Peak systolic velocity of middle cerebral artery in pregnancies complicated by rhesus isoimmunization: prediction of fetal anemia

Pico de velocidade sistólica da artéria cerebral média em gestações complicadas por isoimunização Rh: predição de anemia fetal

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Abstract

Aims: The aim of this study is to analyze the Doppler study of peak systolic velocity of middle cerebral artery (PSVMCA) in the prediction of the anemia grade.
Methods: Retrospective study of 17 gestations with Rh alloimunization diagnosed between 2002 and 2009 and correlation with neonatal anemia grade.
Results: The accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were, respectively, 65%, 50%, 100%, 100% and 45% for diagnosis of fetal anemia and 94%, 83%, 100%, 100% and 92% for severe fetal anemia. The area under ROC curve was not significant for MoM of PSVMCA for gestational age in diagnosing anemia but reached statistical significance (p=0.002) in cases of severe anemia. The cutoff of 0.96 for MoM of PSVMCA represented the best performance of the test in predicting fetal anemia. In severe cases, the cutoff of 1.4 had the best sensivity and specificity.
Conclusions: The study of PSMCA had a significant performance in detecting severe anemia. The test revealed no false positives for anemia. The most accurate cutoff of MoM for PSVMCA in cases of anemia and severe anemia were, respectively, 0.96 and 1.4. Further investigation is required to access adequate cutoffs according to anemia grade.

Keywords: red cell alloimmunization, middle cerebral artery peak systolic velocity, anemia grade.

INTRODUCTION

Rhesus alloimunization has been known since the seventeenth century, although its pathophysiology was only enlightened in the fourth decade of the 20th century, being demonstrated that the hemolytic disease of the newborn was part of an intrauterine process. The disease harbors risks for the fetus (anemia, hydrops and stillbirth) and newborn (anemia, hyperbilirubinemia and Kernicterus).

Widespread use of anti-D immunoprophylaxis has promoted a 100-fold decline in perinatal mortality due to Rh isoimmunization[1]. The sensitization to D-antigen became a preventable disease. Despite this prophylaxis, the problem persists, due to the incapacity to implemente the immunoprophylaxis and also to other antigens capable of producing alloimunization[2]. Presently, Rh alloimunization is still a reality, statistical studies reporting about 6-7 cases per 1000 live births[3].

Serial amniocentesis for amniotic fluid evaluation as an indirect method to assess hemolysis grade and consequent fetal anemia was used over the last decades. The bilirubin level in amniotic fluid, which correlates with the severity of hemolysis, was determined by spectrophotometry and values of the change in optical density at 450nm (OD 450) were plotted on Liley's chart[4]. This procedure has the risk of membrane rupture, fetal loss, infection, preterm labor and worsening of sensitization[4,5].

Since the year 2000, measurement of the peak systolic velocity of middle cerebral artery (PSVMCA) has been applied in face of red cell alloimmunization[6,7]. This non-invasive technique proved to have predictive accuracy in identifying fetal anemia and reduced the need for amniocentesis and collection of fetal blood samples[4,7]. The PSVMCA values were established ac-

cording with gestational age and a cutoff of 1.5 Multiples of Median (MoM) proved to have a good sensitivity in predicting moderate to severe fetal anemia[7-10]. Still, some concerns persist considering this method. The false positive rate reaches 12%[7], representing a group of pregnancies exposed to unnecessary invasive approaches. Also, the values capable of estimating anemia grade are not exactly delineated. Neonatal care and prognosis largely depends on the severity of anemia developed *in utero*.

Our group aimed to evaluate the Doppler study of PSVMCA in a group of gestations complicated by Rh isoimunization, assessing the performance of the test in predicting fetal anemia grade.

MATERIAL AND METHODS

Cases studied

The study was conducted at a tertiary perinatal center of the central region of Portugal. It is a retrospective study of pregnancies complicated by Rh alloimmunization (N=17) diagnosed and followed in our Unit from January 2003 to December 2009. In all cases D-type antibodies were detected, maternal serum antiglobulin titers were measured in face of positive indirect Coombs test. Facing an increased antiglobulin titer, Doppler ultrasound studies were conducted.

Doppler studies

The PSVMCA was measured according to the technique described by Mari *et al*[7]. An axial section of the fetal brain inferior to the plane for measurement of biparietal diameter was obtained. Middle Cerebral Artery (MCA) was identified using color Doppler ultrasound, in the absence of fetal breathing movements. The angle of insonation was inferior to 30 degrees, preferentially around 0 degrees. The caliper was placed on the peak of Doppler waveform after uniform waveforms were obtained.

Doppler evaluation of PSVMCA was repeated every one or two weeks by the specialists of obstetrical ultrasound of the Service. Measurements were plotted against a reference chart[7]. The reference cutoff for intervention (cordocentesis for fetal haemoglobin determination) was 1.5MoM. In all cases, a final Doppler examination of MCA was performed less than 24 hour from delivery, without any type of intervention in this period.

Data from clinical files was collected focusing on fetal and neonatal results (stillbirth, hydrops fetalis, gestational age at delivery, intrauterine transfusions, haemoglobin and bilirrubin levels in the newborn. The haemoglobin concentration in umbilical blood obtained immediately after delivery was used to define anemia and its severity, categorizing anemia grade according to the criteria published by Nicolaides *et al*[11].

Severe anemia was defined as a deficit of hemoglobin above five standard deviations[4].

Statistical analysis

Descriptive statistics used for continuous variables were means and standard deviation, and for categorical variables relative or absolute frequencies.

The performance of Doppler ultrasound in detecting fetal anemia was evaluated considering sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The accuracy was calculated by summing the number of true positives and true negatives and dividing by the total number of cases evaluated.

Receiver operating characteristic (ROC) curves were used to assess the efficiency of MoM of PSVMCA for gestational age in predicting the presence and severity of fetal anemia. The results are represented as area under the curve (AUC) and standard error (S.E.) and considered significant for a p value <0.05. For these statistical studies SPSS version 16.0 was used.

RESULTS

A total of 17 gestations were included in the study. The diagnosis of Rh isoimmunization was made in the first trimester in 2 cases, in the second trimester in 2 cases and in the third trimester in 13 cases. In 2 cases Rh isoimmunization in previous gestations was reported. Serial ultrasound monitoring of PSVMCA less than 24 hours from delivery revealed values above 1.5 MoM in 6 cases. Gestational age at delivery had the following distribution: 30-33 weeks in 5 cases, 34-36 weeks in 6 cases and ≥37 weeks in 6 cases.

The evaluation of haemoglobin in umbilical cord obtained immediately after delivery revealed normal values in 5 cases and anemia in 12 cases, including 5 cases of severe anemia. Table I shows the value of MoM of PSVMCA less than 24 hours from delivery and the anemia grade in each case studied.

Table II represents the correlation between Doppler study of PSVMCA less than 24 hours before delivery

TABLE I: THE VALUE OF PSVMCA LESS THAN 24 HOURS FROME DELIVERY AND THE ANEMIA GRADE IN EACH CASE

MoM value	Anemia grade
0.72	Moderate
0.74	Normal
0.8	Moderate
0.8	Moderate
0.93	Normal
0.93	Normal
0.95	Normal
0.97	Moderate
1.1	Normal
1.12	Moderate
1.25	Moderate
1.55	Severe
1.79	Moderate
1.8	Severe
1.88	Severe
1.9	Severe
1.9	Severe

in the presence of any grade of anemia. Considering a cutoff of 1.5 MoM, the PSVMCA had an accuracy of 65%, a sensitivity of 50%, a specificity of 100%, a PPV of 100% and a NPV of 45%.

In Table III the cases are analyzed according with the diagnosis of severe anemia and correlated with Doppler study of PSVMCA less than 24 hours before delivery. Also for a cutoff of 1.5 MoM, the PSVMCA had an accuracy of 94%, a sensitivity of 100%, a specificity of 92%, a PPV of 83% and a NPV of 100% in predicting severe anemia.

Figure 1 describes the ROC curve for MoM of PSVMCA considering the prediction of anemia in general, the AUC did not reach statistical significance (0.767±0.116 p=0.092). Table IV lists the MoM of PSVMCA and the corresponding sensitivity and specitivity.

Analyzing Table IV, the cutoff of MoM of PSVMCA less than 24 hours before delivery that correlates the best sensitivity (0.75) and specificity (0.80) in the diagnosis of any grade of anemia is 0.9600.

Figure 2 describes the ROC curve for MoM of PSVMCA considering only the prediction of severe anemia, the performance of the test is significantly better (0.983±0.026 p=0.002). The Table V lists the MoM of PSVMCA and the corresponding sensitivity and specitivity.

TABLE II: RESULTS CONSIDERING THE PRESENCE OF ANEMIA AND THE RESPECTIVE VALUE OF PSVMCA

PSVMCA	Normal haemoglobin (n)	Anemia (n)
<1.5 MoM	0	6
≤1.5 MoM	5	6

TABLE III: RESULTS CONSIDERING THE PRESENCE OF SEVERE ANEMIA AND THE RESPECTIVE VALUE OF PSVMCA

PSVMCA	Without severe anemia (n)	Severe anemia (n)
<1.5 MoM	1	5
≤1.5 MoM	11	0

Analyzing Table V, the cutoff of MoM of PSVMCA that correlates the best sensitivity (1.00) and specificity (0.917) in the diagnosis of severe anemia is 1.4000.

DISCUSSION

The study of PSVMCA is a non-invasive technique that proved to be at least equal to traditional amniotic fluid analysis in assessing fetal anemia[12]. The invasive procedure and the associated risks of amniocentesis, progressively supported the application of Doppler study of MCA in the evaluation and monitoring of pregnancies complicated by Rh isoimmunization. It is also important to notice that the spectrophotometry evaluation OD 450 may be falsely elevated in the presence of meconium or blood[13] and the exposition to light and cases of Kell alloimmunization can originate low values[1,14]. In 2- 11% of the cases worsening of sensitization was reported[15,16].

In the present study, the anemia diagnosis was based in the blood cord analysis immediately after delivery and correlated with the previous PSVMCA determination (within 24 hours and without intervention). With this approach, biases of the cordocentesis procedure to obtain fetal blood were obviated, particularly the risk of sample dilution. Considering the anemia prediction, Doppler study of PSVMCA had accuracy of 65%, sensitivity of 50%, specificity of 100%, PPV of

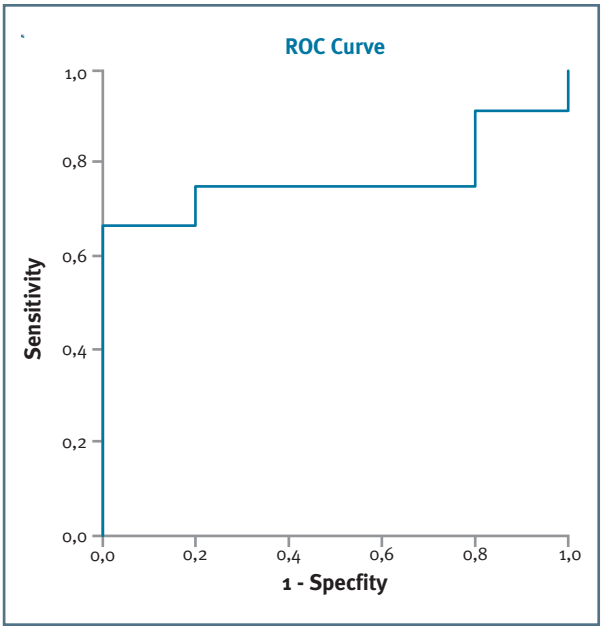

FIGURE 1. ROC curve for the prediction of anemia by PSVMCA

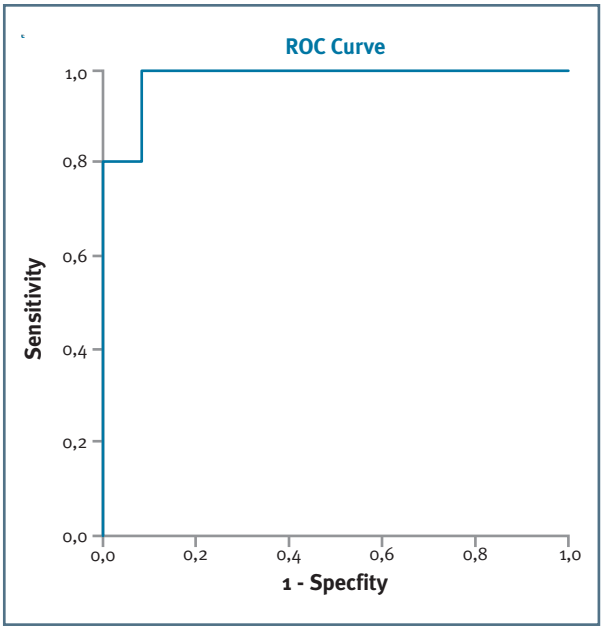

FIGURE 2. ROC curve for the prediction of anemia by PSVMCA

TABLE IV: ROC ANALYSES OF MoM VALUE OF PSVMCA AND RESPECTIVE SENSITIVITY AND SPECIFICITY IN THE DETECTION OF ANY GRADE OF ANEMIA

MoM value[a)]	Sensitivity	Specificity
0.2800	1.000	0.000
0.7300	0.917	0.000
0.7700	0.917	0.200
0.8650	0.750	0.200
0.9400	0.750	0.600
0.9600	0.750	0.800
1.0350	0.667	0.800
1.1100	0.667	1.000
1.1850	0.583	1.000
1.4000	0.500	1.000
1.6700	0.417	1.000
1.7950	0.333	1.000
1.8400	0.250	1.000
1.8900	0.167	1.000
2.9000	0.000	1.000

a) The smallest cutoff value is the minimum observes test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All other cutoff values are the averages of two orders observed test values.

TABLE V: ROC ANALYSES OF MoM VALUE OF PSVMCA AND RESPECTIVE SENSITIVITY AND SPECIFICITY IN THE DETECTION OF SEVERE ANEMIA

MoM value[a)]	Sensitivity	Specificity
0.2800	1.000	0.000
0.7300	1.000	0.083
0.7700	1.000	0.167
0.8650	1.000	0.333
0.9400	1.000	0.500
0.9600	1.000	0.583
1.0350	1.000	0.667
1.1100	1.000	0.750
1.1850	1.000	0.833
1.4000	1.000	0.917
1.6700	0.800	0.917
1.7950	0.800	1.000
1.8400	0.600	1.000
1.8900	0.400	1.000
2.9000	0.000	1.000

a) The smallest cutoff value is the minimum observes test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All other cutoff values are the averages of two orders observed test values.

100% and NPV of 45%. These results reflect a false negative rate of 50% but with no false positives. A screening procedure typically involves the use of a confirmatory test prior to institution of therapy. The false negative rate is far from a good screening test, emphasized by a NPV of 45%. Other studies reported a sen-

sitivity ranging from 73-100% and specificity of 71--87% of PSVMCA in the prediction of fetal anemia[4,8-10,17]. The PSVMCA is considered an efficient method to monitor Rh alloimmunized fetuses up to 34 weeks[18]. Advanced gestational age is associated with a decrease in cerebral arteries resistance, justifying the inferior sensitivity of Doppler study in detecting anemia cases. In this study, 12 cases have 34 weeks ou more, fact that can justify the low sensibility.

The fetal and early neonatal outcome is significantly dependent of anemia grade. Considering this fact, we intended to evaluate the screening capacity of Doppler study relating to severe anemia. For this particular diagnosis, the Doppler performance improved, with accuracy of 94%, sensitivity of 100%, specificity of 92%, PPV of 83% and NPV of 100%. In previous works, the performance of Doppler study prior to 34 weeks had a sensitivity of 100% in predicting moderate to severe anemia[18]. Our results reflect the performance of PSVMCA in the identification of severe anemia, emphasizing its role in screening for this special outcome. Only one case was submitted to an unnecessary intervention and all the other positive Doppler studies corresponded to severe anemia.

The normal PSVMCA increases with advancing gestational age, so data are adjusted for the time of pregnancy. A cutoff value of 1.5 MoM above the mean for gestational age is considered to define a positive test[4,13,19]. Measurements can be initiated as early as 18 weeks[3]. For the diagnosis of anemia, cutoff of 0.96 MoM improved the sensitivity to 0.75, but the specificity decreased to 0,80. This cutoff decreases the false negative results verified with the standard cutoff (1.5MoM), improving the test performance considering a screening procedure. Regarding the cases of severe anemia, a cutoff of 1.4 MoM, approximate to the reference value, proved a sensitivity equal to unit and a specificity of 0.917. The cutoff value of 1.79 MoM would lead to the elimination of false positive results but the loose of sensitivity would lead to undiagnised anemia cases. Previous studies define serial MoM values to define anemia grade[3]. The threshold inferior to median define normal range hematocrit >1.29MoM correspond to mild anemia and >1.5 MoM is the cutoff for moderate to severe anemia. Alshimmiri *et al.* described better sensitivity results for a cutoff of 1.3MoM despite a decrease in specificity compared with 1.5MoM[19]. Despite our limited sample, these results emphasize the screening capacity of PSVMCA in predicting fetal severe anemia.

The initial screening test in face of Rh isoimmunization is the noninvasive ultrasound study of PSVMCA and positive cases require a confirmatory hemogram obtained through cordocentesis. On one hand false positives originate unnecessary invasive procedures, on the other hand the false negatives leads to relevant morbidity by missing this diagnosis. The NPV superior to PPV seems to be less worrying in clinical practice.

The false-positive rate of the Doppler study appears to be higher after 34 weeks of gestation[3,10]. This fact contributes to some problems regarding the surveillance after this gestational age. In our data, the only false positive result for severe anemia represented a case of 35 weeks with hemoglobin levels reaching criteria of anemia, but not severe anemia, leading to intervention in a fetus that could decompensate in a near future. These results emphasize the need to clarify the role of PSVMCA in late preterm gestations and the adequate reference charts.

CONCLUSIONS

Doppler study of PSVMCA is a screening procedure for fetal anemia in face of Rh alloimunization. Despite the limitations, our study suggests that PSVMCA has a good performance in predicting severe fetal anemia, with significant results by ROC curve analyzes. The cutoff of 0.96 for MoM of PSVMCA for gestational age correlated the best sensitivity and specificity for fetal anemia in general, limiting the number of false negatives. Considering cases of severe anemia, the cutoff of 1.4 showed the best performance, similar to the reference cutoff 1.5MoM. Our study emphasizes the need to establish accurate cutoff of PSVMCA for gestational age as a screening procedure, particularly in gestational ages after 34 weeks.

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