Diagnostic procedures for assessing the severity of alloimmune fetal anemia

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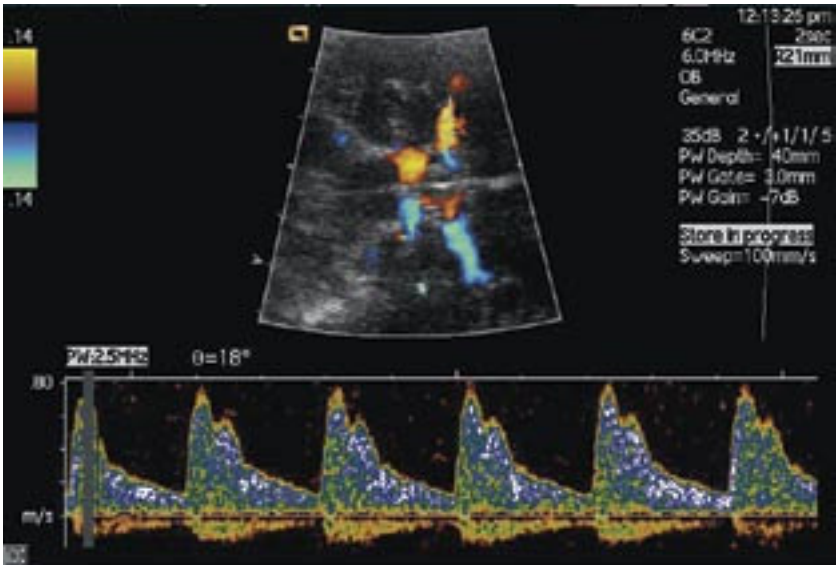
Part 2: Ultrasonographic approach



Effect of an increase of the hematocrit on middle cerebral artery peak and umbilical vein maximum velocities in anemic fetuses

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Abstract

ObjectIve: To measure the effects of acute large increases of the hematocrit on fetal peak arterial and maximum venous blood flow velocities.
Methods: Middle cerebral artery peak flow velocities and umbilical vein maximum flow velocities were measured before, immediately after, and 12-24 h after intrauterine transfusions. All measurements were standardized for gestational age.
Results: Complete measurements were obtained at 60 intrauterine transfusions. The mean hematocrit before intrauterine transfusion was 0.19 l/l and after 0.40 l/l. The middle cerebral artery peak flow velocity decreased immediately after transfusion in 59 of the 60 cases. There was a rise in umbilical vein maximum flow velocity immediately after intrauterine transfusion in 37 of the 60 cases. The sensitivity of middle cerebral artery peak flow velocity for severe anemia before intrauterine transfusion was 54% and the specificity 57%. The sensitivity of umbilical vein maximum flow velocity for severe anemia before intrauterine transfusion was 67% and the specificity 57%.
Conclusions: An acute large increase of the fetal hematocrit significantly decreases middle cerebral artery peak flow velocity. The effect on umbilical vein maximum velocity is, however, unpredictable.

Introduction

Middle cerebral artery peak velocity (MCA peak) and umbilical venous maximum velocity (UV max) increase in fetuses when anemia develops.[1-7] Measurements of arterial and venous flow velocities have even been described as sensitive indicators of the degree of fetal anemia in red cell alloimmunization.[3-7] As such, these measurements have recently been proposed as an alternative diagnostic method to invasive procedures like amniocentesis and cordocentesis.[3-7] A lowered blood viscosity due to a decrease in hematocrit is an obvious explanation for raised blood velocities in anemia. However, flow velocities are influenced by many other factors, and normal values change during gestation: in uncomplicated pregnancies, the MCA peak increases from 23 cm/s at 18 weeks to 58 cm/s at 38 weeks[5], and the UV max increases from 15 cm/s at 18 weeks to 24 cm/s at 38 week.[3,8] Arterial flow velocities depend on blood viscosity, cardiac output, vessel diameter, and peripheral resistance.[9,10] Venous flow velocities depend on blood viscosity, vessel diameter, right atrial pressure, and ductus venosus function.

We wanted to study the effects of acute changes in hematocrit on fetal maximum blood flow velocities. Therefore, we measured the hematocrit before and after intrauterine transfusion (IUT), and MCA peak and UV max before, immediately after, and the day after transfusion.

Methods

Setting and Patients

The Leiden University Medical Center is the national referral center for the treatment of fetal anemia in the Netherlands. Our methods for diagnosis and treatment of severe fetal alloimmune anemia have been described previously.[11] Between November 2000 and August 2001, Doppler ultrasound was performed before, immediately after, and 1 day after all IUTs. The study was approved by the institutional review board, and all women gave oral informed consent. Fetal blood sampling was performed either at the intrahepatic vein or at the placental cord insertion (at the

discretion of the operator), and packed red cells with a hematocrit of around 80% were given intravenously. Before the procedure, meperidine (75 mg), promethazine (25 mg) and indomethacin (50 mg) were given to the mother. Atracurium (0.4 mg/kg) was given to the fetus immediately after the initial blood sample was taken.

Measurements

MCA peak and UV max measurements were performed before IUT (0-6 h), immediately after (within 30 min) and the day after (12-24 h). 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. Flow velocity waveforms were obtained in the absence of fetal breathing and body movements. Doppler studies were done by one of five experienced operators (E.S., K.T., R.H.M., F.K., F.V.) using an Acuson Sequoia (Acuson, Mountain View, CA) ultrasound machine with a 6.0 MHz probe. The circle of Willis was visualized and the middle cerebral artery was examined close to its origin from the internal carotid artery. In the absence of visible changes in the waveform patterns, we measured for at least five waveforms. The highest point of the flow velocity waveform (peak systolic velocity) was measured. The UV max was measured in the straight intrahepatic portion of the umbilical vein. The maximum velocity was measured when a uniform Doppler signal of at least 3 seconds was obtained. The hematocrit was measured in the initial fetal blood sample and at the end of the transfusion.

Normal and standardized values

Normal hematological and blood flow velocity values change during gestation, and we therefore standardized all measurements (hematocrit, MCA peak, and UV max) for gestational age. For fetal hematocrit, we used the reference values proposed by Forestier et al.[12,13]Standardized fetal hematocrit (z_Ht) was defined as the number of SDs that the actual value deviated from the normal mean for gestational age. Severe fetal anemia was defined as z_Ht ≤ -5, moderate fetal anemia as -2 ≥ z_Ht > -5. For MCA peak, we used the reference values proposed by Mari et al. [5], and the

standardized MCA peak velocity was expressed as multiples of median (MoM_MCA) for gestational age. An abnormally increased MCA peak was defined as a MoM_MCA ≥ 1.5. For UV max, we used the reference values proposed by Oepkes et al.[3,8] The standardized UV max velocity (z_UV) was defined as the number of SDs the measured value deviated from the normal mean for gestational age. An abnormally increased UV max was defined as z_UV ≥ 2. Hydrops was classified as mild when a distinct rim of ascites was present with or without pericardial effusion.[14] Hydrops was classified as severe when ascites was abundant (free-floating intra-abdominal organs) with or without pericardial effusion, skin edema, and pleural effusion.[14]

Statistics

To compare the changes in Doppler velocities (MoM_MCA and z_UV) between the three different time points and changes in z_Ht between the two different time points, repeated measures analysis of variance was used. A value of $p < 0.05$ was considered significant. Comparison between subgroups at each time point was performed using t-test (puncture site, type of immunization) and ANOVA (presence of hydrops). Comparison of the changes between the three different time points in the subgroups (according to puncture site, type of immunization and presence of hydrops) was done using repeated measures analysis of variance. Because of multiple testing, a Bonferoni correction was used for comparison of subgroups, and only p-values < 0.01 were considered significant. We thereby assumed that z_Ht on the day after transfusion was identical to the value obtained immediately after IUT [15]. Sensitivity and specificity of MoM_MCA and z_UV before IUT in the prediction of severe fetal anemia were calculated using 2x2 tables. Comparison between sensitivities and specificities of the subgroups (puncture site, type of immunization, presence of hydrops, order of IUT) before transfusion was performed using χ^2 tests. Statistical analysis was performed uing SPSS version 10.0 (SPSS, Chiago, IL.).

Results

During the study period, 80 IUTs were performed in 30 pregnancies. Twenty procedures were excluded from analysis because of incomplete measurements due to fetal breathing, body movements, or fetal position in utero (n=16), or because the final fetal blood sample could not be obtained (n=3). In 1 case, there was persistent bleeding from the puncture site (after the final fetal blood sample) and the fetus was found to be severely anemic again 7 days later (n=1). Complete measurements were obtained from 60 IUTs in 30 fetuses in 29 women (1 with a twin pregnancy). Characteristics of these 60 IUTs are shown in Table 1.

Table 1 - Characteristics of the 60 IUTs

Gestational age (completed weeks), median (range)	29 (19-35)
Type of alloimmunization:	
D	45 (of which 6 mildly hydropic)
Kell	14 (of which 3 mildly and 3 severely hydropic)
c	1
Order of IUTs:	
first	22
second	15
third	11
fourth	7
fifth	4
sixth	1
Degree of anemia:	
moderate (-2 SD ≥ Ht > -5 SD)	14
severe (Ht ≤ - 5 SD)	46
Puncture site:	
placental cord insertion	44
intrahepatic vein	16

IUT: intra uterine transfusion; Ht: Hematocrit

There were no complications following IUT in our study population. The MCA peak decreased immediately after transfusion in 59 of the 60 cases. There was a rise in UV max immediately after the procedure in 37 of the 60 cases. Table 2 shows the mean values (range) of MCA peak, UV max

and Ht and their standardized values before, immediately after and 1 day after IUT. MCA peak and MoM_MCA as well as UV max and z_UV differed significantly between the three time points (p<0.0001). Ht and z_Ht differed significantly between the two time points (p<0.0001). One day after IUT, z_UV was significantly (p < 0.003) higher in severely hydropic fetuses than in mildly hydropic or non-hydropic fetuses. There were no other significant differences at any of the time points in z_Ht, MoM_MCA, and z_UV between the subgroups (according to puncture site, presence of hydrops, type of immunization). In addition, there were no significant difference in the change that occurred after IUT in the subgroups.

Table 2 - Actual and standardized measurements (mean (range)) before, immediately after and 1 day after 60 IUTs

	before IUT	*after IUT*	*1 day after IUT*	*repeated measures ANOVA between time points*
Actual values				
MCA peak (cm/s)	61.87 (26-109)	32.68 (10-60)	44.40 (18-73)	p < 0.0001
UV max (cm/s)	29.72 (15-58)	36.32 (14-101)	22.75 (10-35)	p < 0.0001
Ht (l/l)	0.19 (0.04-0.28)	0.40 (0.22-0.47)		p < 0.0001
Standardized values				
MoM_MCA	1.59 (0.94-2.68)	0.83 (0.26-1.75)	1.13 (0.67-1.62)	p < 0.0001
z_UV	3.09 (-1.38-11.35)	5.19 (-2.6-25.53)	0.87 (-2.92-5.08)	p < 0.0001
z_Ht	5.79 (3.13-8.76)	0.43 (-1.79-3.93)		p < 0.0001

IUT: intrauterine transfusion; MCA: Middle cerebral artery; UV: Umbilical vein; Ht: Hematocrit; MoM_MCA: Multiples of median of MCA for gestational age; z_UV: Number of SDs the actual value of UV deviated from normal mean for gestational age; z_Ht: Number of SDs the actual value of Ht deviated from normal mean for gestational age

Figure 1 plots hematocrit values of 30 fetuses at 60 IUTs against their gestational age. Figure 2 shows the relation between MoM_MCA and z_Ht before, immediately after, and 1 day after IUT. A horizontal line is drawn at MoM_MCA of 1.5 to separate test-positives (above) from test-negatives (below). A vertical line is drawn at the threshold of severe anemia: cases on the left of the vertical line were severely anemic, and cases on the right were moderately anemic. Figure 3 shows the relation between z_UV and z_Ht before, immediately after, and 1 day after IUT. A horizontal

line is drawn at z_UV of 2.0 to separate test-positives (above) from test-negatives (below). A vertical line is again drawn at the threshold of severe anemia: cases on the left of the vertical line were severely anemic and cases on the right were moderately anemic. As such, Figure 2 and 3 can be read as two-by-two tables. Table 3 lists sensitivities and specificities of MoM_MCA and z_UV before IUT in the prediction of severe fetal anemia in the study population. There were no significant differences in sensitivity and specificity before IUT between subgroups (according to puncture site, presence of hydrops, type of immunization, order of IUT).

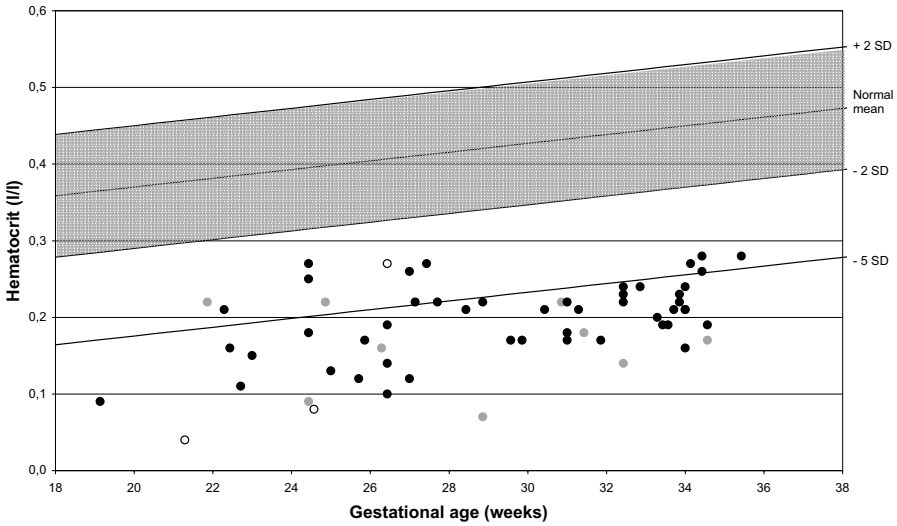

Figure 1 - Hematocrit values before 60 intrauterine transfusions (in 30 fetuses), plotted against gestational age at blood sampling. The grey zone marks the limits of normal (mean ± 2 SD) fetal hematocrit concentrations [13]. The lower line separates moderate (between –2 and -5 SD) from severe (≤ –5 SD) fetal anemia. Dark circles represent non-hydropic fetuses, grey circles represent mildly hydropic fetuses, and open circles represent severely hydropic fetuses.

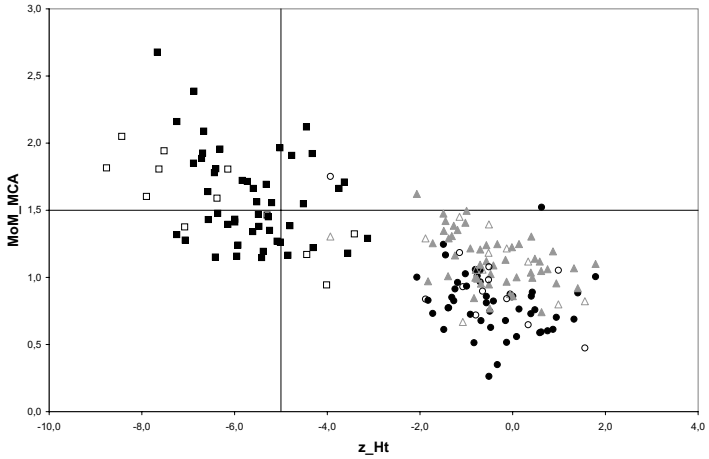

Figure 2 - Relation between standardized middle cerebral artery peak velocity (MoM_MCA) and standardized hematocrit (z_Ht) before (squares), immediately after (circles), and 12-24 h after (triangles) 60 intrauterine transfusions. Closed symbols represent non-hydropic fetuses, and open symbols represent hydropic fetuses. z_Ht was defined as the number of SDs that the actual value deviated from the normal mean for gestational age. MoM_MCA was expressed as multiples of median for gestational age. The horizontal line drawn at 1.5 MoM_MCA seperates test-positives (above) from test-negatives (below). The vertical line drawn at -5 z_Ht is the threshold for severe anemia. Cases on the left of the vertical line were severely anemic, cases on the right were non-anemic or only moderately anemic.

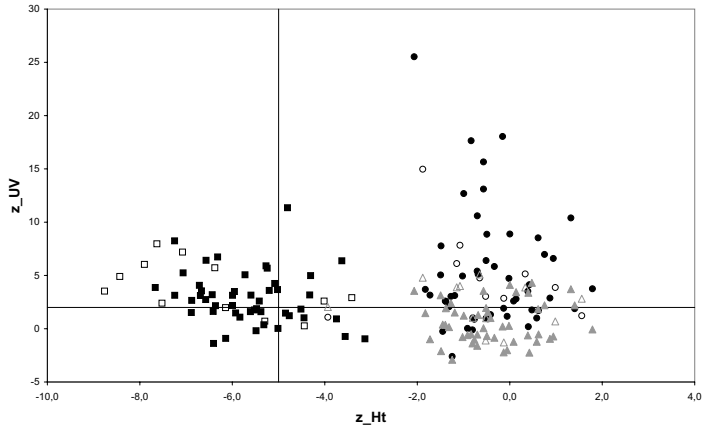

Figure 3 - Relation between standardized umbilical vein maximum velocity (z_UV) and standardized hematocrit (z_Ht) before (squares), immediately after (circles), and 12-24 h after (triangles) 60 intrauterine transfusions. Closed symbols represent non-hydropic fetuses, and open symbols represent hydropic fetuses. z_Ht was defined as the number of SDs that the actual value deviated from the normal mean for gestational age. z_UV was defined as the number of SDs the measured value deviated from the normal mean for gestational age. The horizontal line drawn at 2 z_UV seperates test-positives (above) from test-negatives (below). The vertical line drawn at –5 z_Ht is the threshold for severe anemia. Cases on the left of the vertical line were severely anemic, cases on the right were non-anemic or only moderately anemic.

Table 3 - Sensitivity and specificity of MoM_MCA and z_UV before IUT in the prediction of severe (Ht ≥ -5 SD) fetal anemia

	total study population (n = 60)		*untransfused fetuses (n = 22)*		*previously transfused fetuses (n = 38)*	
	MoM_MCA	z_UV	MoM_MCA	z_UV	MoM_MCA	z_UV
Sensitivity % (95% CI)	54 (39-69)	67 (52-80)	58 (33-80)	74 (49-91)	52 (32-71)	63 (42-81)
Specificity % (95% CI)	57 (29-82)	57 (29-82)	0 (0-71)	33 (1-91)	73 (39-94)	64 (31-89)

MoM_MCA: Multiples of median of MCA for gestational age; z_UV: Number of SDs the actual value of UV deviated from normal mean for gestational age; IUT: Intrauterine transfusion; Ht: Hematocrit; SD: Standard deviation; 95% CI: 95% Confidence Interval.

Discussion

As expected, we found a substantial decrease in MCA peak immediately after IUT. Quite unexpectedly, however, we found a steep, though temporary, increase in UV max immediately after IUT in 37 of our 60 cases. The latter finding challenges the view that the fetal hematocrit is the main determinant of the blood flow velocity. Furthermore, sensitivities of MCA peak and UV max in the prediction of severe fetal anemia were disappointing in our study. There were no significant differences in these sensitivities between untransfused and previously transfused fetuses.

We think that the strength of our study lies in the fact that it resembles a laboratory setting: we measured fetal blood flow velocities in the same fetus before and after substantially changing its hematocrit. There are, however, several shortcomings too. First, we not only changed hematocrit during IUT, but also infused a relatively large volume into the fetal vascular space. It is known from animal experiments, though, that through loss of plasma from the fetal circulation after packed red cell transfusion, the fetal blood volume increases by only half of the transfused volume.[15] It is also known from these experiments, that the fetal blood volume after IUT remains at the same level for 24 h.[15] Second, our standard medication before IUT was another factor that, in addition to the changes in hematocrit and vascular volume, may have influenced the fetal response to IUT. Third, in individual cases, small hematomas or edemas at the

intrahepatic course of the umbilical vein may have influenced UV max near the puncture site. However, we did not find a significant difference in post IUT standardized UV max between procedures according to puncture site.

Mari et al. measured MCA peak before and immediately after IUT in 17 procedures.[10] These authors used no tocolytic agents and did not puncture intrahepatically. They found a significant decrease in MCA peak following IUT. Delle Chiaie et al. measured MCA peak before and after 39 IUTs.[2] They found a significant reduction of post transfusion MCA peak values. Oepkes measured UV max before and 12 -20 h after the first IUT in 36 fetuses.[16] This author found a significant decrease in UV max the day after IUT. Recently, Stefos et al. measured MCA peak before and immediately after 54 procedures.[17] They found a decrease in MCA peak in all but 1 fetus. Our findings on the effect of IUT on MCA peak and UV max are consistent with those of these four studies. The rise in UV max that we found immediately after IUT, however, has not been reported previously.

In an often cited study, Mari et al. have proposed a cut-off value for MoM_MCA of 1.5 for moderate anemia, and one of 1.55 for severe anemia, as these cutoff values resulted in 100% sensitivity, based on their retrospective analysis of 111 fetuses.[5] Recently, Detti et al. proposed a cutoff value for MoM_MCA of 1.69 for severe anemia in previously transfused fetuses, again based on 100% sensitivity in their retrospective series of 64 fetuses.[18] Delle Chiaie et al. found 73% sensitivity of MCA peak before 108 fetal blood samples by using a MCA peak threshold value of 1.29 multiples of median in the prediction of anemia (Hb ≤ 0.84 MoM).[2] Texeira et al. prospectively studied 26 alloimmunized fetuses and found a sensitivity of 67% of an MCA peak of > 2 SD above the mean in predicting severe anemia (z_Ht < -4).[7] In the present study, we found a sensitivity of 54% for MCA peak (MoM_MCA ≥ 1.5) in the prediction of severe anemia (z_Ht ≤ -5). This may seem disappointing. However, it is well known that sensitivities are always lower in prospective series than in the retrospective series from which the cut-off values have been derived.

Our study probably raises more questions than that it gives answers. First, there was the unexpected rise in UV max immediately after IUT.

From this finding, it seems obvious that the fetal hematocrit is not the only and probably not the most important factor determining maximum umbilical venous blood flow velocities. The venous pressure may be far more important in this matter. Second, the wide range in pre-transfusion MoM_MCA for fetuses with identical z_Ht points to the fact that, besides hematocrit, other factors such as cardiac output and peripheral resistance must play an important role in determining MCA peak. Finally, the protein concentration is also a major determinant of blood viscosity.[19]

In conclusion, an increasing fetal hematocrit has a clear effect on maximum arterial and venous flow velocities. This effect is partly the result of the changes in blood viscosity, but other factors are certainly involved. Given the potential consequences of a false negative result, the sensitivities of MCA peak and UV max for severe fetal anemia were disappointing in our study. Therefore, monitoring of pregnancies at risk of fetal anemia should not rely solely on measuring blood flow velocities.

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