificant correlations between the mean decline rate in fetal Hct levels and measurements MCA-MoM-2 r = 0.109; p = 0.469 after the first IUIVT as so as between the mean decline rate in fetal Hct levels and MCA-MoM-3 before second IUIVT r = - 0.038; p = 0.804.

The study documented significant negative correlation between the interval between both procedures (expressed in day) (T) and measurement MCA MoM-3 before the second IUIVT r = - 0.284; p = 0.029 (Figure 1).

There were also insignificant correlations between mean decline rate in fetal Hct levels between first and second IUIVT and the volume of blood to be transfused r = 0.276; p = 0.063, as well as between interval between both procedures (T) r = 0.169, p = 0.261.

Discussion

IUIVTs for severe fetal anemia are performed between 19 to 34 weeks of gestation in the present Department (Table 1). Before 18 weeks, fetal transfusions are rarely successful due to limited visualization and small size of the relevant anatomic structures [11]. As Plećaš *et al.* have reported [10] after 34 weeks, the procedure is generally considered riskier than late preterm delivery and neonatal treatment of severe anemia.

As Egberts *et al.* reported [12], significant correlation of mean decline rate and time interval between both procedures mean the study was not determinated. In fact, it has been shown that transfused adult red cells destruction in nonlinear fashion, possibly reflecting mechanical effects of hemoconcentration, and biochemical effects of a fetal circulation on transfused adult red cells and membranes. Besides this, adult red cells are supposed to have a reduced lifespan in more severely anemic fetuses. Sumacher *et al.* [13], and Egberts *et al.* [14] have hypothesized that more fetal red cells disappear during the first days after the transfusions.

The most centers that treat alloimunized pregnancy consider an average decline rate of 0.3 to 0.4g/dl Hb per day, to calculate the expected fetal hemoglobin concentration at the time of the second transfusion [9]. In the present study, the authors notice that the average decline rate of 1.13 %/ Hct per day. Scheier *et al.* [3] have reported that MCA PSV exhibits a significant correlation with fetal hemoglobin concentration before the first and before the second IUIVT. However, the present study confirmed statistical signifi-

cant correlations between MCA MoM-1 and fetal Hct-1 before the first IUIVT, as so as between MCA MoM-3 and fetal Hct-3 before the second IUIVT. It can be useful to time subsequent transfusions. As Nishie *et al.* [15] have reported studies on the evaluation of fetal myocardial performance may help establish the best moment for fetal treatment before myocardial function is affected. Radunović *et al.* [16] previously reported that volume of blood to be transfused could increase fetal Hct and viscosity. The fetal cardiac failure would be eventually developed [17]. Nishie *at al.* [15] showed that MCA Doppler prediction performance is slightly lower in subsequent transfusions compared with first time transfusions. In the present study, the authors found a sensitivity of pre-transfusion MCA-MoM-1 for severe anemia before the first IUIVT was 100% and the sensitivity of pre-transfusion MCA-MoM-3 before the second IUIVT was 88.89% in the prediction of severe fetal anemia (cut-off value for severity anemia MCA MoM $\geq$ 1.5). The present study documented that MCA Doppler can be useful to predict severity of fetal anemia before the first as before the second IUIVT. The sensitivities are always lower in the prospective than in the retrospective series.

The study did not demonstrate statistical significance between MCA-MoM-3 before the second IUIVT and the mean decline rate in fetal Hct levels between first and second IUIVT (expressed in percentage/day). Hct value is described as relative number or percentage, as well time interval in day. The present authors documented that all of three variables are correlated (Hct-2), (Hct-3), and the third MCA MoM insignificant correlate to interval between both procedures (expressed in day), as well as the fetal hematocrit mean decline rate insignificant correlate to interval between transfusions. During IUIVT we documented changes only in fetal hematocrit, although after IUIVT a relatively large fetal blood volume exists in the vascular space, the fetal blood volume does not change in 24 hours. It is known though, that through loss of plasma from fetal circulation, after packed red cell transfusion, the fetal blood volume increases by only half of the transfused volume As Loboto *et al.* reported [9] in cases of severe fetal anemia, the amount of blood that can be transfused is limited by fetal tolerance to volume overload and fetal hemoglobin concentration after treatment may still be below the optimal level The mean decline rate in fetal Hct levels between first and second IUIVT and the volume of blood to be transfused insignificant correlate as well as the volume of blood to be transfused and interval between both procedures (T).

Although an estimated fetal Hct decline of approximately 1% / day, as in the present study, this parameter is quite variable and independent of the volume of blood to be transfused. The present authors supported the results of study Mary *et al.* [6] that the optimal interval between Doppler examination for MCA PSV has not been determined, but appears to be one or two weeks. As Steel *et al.* [18] reported, the wide range in pre-transfusion MCA MoM for fetuses with identical Hct, other factors such as, blood viscosity, cardiac output, and peripheral resistance must play an important role in determination MCA peak. Scheier *et al.* [3] confirmed that more precise predictive models have been investigated. Unexpectedly, the present study showed the significant negative correlation between MCA-MoM-3 before the second IUIVT and the time interval between both procedures (T) (Figure 1). The present authors think that may not be a rule, but it can be useful for future investigations. The present study involving 47% hydropic fetuses with low pre-transfusion Hct and high post-transfusion Hct values, should be candidates for shorter interval between two measurements MCA-MoM than the timing approximately 12 days between the first and second IUIVT, as we documented in the study.

In the present Department, in cases of severe fetal anemia, ultrasound-directed fetal blood sampling (ie, cordocentesis) allows direct access to the fetal circulation to obtain important laboratory values such as Hct, fetal blood type, reticulocyte count, and platelet count. Doppler velocimetry were performed initially before the first and before each IUIVT can be usefully for determinate the best timing for the next procedure.

Conclusion

An increasing fetal Hct after IUIVT has effect on maximum MCA PSV. The present study documented high sensitivity MCA-MoM before the first and second IUIVT in predicting severity of the fetal anemia. The wide range in pre-transfusion MCA-MoM, for fetuses with identical Hct, exist, besides other factors must determine MCA peak or MoM-MCA. The measurements MCA MoM before every IUIVT can be useful for determining severity of fetal anemia as well in predicting the best time interval for the next IUIVT.

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Address reprint requests to:
I. BABOVIC, M.D.
63/27 Luke Vojvodica Street
Belgrade (Serbia)
e-mail: ivana.r.babovic@gmail.com