Doppler middle cerebral artery peak systolic velocity measurement as diagnostic tool for fetal anemia after *in utero* transfusions in red blood cell alloimmunisation

Dopler merenje maksimalnog protoka u sistoli arterije cerebri medije kao dijagnostičko sredstvo u proceni fetalne anemije nakon intrauterine transfuzije kod bolesnica sa Rhesus aloimunizacijom

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Abstract

Background/Aim. Doppler sonography of fetal middle cerebral artery peak systolic velocity (MCA-PSV) can be used to predict fetal anemia and the need for *in utero* intravascular transfusion (IUIT) in red blood cell (RBC) alloimmunisation pregnancies. The aim of this study was to evaluate whether measurement of MCA-PSV in fetuses that had undergone one to three transfusions is a good diagnostic tool for fetal anemia. **Methods.** Study included 36 pregnancies treated due to RBC alloimmunisation in our tertiary referral center during the 5-year period (2012–2017). We measured MCA-PSV and hematocrit (Hct) in all patients. In seven pregnancies there was a need to perform sequential IUITs for correction of fetal anemia. In these patients we compared MCA-PSV and Hct values before and after every transfusion. **Results.** Hct and MCA-PSV correlated negatively before transfusion therapy ($p = 0.035$) and after the second transfusion ($p = 0.046$). Contrary, after the first

($p = 0.954$), before the second ($p = 0.738$), as well as before ($p = 0.092$) and after ($p = 0,741$) the third transfusions there were no significant correlations between Hct and MCA-PSV values. Hct values before and after transfusions were positively associated ($p = 0.001$), but MCA-PSV were not ($p = 0.296$). According to performed receiver operating characteristic (ROC) analysis the cut-off point of MCA-PSV for investigated patients was 1.22 multiples of its median (MoM). **Conclusion.** There is a reduction in MCA-PSV accuracy for assessing fetal anemia in previously transfused fetuses. Larger studies are needed to explain the reasons for these findings and potentially set new referral values of MCA-PSV for better diagnostics of fetal anemia.

Key words:
fetus; anemia; blood transfusion, intrauterine; blood group incompatibility; rh-hr blood group system; ultrasonography; hematocrit.

Apstrakt

Uvod/Cilj. Dopler sonografsko merenje maksimalnog protoka tokom sistole u fetalnoj arteriji cerebri mediji (MCA-PSV) može da se koristi u predikciji fetalne anemije kao i za određivanje potrebe za intrauterinom transfuzijom (IUIT) kod bolesnica sa Rhesus (Rh) aloimunizacijom. Cilj studije bio je ispitati da li je merenje MCA-PSV kod fetusa koji su prethodno imali jednu do tri transfuzije dobar dijagnostički pokazatelj za fetalnu anemiju. **Metode.** Ova prospektivna studija obuhvatila je 36 trudnica koje su ispitivane i lečene zbog Rh aloimunizacije u tercijarnom centru tokom petogodišnjeg perioda (2012–2017). Merili smo MCA-PSV i hematokrit (Hct) kod svih fetusa. Kod sedam trudnica bilo

je neophodno primeniti intrauterino transfuzije radi korekcije anemije. Kod ovih bolesnica poredili smo vrednosti MCA-PSV i hematokrita pre i posle svake transfuzije. **Rezultati.** Hct and MCA-PSV su u negativnoj korelaciji pre terapije *in-utero* transfuzijom ($p = 0,035$) i pre i posle druge transfuzije ($p = 0,046$). Obratno, posle prve ($p = 0,954$), pre druge ($p = 0,738$), kao i pre ($p = 0,092$) i posle ($p = 0,741$) treće transfuzije nije nađena statistički značajna povezanost između vrednosti Hct i MCA PSV. Vrednosti hematokrita pre i posle transfuzije bile su u pozitivnoj korelaciji ($p = 0,001$), ali vrednosti MCA PSV nisu ($p = 0,296$). Prema ROC analizi granični skor za MCA PSV za ispitivane bolesnice bila je 1,22 MoM. **Zaključak.** Tačnost MCA PSV kao pokazatelja fetalne anemije redukovana je kod fetusa koji su

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prethodno imali transfuziju. Veće studije su potrebne da bi se objasnili uzroci ove pojave i da bi se potencijalno postavile nove referentne vrednosti MCA-PSV, a u cilju bolje dijagnostike fetalne anemije.

Introduction

Red blood cell (RBC) alloimmunisation develops when a pregnant woman has an immunological response to a paternally derived red-cell antigen that is foreign to the mother but inherited by the fetus [1–3]. Maternal antibodies produced as immunological reaction can cross the placenta, bind to antigen present on the fetal erythrocytes and cause fetal anemia due to their hemolytic properties [1–3]. The treatment of choice for this condition is *in utero* intravascular transfusion (IUIT) of red blood cells that was first proposed by Liley [4]. A blood transfusion is indicated if fetal anemia is moderate (hemoglobin deficit 2–7 g/dL, i.e. < 0.65 multiples of its median – MoM) or severe (deficit greater than 7 g/dL, i.e. < 0.55 MoM) [5, 6].

The only absolutely accurate test to assess the degree of fetal anemia is fetal blood sampling. Another standard method for evaluation of fetal anemia is serial amniocentesis for the determination of bilirubin levels in amniotic fluid [7, 8]. Hemolysis leads to the accumulation of bilirubin in amniotic fluid, so its level correlates with the severity of hemolysis [9]. Spectrophotometry is used to quantify bilirubin level which is expressed as the change in optical density at a wave length of 450 nm. Those values are then plotted on a Liley chart to estimate the severity of anemia [4, 10]. However, both of these diagnostic procedures are invasive and consequently can cause miscarriage, premature rupture of membrane, preterm delivery as well as feto-maternal hemorrhage, thereby exacerbating the severity of the disease [11, 12].

Therefore, non-invasive methods for the prediction of fetal anemia and timing of its therapy have been assessed lately [6, 13]. Several studies have established that fetal anemia is associated with increased arterial and venous blood flow velocities. Fetal anemia causes decreased blood velocity leading to increased venous return and preload with consequent increase in cardiac output which is manifested as hyperdinamic circulation. These changes start occurring after the 16th gestational week when fetal reticulo-endothelial system is mature enough to destroy antibody-coated erythrocytes [1–3]. It is well known that hemodynamic changes can be assessed by Doppler ultrasound and therefore authors have proposed measuring the fetal middle cerebral peak systolic velocity (MCA-PSV) as the most accurate for potential prediction of alloimmunisation complications [9, 12].

Moreover, MCA-PSV is also generally used to decide when to perform IUIT and delivery. Nevertheless, there are still few studies regarding the non-invasive alloimmunisation diagnostics and their results regarding the reliability of MCA-PSV measurements are conflicting. The major issue presents the reliability of MCA-PSV for anemia diagnostics in previously transfused fetuses [7, 8].

Therefore, the aim of this study was to evaluate whether Doppler measurement of MCA-PSV of the fetus who has undergone one, two or three *in utero* transfusions due to correction of anemia, is a good diagnostic tool for timing next transfusion.

Methods

This prospective cohort study included all consecutive singleton pregnancies with RBC alloimmunisation that were checked-up, treated and delivered in our tertiary referral centre during the 5 year period (2012–2017).

Detailed history data were taken from every woman, such as age, BMI, obstetrical history and neonatal outcome for all previous pregnancies. Patients were regularly checked-up (laboratory and sonography) throughout pregnancy. After the 24th week of gestation examinations were performed every fortnight. We measured fetal MCA-PSV and hematocrit (Hct) in all patients.

The method of evaluation the MCA-PSV was classic. First, in transverse section of the fetal head we ultrasonographically identified the circle of Willis and the middle cerebral artery. Then, applying pulsed-wave Doppler on the proximal one-third of the MCA at the angle of < 20 degrees, three consecutive waveforms, in the absence of fetal body or breathing movements, were recorded. The highest level was considered as the PSV (cm/s). Finally, the PSV was recalculated in multiples of its MoM and this value was used in further analysis.

Cordocentesis was performed for fetal blood sampling and measurement of hemoglobin and hematocrit levels during pregnancy. Upon birth, blood sample from the umbilical cord was taken for laboratory testing.

In utero intravascular transfusion was indicated in cases of ultrasonographically suspected imminent or existing fetal hydrops (ascites and dilated heart) or whenever the optical index at 450 nm (DOD450) showed Liley zone B2 i.e. III. Fetal anemia was also considered if hemoglobin levels were < 7.0 g/dL and/or hematocrit $< 30\%$. Moreover, severe fetal hemolysis was considered if antibody levels were > 35 IU/mL. If indicated transfusions were administered between the 28th, and 32nd gestational week in our study.

We compared MCA-PSV and Hct values before and after every transfusion and assessed their relationship. The extent of MCA-PSV change after IUIT treatment was calculated. Finally, we assessed the impact of MCA-PSV and Hct values before and after every transfusion on neonatal outcome.

As main neonatal outcomes of the examined pregnancy we considered gestational age at birth, birth weight and Ap-

gar score. We also measured hemoglobin and hematocrit levels at birth for every live-born child.

Obtained data were statistically analyzed by methods of descriptive and analytical statistics (percentages, mean, median, standard deviation, ANOVA, Wilcoxon Z test, Kruskal Wallis χ^2) and a computer program SPSS 20. Spearman correlation was applied to assess the relationship between parameters of fetal condition before and after transfusion treatment. To examine to which extent MCA-PSV measurement can be used to differentiate the fetal anemia patients and cases that do not need to have IUIT we calculated the tests accuracy (number true positives + true negatives/all examined patients number). As the cut-off level we used the generally accepted 1.5 MoM MCA-PSV. Finally, we performed the receiver operating characteristic (ROC) analysis to assess the best cut-off values of MCA-PSV timing first, second and third IUIT based on Hct values.

Results

Study included 36 pregnant women with RBC alloimmunisation. The mean age of these women was 31.14 ± 5.49 years. In their previous pregnancies two women had already had RBC alloimmunisation. In the monitored pregnancy IUIT for correction of fetal anemia had to be performed in seven cases. There were significant differences in the second trimester MCA-PSV ($p = 0.016$) and Hct ($p = 0.020$) between patients who needed and those who did not need IUIT. In one case we registered the presence of different antibody types while the remaining six patients had only RhD antibodies in the examined pregnancy. According to Liley score

four women were in A, i.e. I zone, two in B1, i.e. II and one had B1/B2, i.e. II/III Liley zone.

We registered fetal hydrops in four cases and polycythemia in one case of the seven pregnancies that needed IUIT for fetal anemia treatment. Investigated patients were delivered in average in the 35th gestational week (34.5 ± 0.87 weeks). The mean birth-weight of their children was 2633.33 ± 246.64 grams while their Apgar score was quite low ranging from 1 to 7 (4.5 ± 2.38). Average hemoglobin level upon birth was 14.7 ± 1.5 g/dL (minimum 13.6, maximum 16.4 g/dL) indicating adequate treatment of fetal anemia. Still, we had one case with adverse pregnancy outcome in which IUIT did not mend the hemolysis.

Transfusion was performed three times in four and four times for remaining three patients. We presented the parameters of fetal condition (Hct and MCA-PSV) before and after every IUIT in Table 1. There were significant differences between levels of both Hct and MCA-PSV before and after the completion of transfusion treatment. The Hct levels were increased almost twice after administered IUITs (Table 1).

We also examined the relationship of Hct and MCA-PSV before and after each of the administered transfusions (Tables 2 and 3). It can be seen that levels of Hct and MCA-PSV (MoM) negatively correlated before the commencement of transfusion treatment as well as after the first and second transfusions. However, before the second as well as before and after the third transfusions there were no significant correlations between Hct and MCA-PSV (MoM) values. This can be explained by reduction in accuracy of MCA-PSV for assessing fetal anemia in previously transfused fetuses.

Table 1

Investigated parameters of fetal condition (Hct and MCA PSV) in pregnancies with red blood cell alloimmunisation before and after IUIT

Parameters	Min	Max	Mean ± SD	Z	p
Hct before transfusion	0.10	0.50	0.27 ± 0.09	-5.234	0.001
Hct after transfusion	0.26	0.55	0.42 ± 0.07		
MCA PSV MoM before IUIT	0.62	1.60	1.17 ± 0.24	-4.577	0.001
MCA PSV MoM after IUIT	0.44	1.07	0.79 ± 0.16		

Hct – hematocrit; IUIT – *in utero* intravascular transfusion; MoM– multiples of median, MCA-PSV – fetal middle cerebral artery peak systolic velocity; Min – minimum; Max – maximum; SD – standard deviation.

Table 2

Correlations of Hct and MCA-PSV before each of the administered transfusions

Parameters		MCA-PSV MoM before transfusion I	MCA-PSV MoM before transfusion II	MCA-PSV MoM before transfusion III
Hct before transfusion I	Ro	-0.929	-0.414	-0.290
	p	0.003	0.355	0.577
Hct before transfusion II	Ro	-0.378	-0.345	-0.809
	p	0.403	0.448	0.051
Hct before transfusion III	Ro	-0.609	-0.265	-0.088
	p	0.200	0.612	0.868

Ro – Spearman`s coefficient of correlation.
For other abbreviations see under Table 1.

Table 3

Correlations of Hct and MCA-PSV after each of the administered transfusions

Parameters		MCA-PSV MoM before transfusion I	MCA-PSV MoM before transfusion II	MCA-PSV MoM before transfusion III
Hct before transfusion I	Ro	-0.893	-0.179	-0.600
	p	0.007	0.702	0.208
Hct before transfusion II	Ro	0.631	-0.912	0.290
	p	0.129	0.011	0.577
Hct before transfusion III	Ro	-0.232	-0.696	-0.348
	p	0.658	0.125	0.499

For abbreviations see under Tables 1 and 2.

Finally, we determined the MCA-PSV measurement accuracy prior to IUIT treatment in detecting fetal anemia. For our sample it was rather low – 14.29%. So, it can be seen that the indication for IUIT has to be made in accordance with laboratory testing and not only MCA-PSV measurements.

Performed ROC analysis shows that MCA-PSV (with standard cut-off level on 1.5 MoM) adequately predicts 83.3% of fetal anemia cases prior to transfusion therapy. On the other hand, in our sample MCA-PSV predicts 50% before second transfusion and only 25% of anemic fetuses before the third transfusion. For investigated pregnancies the optimal MCA-PSV cut-off for timing the second as well as the third transfusion was 1.22 MoM (II IUIT sensitivity = 100%; specificity = 50%; III IUIT sensitivity = 100%; specificity = 33.3%).

Discussion

In the clinical management of RBC alloimmunization the major concern is to identify the affected fetus and to correct the fetal anemia by intrauterine blood transfusion, on time [1, 14]. The timing of IUIT is important because each procedure bears a 1.5–3% risk of fetal morbidity and mortality. The adequate evaluation of fetal anemia severity should enable clinicians to avoid unnecessary interventions. However, when to perform a subsequent transfusion is a subject of ongoing debate [8, 15].

There are several methods for timing of the first, second, and subsequent transfusions. Some centers still perform IUIT empirically every 7–10 days for gestations ≤ 24 weeks, 15 days for subsequent transfusions after the second one or 21 days once fetal erythropoiesis has been suppressed [11, 16]. Others use the decline in fetal hemoglobin of 0.4, 0.3 and 0.2 g/dL/day for calculating the intervals between first, second and third transfusion [4, 17]. It is known that after sequential transfusions decrease in hemoglobin concentration becomes almost regular at about 1% hematocrit point per day due to suppression of fetal red cell production and predomination of donor adult blood cells [5]. However, if fetal hydrops occurs, decline in fetal Hct is more rapid mostly requiring a shorter period between the transfusions [16].

Currently, it is widely accepted that MCA-PSV is not only accurate test for fetal anemia prediction before first transfusion treatment, but that it is also useful for timing sub-

sequent transfusions [10, 18, 19]. The reliability of MCA-PSV in detecting fetal anemia was established 17 years ago in the initial report by Mari et al. [1] which gave a normative data for gestational age and threshold values for predicting moderate to severe anemia. Other investigators determined that accuracy of MCA-PSV Doppler assessment is better (85%), than the Liley curve (76%) and the Queenan curve (81%) [8]. These data have led to acceptance of using Doppler assessment of MCA-PSV in detecting fetal anemia in RBC alloimmunization as the primary diagnostic tool [6, 20]. When the critical antibody titer above 32 is detected, Doppler assessment of MCA–PSV should be performed serially every 1–2 weeks [14, 15]. Literature data have proved the association of MCA–PSV and fetal hemoglobin in fetuses before and after first transfusion [13, 20]. It is mostly accepted that MCA–PSV greater than 1.5 MoM is an indication for cordocentesis for fetal hematocrit determination and decision whether IUIT is needed [1, 2, 9].

However, cut-off levels of MCA-PSV for diagnosis of fetal anemia following serial transfusions have not yet been precisely defined [16, 17]. According to some literature data the overall performance of MCA-PSV in predicting severe anemia is almost unchanged from the first to the last IUIT, suggesting the usefulness of MCA-PSV in cases of serial transfusions [10, 21]. Conversely, other investigations found that reliability of MCA-PSV in serial transfusions is decreased necessitating higher cut-off levels upon which a decision to perform IUIT should be made [13, 20]. The reasons for a decreased predictive value of MCA-PSV following IUIT are still not completely understood, but relate with lower fetal blood viscosity after IUIT. This can be explained by changing fetal blood with donor adult red cells, which, when compared to fetal red cells, are smaller, have decreased cellular rigidity and increase erythrocyte aggregation [17, 22]. After couple of IUITs most of the circulating red cells in the fetal circulation are donor cells that contain adult hemoglobin which can decrease the delivery of oxygen at the fetal tissues. This could affect the cerebral vascular regulation and account for increases in MCA-PSV [19]. Moreover, IUITs are associated with higher fetal hematocrit levels, which consequently increases the whole blood viscosity [14, 15]. Both of these will slow the speed at which blood moves through the fetal circulation. Thus, it was demonstrated that detection rate of MCA-PSV with cut-off for anemia set on 1.5 MoM does not go above 64% [10, 18].

Therefore, as the positive predictive value of MCA-PSV decreases with each subsequent IUIT numerous authors believe that modified threshold for detecting moderate-severe anemia should be used. Still, results on the new cut-off levels are conflicting and while some studies report increased cut-off for transfusion (> 1.7 MoM) others propose to use the decreased levels (1.2 to 1.3 MoM) of MCA-PSV when deciding about the second and/or third IUIT [15, 19].

It is still not certainly whether transfusion interval between the second and the third IUIT can be accurately assessed by MCA-PSV and more studies on larger samples are needed [11, 15]. Therefore, in literature several normal reference ranges of MCA-PSV are mentioned and it is thought that any of them could be used if cut-offs are reviewed and patients serial measurements are monitored on an individual basis [2, 5]. Still, other investigations indicate that discriminatory power, sensitivity and specificity of Mari's curve and its given cut-offs are still the optimal for prediction of fetal anemia [10, 21].

We found that MCA-PSV and fetal Hct are significantly correlated before and after the first and after the second IUIT. However, before the second as well as before and after the third IUIT, in our study, there was no association of MCA-PSV and fetal Hct. These findings correspond to those reported in some previous studies that the MCA-PSV in not completely reliable in predicting severe anemia in fetuses that already had two previous transfusions [3, 8, 9, 20]. Moreover, our findings support the application of decreased cut-off MCA-PSV levels for timing subsequent transfusions. We suggest that the use of 1.22 MoM threshold in our population should be reconsidered.

The main limitation of our study is the very small sample. The available data do not allow us generalizing specificity of MCA-PSV assessment in the diagnosis of fetal anemia after two or more transfusions. However, RBC alloimmunisation nowadays is quite rare condition due to adequate prevention. The incidence of red blood cell alloimmunisation in literature is only 0.6%. There are even less fetuses that have such severe anemia that they need the transfusion treatment. Nevertheless, RBC alloimmunisation is not only extremely important, but also very current as it is still both diagnostic and therapeutic challenge. Consequently, other studies have also been performed on 10 cases, which for this rare condition is considered as an adequate sample and any study of RBC alloimmunisation presents valuable data for perinatologists. Therefore, we wanted not only to present a case-series of our patients, but also to statistically analyze obtained data and give some new perspectives on the condition. Still, we do believe that further studies on more patients or potentially some meta-analyses should be performed to test the accuracy of the newly established MCA-PSV cut-off for our population and make more reliable statistical conclusions.

Conclusion

In this study we proved that there is a reduction in accuracy of middle cerebral artery peak systolic velocity for assessing fetal anemia in previously transfused fetuses. Therefore, Doppler measurement of middle cerebral artery peak systolic velocity of the fetus who has undergone two or more *in utero* transfusions for anemia correction cannot be the only diagnostic tool for timing of serial transfusions. It has to be assessed together with the mean projected daily decrease in fetal hemoglobin. We suggest that new cut-off levels should be created that might enable better accuracy of middle cerebral artery peak systolic velocity in prediction of fetal anemia severity. Accordingly, further studies on larger samples are needed for such calculations.

Conflict of interest

Authors declare no conflict of interest.
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