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Diagnosis of fetal anemia with Doppler ultrasound in the pregnancy complicated by maternal blood group immunization

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ABSTRACT

We investigated whether Doppler measurement of the fetal middle cerebral artery peak systolic velocity can be used to detect fetal anemia in pregnancies complicated by maternal blood group immunization. We first studied normal values for the middle cerebral artery peak systolic velocity in 135 fetuses (Group A), and also in 23 fetuses at risk for anemia who underwent 56 cordocenteses to assess the fetal hematocrit (Group B). A test to detect fetal anemia, based on the middle cerebral artery peak systolic velocity, was developed by using the data of the fetuses of Group A and Group B. Successively, the middle cerebral artery peak systolic velocity was prospectively determined in 16 fetuses at risk for anemia who underwent 42 cordocenteses (Group C) to assess the test developed, in a multicenter prospective fashion, by using the data of Group A and Group B.

In the normal fetuses an exponential model expressed the increase of the middle cerebral artery peak systolic velocity values with advancing gestation. By using the data of the fetuses of Group A and Group B, four zones of anemia risk were identified. In Group C, none of the anemic fetuses had the middle cerebral artery peak velocity below the normal mean value, whereas all of the anemic fetuses had the peak velocity above the normal mean.

The middle cerebral artery blood velocity increases with advancing gestation and is a non-invasive method of detecting anemia in pregnancies complicated by maternal blood group immunization.

INTRODUCTION

In the United States, fetal anemia is commonly the consequence of maternal aloimmunization, in which maternal antibodies cross the placenta, with resultant destruction of red blood cells. Since the introduction of rhesus factor immune globulin in North America in 1968, the frequency of rhesus isoimmunization has decreased[1], but fetal anemia as a consequence of alloimmunization still occurs in patients who do not respond to treatment or who receive inadequate treatment, or due to other 'irregular' antibodies. The diagnosis of fetal anemia is achieved either indirectly by amniocentesis, with the bilirubin level measured in the amniotic fluid[2], or directly by cordocentesis[3] through measurement of fetal hemoglobin concentration. Although cordocentesis accurately reflects the fetal status, it is an invasive procedure associated with complications such as infection, bleeding from the cord puncture site, transient fetal bradycardia and fetal demise[4], and worsening of the maternal immunization. Furthermore, if an initial sample does not yet demonstrate anemia, the timing of repeat cordocentesis is arbitrarily determined.

The diagnosis of fetal anemia with non-invasive procedures has been the goal of many investigators. Nicolaides and co-workers[5] measured six ultrasonographic parameters in 50 rhesus-isoimmunized pregnancies at 18–26 weeks' gestation. They reported that, in the absence of fetal hydrops, none of these parameters reliably distinguished anemic from non-anemic fetuses. This group also compared the mean aortic velocity obtained by Doppler to the hemoglobin concentration in fetuses undergoing cordocentesis[6], and concluded that Doppler measurements of the fetal aortic mean velocity are not accurately predictive of the degree of fetal anemia. Rightmire and associates[7] reported Doppler ultrasonographic results from the aorta, inferior vena cava and umbilical vein of isoimmunized fetuses. They presented a model that predicted the level of fetal hematocrit with

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a mean error of 3.8%, although this model has never been tested prospectively.

Our hypothesis in the current study was that a decreased fetal hematocrit in pregnancies complicated by maternal blood group immunization is associated with an increased blood flow velocity. Doppler ultrasound allows measurement of values close to the real blood flow velocity if the angle between the ultrasound beam and the blood flow is close to 0°. The fetal middle cerebral artery may be assessed with pulsed Doppler with an angle close to 0° and the real velocity may be determined. Therefore, we studied the fetal middle cerebral artery in normal fetuses and in fetuses at risk of anemia, to assess whether Doppler measurement of fetal middle cerebral artery blood velocity can be used in the diagnosis of fetal anemia.

METHODS

This was a retrospective study (Groups A and B), complemented by a prospective study (Group C). All the pregnant women gave oral informed consent for the study. The study was carried out at Yale New Haven Hospital, Baylor College of Medicine and Pennsylvania Hospital. It was approved by the Human Investigational Review boards.

Group A comprised 135 normal fetuses evaluated cross-sectionally. No fetus was included more than once. Gestational age was determined by menstrual history confirmed by sonographic fetal biometry. The mean age was 28.9 ± 7.2 weeks (mean ± SD), with a minimum of 15 weeks and maximum of 42 weeks.

Group B comprised 23 fetuses undergoing 56 cordocenteses for evaluation of fetal hematocrit. Twenty pregnancies were complicated by anti-D isoimmunization, one involved c disease, and the remaining two pregnancies involved Kell isoimmunization. In Group B, the number of procedures during which hemodynamic studies were undertaken was as follows: one cordocentesis, nine fetuses; two cordocenteses, four fetuses; three cordocenteses, four fetuses; four cordocenteses, three fetuses; five cordocenteses, three fetuses. The mean gestational age at the time of the first procedure was 25 ± 3.5 weeks (range 21–32 weeks); it was 28.4 ± 3.3 weeks at the time of the following procedures (range 23–35 weeks). The hematocrits of the fetuses at their first cordocentesis ranged from 10 to 28% (mean ± SD, 25.1 ± 8.3%). The hematocrits at their subsequent cordocenteses ranged between 15 and 43% (mean ± SD, 29.7 ± 6.7%).

Group C comprised 16 fetuses undergoing 41 cordocenteses for evaluation of fetal hematocrit. Fourteen pregnancies were complicated by anti-D isoimmunization, and two involved c disease. The numbers of procedures during which hemodynamic studies were undertaken were as follows: one cordocentesis, seven fetuses; two cordocenteses, one fetus; three cordocenteses, four fetuses; four cordocenteses, two fetuses; five cordocenteses, one fetus; seven cordocenteses, one fetus. The mean gestational age at the time of the first procedure was 26 ± 6 weeks (range 18–35 weeks); it was 28.4 ± 4.2 weeks at the time of the following procedures (range 19.3–35 weeks). The hematocrits of the fetuses at their first cordocentesis ranged from 7.8 to 38% (mean ± SD, 26.1 ± 10.9%). The hematocrits at their following cordocenteses ranged between 14.7 and 36% (mean ± SD, 27.6 ± 5.6%).

Fetal anemia was defined as a hematocrit value of more than 2 standard deviations below the mean for gestational age, as determined by Daffos and Forestier[8,9].

The fetal middle cerebral artery peak systolic velocity was studied by Doppler ultrasound as previously reported[10]. An axial section of the brain, including the thalami and the cavum septum pellucidum, was obtained. By moving the transducer caudally, toward the base of the fetal skull, the circle of Willis was visualized. The middle cerebral artery was insonated through an anterior (temporo-occipital) or a posterior (occipito-temporal) window. The angle between the ultrasound beam and the blood flow was close to 0° (Figure 1). The angle was not measured and its accuracy was based on the experience of the operator. The highest point of the waveform (peak systolic velocity) was measured. The fetuses were examined when the mothers were in a semirecumbent position. Two-dimensional directed pulsed Doppler ultrasound (GE PASS II, General Electric Medical Systems, Milwaukee, WI) and/or color Doppler imaging (Acuson 128 XP, Mountain View, CA) were used for Doppler studies. All the studies in Group A and Group B were performed by one of the authors (G.M.); the studies in Group C were performed by seven experienced sonologists who were not aware of the fetal hematocrit when the Doppler samples were obtained.

The normal values for the middle cerebral artery peak systolic velocity as a function of gestational age were determined by using the data of Group A. Analysis was based on the following factors: (1) *t*-values of the coefficients statistically different from zero; (2) correlation coefficient (R^2) applied to the best regression line; and (3) residuals well distributed and around zero and approximating a normal distribution. Analysis of variance was used to determine whether the regression accounted for a significant part of the variability in the dependent variable. A p-value of < 0.05 was selected to indicate statistical significance. There was only one measurement of middle cerebral artery peak systolic velocity per fetus in this portion of the data.

The intraobserver error for the middle cerebral artery peak velocity was determined by examining ten normal fetuses; five measurements were taken from each fetus during a 1-h period. The interobserver error was determined by two of the authors (G.M. and A.Z.A.) examining ten normal fetuses.

The receiver operating characteristic curve (ROC)[11] was used to select a cut-off point that would distinguish between anemic and non-anemic fetuses in Group B using the middle cerebral artery peak systolic velocity. A test for detecting fetal anemia, based on this velocity, was developed by using the data of the fetuses of Group A and Group B. The middle cerebral artery peak systolic

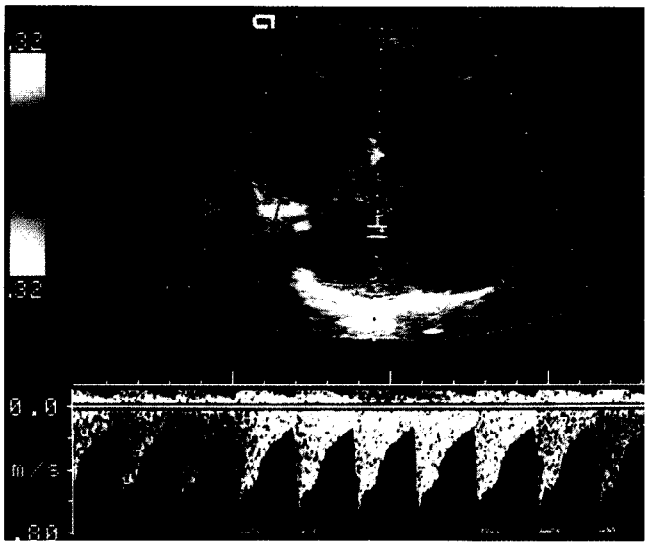

Figure 1 Transverse sonogram of the fetal head which shows how flow velocity waveforms of the middle cerebral artery are obtained

velocity was determined in the fetuses of Group C to assess the results obtained by using the data of Groups A and B in a multicenter prospective fashion.

RESULTS

All fetuses of Group A were delivered after 37 weeks of gestation; comprehensive pediatric assessment revealed no evidence of growth or congenital abnormalities. There were no anemic fetuses in this group. The intraobserver error for the middle cerebral artery peak systolic velocity was 2.3%. The interobserver error was 2.5%. The fetuses of Group B were anemic on 42 occasions and non-anemic on 14 occasions. The fetuses of Group C were anemic on 31 occasions and non-anemic on ten occasions.

In Group A, the data were best fitted to an exponential model (Figure 2). The middle cerebral artery peak systolic velocity was converted into multiples of the standard error of estimation, as determined from the exponential model parameters. The multiples of the standard error of estimation were used because standard deviation does not apply in an exponential model. Table 1 demonstrates the mean and the multiples of the standard error of estimation values based on gestational age.

By use of the data of Groups A and B, the ROC allowed us to select a cut-off value at 0.80 multiples of the standard error of estimation above the mean as best differentiating between anemic and non-anemic fetuses. There were two false-negative fetuses of Group B on the non-anemic side with the use of the ROC. One fetus at 35 weeks had a hematocrit of 34.4%, while the middle cerebral artery peak systolic velocity was 0.4 multiples of the standard error of estimation below the mean; a second fetus had a hematocrit of 26% at 24 weeks, and the middle cerebral artery peak systolic velocity was 0.1 multiples of the standard error of estimation above the mean. We did not want to miss any anemic fetuses; therefore, two other cut-off points at 0.0 and at 0.5 multiples of the standard error of estimation below the

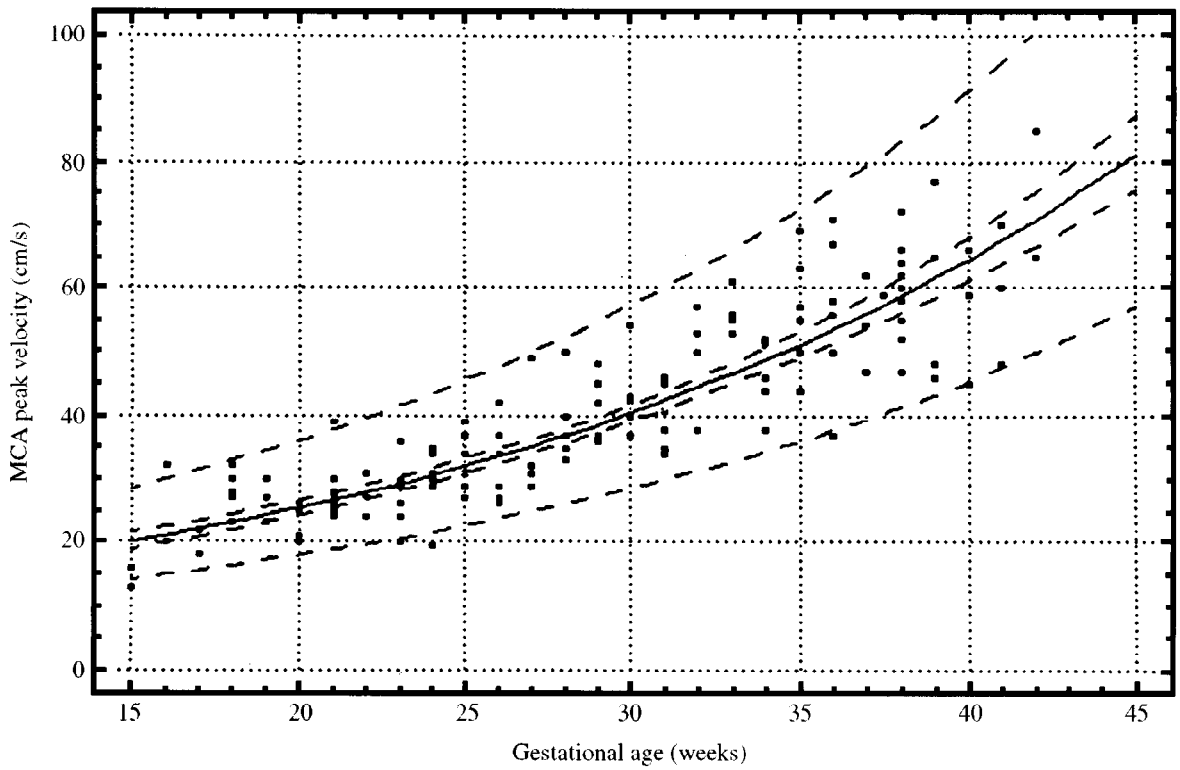

Figure 2 Normal range of middle cerebral artery (MCA) peak velocity as a function of gestational age, constructed from a study of 135 normal fetuses. Inner dashed lines represent 95% confidence intervals and outer dashed lines represent 95% prediction intervals. See Table 1 for equation. R^2 = 78.7; standard error of estimation = 0.174782

mean, respectively, were identified. Four zones of anemia risk were identified: (1) A-zone; (2) B-zone; (3) C-zone; (4) D-zone.

Table 1 Values of the middle cerebral artery peak velocity (MCA-PV) as a function of gestational age at different multiples of the standard error of estimation (MSEE). MCA-PV (MSEE) = [ln(MCA-PV – 2.30921 – 0.0463954 × age (weeks)]/ 0.174782

	MSEE				
Weeks	*–2*	*–0.5*	*0*	*0.8*	*2*
15	14.2	18.5	20.2	23.2	28.6
16	14.9	19.4	21.1	24.3	30.0
17	15.6	20.3	22.1	25.5	31.4
18	16.4	21.3	23.2	26.7	32.9
19	17.1	22.3	24.3	27.9	34.5
20	17.9	23.3	25.5	29.3	36.1
21	18.8	24.4	26.7	30.7	37.8
22	19.7	25.6	27.9	32.1	39.6
23	20.6	26.8	29.3	33.6	41.5
24	21.2	28.1	30.6	35.2	43.5
25	22.6	29.4	32.1	36.9	45.5
26	23.7	30.8	33.6	38.7	47.7
27	24.8	32.3	35.2	40.5	50.0
28	26.0	33.8	36.9	42.4	52.3
29	27.2	35.4	38.9	44.5	54.8
30	28.5	37.1	40.5	46.6	57.4
31	29.9	38.9	42.4	48.8	60.2
32	31.3	40.7	44.4	51.1	63.0
33	32.8	42.6	46.5	53.5	66.0
34	34.4	44.7	48.7	56.1	69.1
35	36.0	46.8	51.1	58.7	72.4
36	37.7	49.0	53.5	61.5	75.9
37	39.5	51.3	56.0	64.4	79.5
38	41.4	53.8	58.7	67.5	83.2
39	43.3	56.3	61.5	70.7	87.2
40	45.4	59.0	64.4	74.1	91.3
41	47.5	61.8	67.4	78.6	95.7
42	49.8	64.7	70.7	81.3	100

This was a retrospective study. The four zones were assessed prospectively in Group C. All of the anemic cases of Group C (n = 31) had the middle cerebral artery peak systolic velocity above the mean (Figure 3); five of the non-anemic cases in Group C (5/10) had values below the mean, and five were above the mean (Figure 4).

Some of the fetuses of Groups B and C were studied on multiple occasions, suggesting the possibility that a bias could have been introduced. To assess if a real correlation did exist between the middle cerebral artery peak systolic velocity and the fetal hematocrit, we carried out the regression of hematocrit multiples of the standard deviation (MSD) and middle cerebral artery peak systolic velocity multiples of the standard error of estimation (MSEE) for the first cordocentesis only in the fetuses of both Groups B and C (n = 39). The results are shown in Figure 5 and they show a good correlation between the hematocrit and the middle cerebral artery peak systolic velocity (MCA-PV): Hct (MSD) = –1.712 – 1.150 (MCA-PV) MSEE (R^2 = 64). The reason for selecting multiples of the standard deviation of the hematocrit was that this parameter is better than the absolute hematocrit value, because the fetal hematocrit increases with advancing gestation.

DISCUSSION

The goal of this project was to develop a non-invasive method for diagnosing fetal anemia, to reduce the risks associated with the use of invasive procedures, such as amniocentesis and cordocentesis. Although many reports have addressed the role of Doppler ultrasound in relation to the anemic fetus and the effects of intravascular transfusion[12–15], they have failed to find a good correlation between Doppler ultrasound examination and fetal

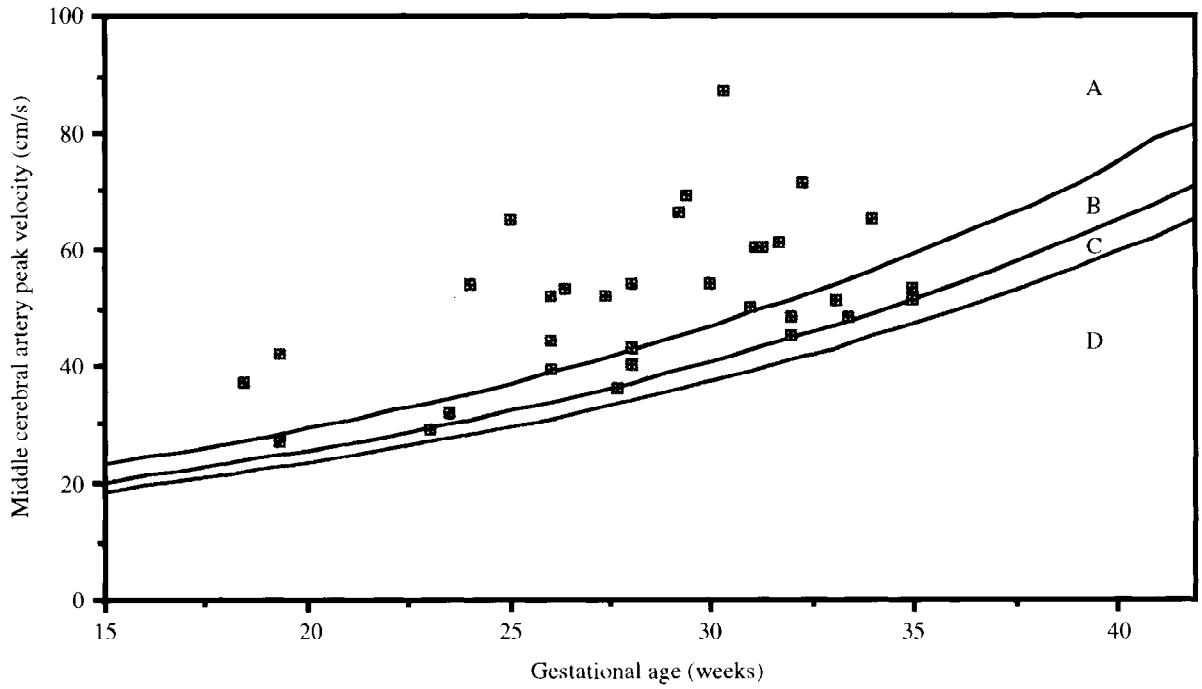

Figure 3 Anemic cases of Group C plotted over the risk zones. Note that the anemic fetuses, who apparently had a value on the mean, in reality had a value just above the mean

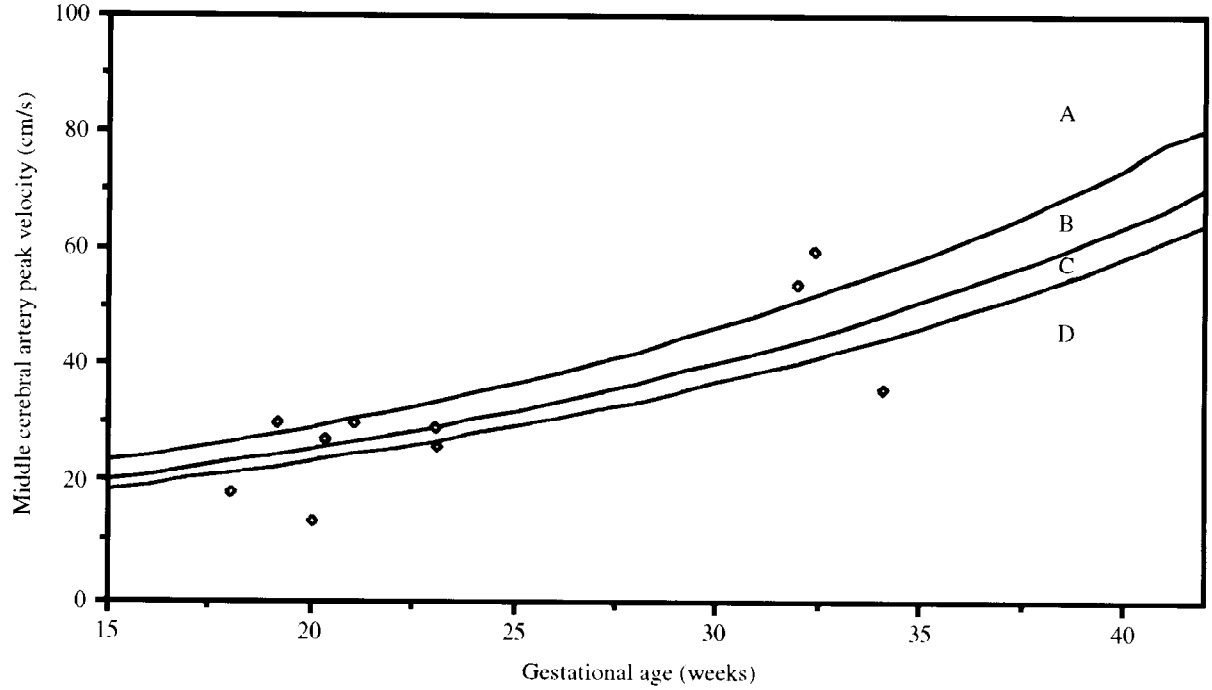

Figure 4 Non-anemic cases in Group C plotted over the risk zones. One fetus apparently had a value on the mean. In reality, its value was just below the mean

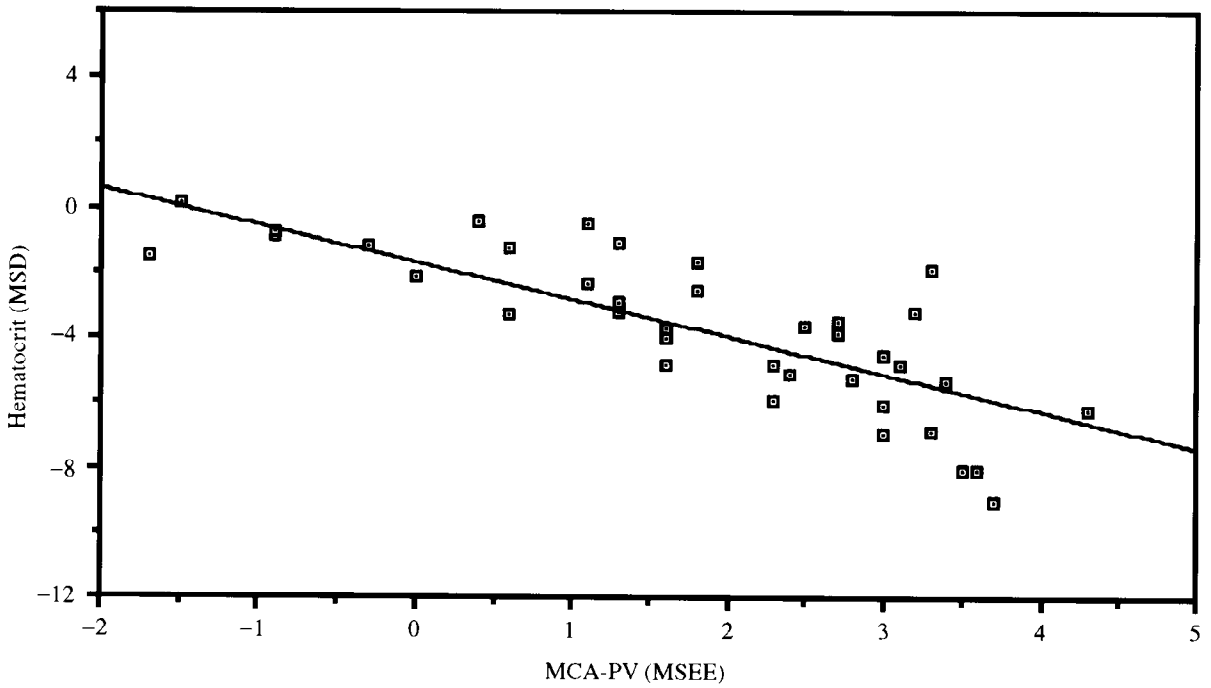

Figure 5 Regression of hematocrit multiple of standard deviation (MSD) on middle cerebral artery peak velocity (MCA-PV) multiple of standard error of estimation (MSEE) for the first cordocentesis in the fetuses of Groups B and C

anemia[6,7]. The reason for previous investigators failing to find such a correlation between fetal Doppler measurements and anemia may be that they used angle-independent indices, which may not be affected by a change in hematocrit, or they measured velocity in vessels requiring angle correction. Our hypothesis was that a decrease in hematocrit could increase the blood velocity, which is most accurately measured when the angle between the ultrasound beam and the direction of blood flow is low. We selected the middle cerebral artery, because this artery may be studied with an angle of insonation between the ultrasound beam and the direction of blood flow close to 0° and, therefore, the peak systolic blood velocity may be measured accurately.

One limitation of our investigation was that we studied some fetuses on more than one occasion. However, Figure 5 shows that the correlation between the hematocrit and the middle cerebral artery peak systolic

velocity was maintained when only first cordocenteses were included.

The strength of the study is that no significantly anemic fetus of Group C had the measured velocity below the mean value. This suggests that up to half of cordocenteses could be avoided, because, when the middle cerebral artery peak systolic velocity was below the mean, no fetus was anemic. These data suggest that measurement of the peak systolic velocity of the fetal middle cerebral artery is a non-invasive method of ruling out fetal anemia in a group at risk for maternal blood group immunization. The test we present in this study has the potential to be used for timing the cordocentesis in the fetus at risk for anemia.

It must be emphasized that, in order successfully to apply our test, the population must be at risk for fetal anemia. The middle cerebral artery peak systolic velocity must also be determined with an incident angle between the ultrasound beam and the vessel close to 0°. The highest peak velocity should be recorded. Table 1 shows the mean and standard error, given the age in weeks. If the peak velocity of the middle cerebral artery is below the mean, the fetus is non-anemic and a cordocentesis may be delayed. Figure 5 shows that there is an inverse relationship between the middle cerebral artery peak systolic velocity and the hematocrit. Therefore, the most severe cases of anemia have the highest velocities.

In summary, the middle cerebral artery peak systolic velocity has the potential to be used in the management of the fetus at risk for anemia. Each sonologist should examine the zones of anemia risk suggested in this study prior to applying the test in practice. This approach, if successful, could reduce the number of invasive procedures needed in the management of the potentially anemic fetus.

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