

The effects of serial intravascular transfusions in ascitic/hydropsic RhD-alloimmunized fetuses

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ABSTRACT

Objective To evaluate the effects of serial intravascular transfusions on RhD-alloimmunized fetuses with ascites/hydrops at the time of the first transfusion by measuring multiple hematological/biochemical blood variables.

Methods Thirty-one singleton pregnancies were referred for management of RhD alloimmunization. Seven fetuses had hydrops on presentation and were transfused immediately. The remainder underwent weekly ultrasound examinations, and fetal blood sampling and transfusion were performed on development of ascites. In the 104 samples collected overall from the 31 fetuses, glucose, uric acid, urea, creatinine, total protein, total and direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyltransferase, alkaline phosphatase, lactic dehydrogenase, amylase, pseudocholinesterase (PCHE), creatine kinase, triglycerides and cholesterol were measured and compared with a reference range for non-anemic fetuses.

Results The median gestational age at first transfusion was 26 (range, 18–34) weeks. There were three fetal losses after the first transfusion, two of which were due to procedure-related complications; one further loss occurred. At the first transfusion fetal hematocrit, pO_2 , total protein, PCHE, creatinine and urea concentrations were significantly decreased compared to reference data, while total and direct bilirubin, AST, ALT, amylase, triglyceride and uric acid concentrations were increased. In all surviving fetuses ascites/hydrops had disappeared by the second transfusion. Fetal pO_2 , total protein, AST, ALT and PCHE concentrations had normalized by the third transfusion. Correction of fetal anemia did not affect the other variables.

Conclusions RhD-alloimmunized fetuses with ascites/hydrops at the time of the first transfusion had a survival

rate of 87%. Alterations of several biochemical fetal blood indices are present at the first sampling/transfusion, but most variables normalize with intravascular transfusions. Copyright © 2005 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Fetal intravascular transfusion is now the standard of care in the management of severe RhD alloimmunization. A review of the literature demonstrates that the overall survival rate is 84% for fetuses managed with intravascular transfusion: 94% for non-hydropsic fetuses and 74% for those fetuses who have developed hydrops¹. This suggests that fetal blood sampling and transfusion should be performed prior to the development of hydrops. Accordingly, several strategies have been developed to try to determine the optimal time for intervention. These strategies, both invasive (amniocentesis) and non-invasive (ultrasound/Doppler studies), have focused on the presence of fetal anemia as the determining point for initiation of transfusion.

However, fetal blood sampling and transfusion are still associated with a significant risk of fetal loss. Additional complications include cord hematoma, vein thrombosis, development of porencephalic cysts and transient or persistent fetal bradycardia resulting in emergency delivery. Therefore, a strategy limiting procedures to only those fetuses that clinically require transfusion would be ideal. In adult medicine, anemia in the absence of clinical symptoms does not always necessitate transfusion. It might be argued that the same approach could be applied to the anemic fetus, although fetal anemia cannot be treated with conventional alternative treatments such as iron supplementation.

In an effort to minimize the number of invasive procedures, a protocol has been established in our unit to follow non-hydrops fetuses with weekly ultrasound examinations. Fetal blood sampling and transfusion are performed only when clear sonographic changes occur.

The purpose of this study was to evaluate the effects of serial intravascular transfusions on RhD-alloimmunized fetuses with ascites/hydrops at the time of the first transfusion by measuring multiple hematological and biochemical blood variables.

METHODS

The series included 31 consecutive singleton pregnancies in 27 women who were referred for antenatal management of Rh-D alloimmunization during a 5-year period (1998–2002), all of whom required intrauterine transfusion. Ten women (37%) had had one or more perinatal deaths during previous pregnancies.

Seven fetuses (23%) presented with hydrops (ascites and subcutaneous edema) and were transfused immediately. The remainder underwent weekly ultrasound examinations from the time of referral. The first transfusion was performed only after detection of ascites, which prompted immediate fetal blood sampling and, after confirmation of anemia, intravascular transfusion. None of these 24 fetuses had subcutaneous edema.

The 31 fetuses underwent a total of 104 blood samplings/transfusions, which were performed by ultrasound-guided puncture of the umbilical vein either at the placental cord insertion or at its intrahepatic portion, with a 20-gauge spinal needle and a free-hand approach. The amount of blood transfused at each procedure was calculated based on the initial hemoglobin/hematocrit and the estimated fetoplacental blood volume², with the aim of achieving a final hematocrit ranging from 40% to 50%.

All procedures were performed in the morning, with the mother not fasting, and no maternal or fetal sedation were used. Prior to each transfusion, 2.5 mL fetal blood was taken: 1 mL for a blood count, 0.5 mL to assess acid–base balance, and 1 mL in plain tubes for later assessment of glucose, uric acid, urea, creatinine, total protein, total and direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyltransferase (γ -GT), alkaline phosphatase (ALP), lactic dehydrogenase, amylase, pseudocholinesterase (PCH), creatine kinase, triglycerides and cholesterol. Standard enzymatic methods were employed for all determinations. Maternal blood was collected immediately before fetal blood sampling and sent for analysis of the same biochemical constituents.

Three intrauterine deaths occurred (two due to cord tamponade, and one unexplained), within 24 h after the first transfusion, which had been performed at the placental cord insertion in all three. Twenty-five fetuses underwent a second transfusion, 2 weeks after the first, when all fetuses showed resolution of ascites/hydrops. A third, fourth, fifth, sixth and seventh transfusion was performed in 20, 14, nine, four and one fetuses, respectively. One fetus died *in utero* a few days after the

sixth transfusion; the postmortem suggested a congenital viral infection. All neonates, whose gestational age at delivery ranged from 31 to 38 weeks' gestation (median, 36 weeks), were discharged home in good condition. Overall survival was therefore 87% (27/31).

Fetal acid–base, hematological and biochemical data were compared with reference ranges reported previously³ and values were expressed as number of SDs from the mean for gestational age (Z-scores). Since fetal and maternal glucose, creatinine, urea and uric acid concentrations were shown to be correlated, but other values were not, we calculated appropriate Z-scores for maternal and fetal values and maternal–fetal differences for these measurements.

Due to the asymmetric distribution of data, the Wilcoxon test was used to estimate deviations from the reference range³. Analysis of variance was used to evaluate changes at subsequent transfusions for all fetuses. Correlations between hematocrit and the other blood parameters were tested by the least-squares method.

RESULTS

The median gestational age at the first transfusion was 26 (range, 18–34) weeks. There was no difference in gestational age at the first transfusion, nor, with one exception, in any of the variables analyzed between the seven fetuses which had been hydropic for an unknown duration of time (median hematocrit Z-score = -8.4 ; 95% CI, -9.1 to -4.7), and the remainder which had had a normal scan a week before (median hematocrit Z-score = -5.0 ; 95% CI, -6.4 to -4.1). The exception was fetal glucose, which was significantly higher in the fetuses which were hydropic at the first referral (median

Table 1 Mean numbers of SDs from the mean for gestational age, and 95% CIs, of the analyzed blood variables of all fetuses at the first transfusion

	Mean	95% CI	P
pH	-0.27	(-0.77 to 0.22)	NS
pO ₂	-0.73	(-1.10 to -0.36)	0.001
Hematocrit	-5.68	(-6.67 to -4.69)	0.0001
Aspartate aminotransferase	5.09	(0.03 to 10.16)	0.05
Alanine aminotransferase	2.33	(0.52 to 4.15)	0.01
γ -glutamyltransferase	0.22	(-0.31 to 0.75)	NS
Total bilirubin	17.81	(1.62 to 34.00)	0.03
Direct bilirubin	1.25	(0.55 to 1.95)	0.001
Lactic dehydrogenase	3.60	(-0.54 to 7.75)	NS
Amylase	1.25	(0.60 to 1.90)	0.001
Total proteins	-0.57	(-1.13 to -0.01)	0.05
Alkaline phosphatase	-0.31	(-0.70 to 0.09)	NS
Pseudocholinesterase	-0.96	(-1.52 to -0.41)	0.01
Creatine kinase	-0.37	(-1.00 to 0.26)	NS
Triglycerides	0.89	(0.30 to 1.49)	0.01
Cholesterol	-0.57	(-1.19 to 0.05)	NS
Urea	-0.52	(-0.88 to -0.17)	0.01
Glucose	0.47	(-0.07 to 1.00)	NS
Creatinine	-1.30	(-1.75 to -0.85)	0.0001
Uric acid	0.77	(0.24 to 1.29)	0.01

NS, not significant.

Z-score = 1.7; 95% CI, 0.7 to 2.4) compared with the remaining 24 (median Z-score = 0.4; 95% CI, -0.5 to 0.7; $P = 0.009$).

Table 1 shows the variables analyzed in all fetuses at the first transfusion. Fetal hematocrit, pO₂, total protein, PCHE, creatinine and urea concentrations were significantly decreased compared to reference data³, while total and direct bilirubin, AST, ALT, amylase, triglyceride and uric acid concentrations were increased. In the mothers, urea (mean Z-score = -0.4; 95% CI, -0.8 to 0;

$P = 0.04$) and creatinine (mean Z-score = -0.6; 95% CI, -1.0 to -0.1; $P = 0.02$) concentrations were significantly decreased, while glucose concentration (mean Z-score = 0.6; 95% CI, 0.1 to 1.1; $P = 0.02$) was increased. The maternal-fetal difference of urea was not different from the reference range³ (mean Z-score = 0; 95% CI, -0.7 to 0.6; not significant); therefore, the low values of fetal urea are attributable to decreased concentrations in the maternal compartment. In contrast, maternal-fetal differences of creatinine (mean Z-score = 1.7;

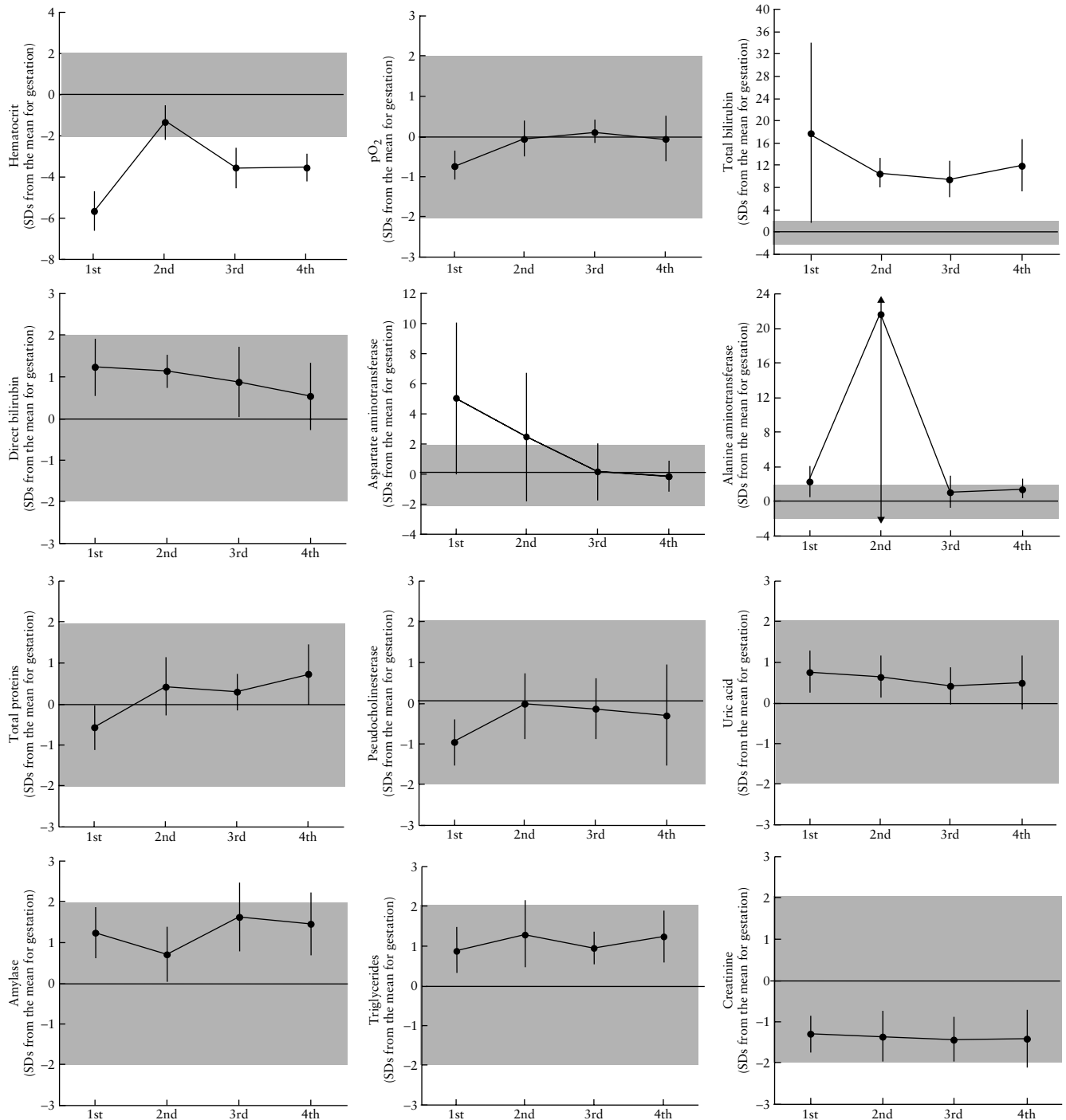


Figure 1 Means and 95% CIs of fetal blood variables at the first, second, third and fourth intravascular transfusion expressed as numbers of SDs from the mean for gestational age. The shaded areas indicate the reference range³ (from -2 to +2 SDs). CIs for the values of alanine aminotransferase at the second transfusion exceed the graph area.

95% CI, 1.2 to 2.1; $P = 0.00001$) and uric acid (mean Z-score = -0.7 ; 95% CI, -1.2 to -0.3 ; $P = 0.005$) concentrations were significant; hence the low values of fetal creatinine and the high concentrations of fetal uric acid cannot be justified by changes in the maternal values alone. At the first transfusion, the initial hematocrit was positively correlated with total protein (Z-score total protein = $1.55 + 0.362$ Z-score hematocrit; $r = 0.72$; $P = 0.0001$), PCHE (Z-score PCHE = $0.442 + 0.24$ Z-score hematocrit; $r = 0.48$; $P = 0.01$) and cholesterol (Z-score cholesterol = $1.53 + 0.366$ Z-score hematocrit; $r = 0.68$; $P = 0.0001$) concentrations, and negatively correlated with γ -GT (Z-score γ -GT = $-1.24 - 0.25$ Z-score hematocrit; $r = -0.52$; $P = 0.007$), and triglyceride (Z-score triglycerides = $-0.43 - 0.23$ Z-score hematocrit; $r = -0.45$; $P = 0.03$) concentrations.

Figure 1 shows changes with subsequent (up to the fourth) transfusions in those pretransfusion blood variables which were significantly different from the reference range³ at the first procedure. Fetal pO₂, total protein, PCHE and AST concentrations had normalized by the second transfusion and ALT by the third. Not surprisingly, fetal hematocrit and total and direct bilirubin remained significantly different from the mean for gestational age throughout the transfusions. The other variables (amylase, triglycerides, creatinine and uric acid) did not change significantly over the four transfusions. Fetal glucose concentration was higher compared with the reference range³ by the second transfusion and remained so at the following procedures; this was unrelated to increased maternal values, but was always within 1 SD from the mean for gestational age.

DISCUSSION

The issue of when to initiate invasive treatment in RhD alloimmunization is controversial. Intravascular transfusion of anemic fetuses before the development of hydrops is widely thought to be associated with increased survival rates. The advantages of early intervention, however, need to be weighed against the risk of procedure-related complications. Despite recent advances in the surveillance of the RhD-sensitized fetus, the critical level of anemia that warrants the inherent risks of intervention is unclear. The protocol followed in this study of weekly ultrasound examinations and of performing the first intravascular transfusion only after the appearance of ascites was associated with a survival rate comparable to that reported in series of non-hydrotic fetuses^{1,4}. Although this was not a controlled study, it is unlikely that a policy of earlier transfusion would have achieved significantly better results, since at least two of the four fetal losses were the result of a procedure-related complication (cord tamponade). It must be emphasized, however, that for the majority of fetuses in the study, intravascular transfusion was performed less than 1 week after the onset of ascites. This might also explain the finding that there was complete reversal of the ascites by the second transfusion in all

fetuses in this group. However, at the time of the first transfusion several variables in fetal blood other than hematocrit were altered. This raises the concern that transfusion should have been performed earlier in the process.

At the time of the first transfusion, hematocrit in the study fetuses had decreased on average to almost -6 SDs from the mean for gestational age. At that level of anemia, fetal pO₂, but not pH, was also significantly decreased, but had normalized by the second transfusion. Although fetal hematocrit did not correlate with pO₂ levels in this series, it was noted in a previous larger series⁵. This indicates that at the time that fetal ascites appears, mild hypoxemia is present, probably as a consequence of increased fetal and placental tissue oxygen extraction, but this is a transitional phenomenon which is corrected by intravascular transfusion.

The liver enzymes AST and ALT and bilirubin were abnormal at the first sample, with normalization of the enzymes by the third transfusion. Median total bilirubin was greater than 17 SDs above the mean for gestational age at the first transfusion and high bilirubin levels persisted until delivery, suggesting that hemolysis persisted to a large degree regardless of the presence of ascites. The degree of anemia at the first transfusion correlated directly with two indices of liver synthesis, protein and PCHE concentrations, which had also normalized by the second transfusion. We have already reported this liver dysfunction in anemic fetuses⁶.

In contrast, fetal amylase remained significantly higher compared with that in controls. This finding is also present in growth-restricted fetuses with abnormal blood flow studies, and might be related to pancreatic hypoxemia, secondary to reduction of perfusion of the splanchnic area⁷. Raised triglyceride levels in growth-restricted fetuses might indicate chronic hypoglycemia with compensatory lipolysis. The anemic fetus, however, is rather chronically hyperglycemic or normoglycemic⁸ and a different mechanism must therefore be postulated. Indeed, the levels of triglycerides were inversely related to fetal hematocrit at the first transfusion. The fact that anemia leads to lipid metabolism disturbances is further supported by the finding that cholesterol correlated directly with fetal hematocrit, suggesting that the anemic fetus synthesizes less cholesterol and more triglycerides, or utilizes more cholesterol and fewer triglycerides for peripheral oxidation. We have reported that growth-restricted fetuses have increased triglyceride levels inversely proportional to the degree of growth restriction and not to the degree of fetal compromise as indicated by blood flow studies⁷. Whether this imbalance in lipid metabolism may be the trigger for the metabolic and circulatory abnormalities found in adults who were growth-restricted *in utero* remains to be clarified. The finding that severe RhD-alloimmunized fetuses share this metabolic abnormality should prompt a long-term follow-up of adults who were anemic *in utero*.

Parallel to this increased availability of substrates, the low concentrations of fetal creatinine suggest that

fetuses with Rh-alloimmunization exhibit increased blood volume, probably related to the larger placental mass and resultant expansion of the fetal vascular bed. This increase may be associated with the expansion of fetal blood volume reported in fetuses with hydrops⁹, but it is likely to precede the hydropic changes, since creatinine remained low throughout gestation despite correction of both anemia and ascites.

Clinical management of the RhD-sensitized fetus has focused on determining the presence of fetal anemia. Mari *et al.* reported on the use of Doppler assessment of the middle cerebral artery to detect moderate to severe fetal anemia in the non-hydropic fetus: although this technique achieved a sensitivity of 86–100%, there was still a 12% false-positive rate¹⁰. This would result in one in ten fetuses being exposed to unnecessary procedures. Additionally, it remains undetermined if the presence of fetal anemia in the non-hydropic fetus necessitates intravascular transfusion.

The recent report of van Kamp *et al.*⁴ demonstrates the important distinction in the outcomes of those fetuses with mild versus those with severe hydrops: in fetuses classified as mildly hydropic, there was reversal of hydrops in 88% of the cases and an overall survival rate of 98%. It is conceivable that the reason a fetus develops severe hydrops is the duration of time that the fetus has been exposed to anemia below a critical level. Early detection and intervention is possible with the implementation of close fetal surveillance with weekly ultrasound examinations. Our current protocol of intervening only if there is clinical evidence of ascites offers some potential benefits. First, it limits invasive intervention to those fetuses that clearly warrant therapy. Second, the recognition of ascites does not require advanced equipment or training, so the patient can be followed locally with transfer to a regional perinatal center only when there is the indisputable need for transfusion. Third, our results suggest that there is no increased risk in perinatal mortality, while fetal exposure to procedure-related risks is limited. It must be acknowledged, however, that this was not a controlled study and it involved only Rh-D alloimmunized women. Other authors have been able to limit the number of unnecessary fetal blood samplings by measuring middle cerebral artery blood flow velocities¹¹. Similar to our study, the recommended interval between scans was 7 days. The advantage of intervening once the peak velocity in the middle cerebral

artery is above the reference range³ for gestational age is that transfusion is performed prior to the development of ascites. Indeed, some alterations of fetal physiology occur with severe anemia as indicated by the abnormalities in the variables that we studied. Our data also indicate, however, that most of these changes are reversible with correction of fetal anemia.

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