Are fetal bilirubin levels associated with the need for neonatal exchange transfusions in hemolytic disease of the fetus and newborn?

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BACKGROUND: Fetal bilirubin is routinely measured at our center when taking a pretransfusion blood sample at intrauterine transfusions in hemolytic disease of the fetus and newborn. However, the clinical value of fetal bilirubin assessment is not well known, and the information is rarely used. We speculated that there could be a role for this measurement in predicting the need for neonatal exchange transfusion.

OBJECTIVE: This study aimed to evaluate the predictive value of fetal bilirubin for exchange transfusions in severe hemolytic disease of the fetus and newborn.

STUDY DESIGN: A total of 186 infants with Rh alloantibody–mediated hemolytic disease of the fetus and newborn treated with one or more intrauterine transfusions at the Leiden University Medical Center between January 2006 and June 2020 were included in this observational study. Antenatal and postnatal factors were compared between infants with and without exchange transfusion treatments. The primary outcome was the fetal bilirubin levels before the last intrauterine transfusion in relation to the need for exchange transfusion.

RESULTS: In a multivariate logistic regression analysis, the fetal bilirubin level before the last intrauterine transfusions (odds ratio, 1.32; 95% confidence interval, 1.09–1.61 per 1 mg/dL) and the total number of intrauterine transfusions (odds ratio, 0.63; 95% confidence interval, 0.44–0.91 per intrauterine transfusion) were independently associated with the need for exchange transfusion. The area under the curve was determined at 0.71. A Youden index was calculated at 0.43. The corresponding fetal bilirubin level was 5 mg/dL and had a sensitivity of 79% and a specificity of 64%.

CONCLUSION: A high fetal bilirubin level before the last intrauterine transfusion was associated with a high likelihood of neonatal exchange transfusion.

Key words: fetal reference values, hematology, intrauterine transfusion

Introduction

Hemolytic disease of the fetus and newborn (HDFN) is a condition in which maternal alloantibodies lead to the destruction of fetal erythrocytes. In the antenatal period, this can lead to severe fetal anemia, fetal hydrops, and ultimately intrauterine demise.[1,2] The mainstay of antenatal treatment in HDFN is intrauterine transfusion (IUT) to correct fetal anemia. In the postnatal period, HDFN can lead to severe hyperbilirubinemia and prolonged anemia. Hyperbilirubinemia may cause kernicterus or chronic bilirubin encephalopathy, which can lead to permanent brain damage.[1] Hyperbilirubinemia is primarily treated by intensive phototherapy and, in case of treatment failure, exchange transfusion (ET) to rapidly excrete excess bilirubin.

ET is an invasive procedure with rates of associated morbidity up to 74%. Complications include central line complications (such as catheter infection, thrombosis, or dislocation), thrombocytopenia, and neutropenia and metabolic derangement.[3] The rate of ET in infants with severe HDFN treated at our center was approximately 15% after more restrictive guideline adaptation from the American Academy of Pediatrics (AAP) in 2005.[4] Although a few potential variables have been identified as risk factors for the need for ET, it is still challenging to predict antenatally which infant will likely require ET. Improved antenatal prediction could help anticipate and improve individualized postnatal care.

As fetal bilirubin levels are routinely measured at our center before each IUT procedure and as high bilirubin levels after birth are indicators for ET, we hypothesized that high levels of fetal bilirubin before birth could predict the need of ET in the neonatal period. The primary aim of this study was to evaluate whether fetal bilirubin could be a predictor for ET in the neonatal period.

Materials and Methods

Study design and population

All children treated with one or more IUTs for severe Rh-mediated HDFN between January 2006 and June 2020 at the Leiden University Medical Centre (LUMC) were included in an observational study. The LUMC is the national referral center for maternal-fetal therapy in the Netherlands for treatment of severe fetal anemia caused by red blood cell alloimmunization. After fetal treatment with IUT, admission of the neonate to the LUMC is highly recommended; therefore, the study was composed nearly of all live-born infants treated with IUT for HDFN in the Netherlands during the study period. Incidentally, infants were admitted elsewhere because of spontaneous preterm delivery. Furthermore, we included only infants treated with IUT as fetal samples of bilirubin were taken only during this procedure and not in cases without IUT. The cohort was selected from 2006 onward as ET and phototherapy thresholds were changed in 2005.

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AJOG MFM at a Glance

Why was this study conducted?

The mechanism of fetal bilirubin metabolism is poorly understood. Fetal bilirubin is routinely measured at intrauterine transfusions in severe hemolytic disease of the fetus and newborn. This study explored its potential clinical implications.

Key findings

This study demonstrated the potential clinical use of fetal bilirubin levels as a predictor for exchange transfusion.

What does this add to what is known?

Fetal bilirubin is a valuable addition in determining a personal risk profile, including the need for exchange transfusion for infants with hemolytic disease of the fetus and newborn.

After the guideline change, the criteria for ET were as follows: (1) total serum bilirubin above thresholds of 0.5 mL/dL/h according to the AAP guideline; (2) rise of bilirubin >0.5 mL/dL/h despite intensive phototherapy; and/or (3) clinical symptoms of acute bilirubin encephalopathy regardless of bilirubin level.[4] Infants born at <35 weeks' gestation were excluded in this study, as well as infants with blood group alloimmunization caused by non-Rh antigens because of different pathophysiological characteristics in terms of bilirubin accumulation and ET risk after birth.[5]

Data collection

Patient data were collected from medical records of all infants and anonymously recorded. Collected data included sex, gestational age (GA) at birth (weeks), birthweight, cesarean delivery, GA at first IUT (weeks), number of IUTs per fetus, type of alloimmunization, number of ETs per infant, hours after birth until first ET, bilirubin level at birth, maximum bilirubin level after birth, and fetal bilirubin level at all IUTs. Because of the noninvasive nature of this study, the medical ethics committee of our center granted a waiver of consent.

Outcome measures

The primary outcome was the bilirubin level before the last IUT of infants treated with ET in the neonatal period compared with the bilirubin level before the last IUT of infants not treated with ET. Secondary outcomes included the number of ETs per infant, time from birth to first ET (hours), and number of days of phototherapy.

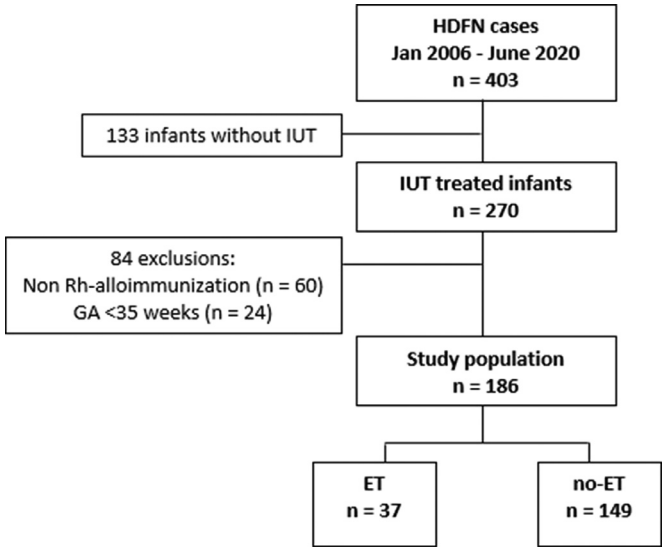
Statistical analysis

The following variables were compared between infants with and without treatments with ET as potential predictors of ET: sex, GA at birth, number of days between birth and last IUT, total number of IUTs, bilirubin at birth, and fetal bilirubin at last IUT. In addition, these variables were included in a multivariable logistic regression model, to correct for potential confounders, except for the bilirubin at birth as this value is highly correlated with the fetal bilirubin at last IUT. Results are presented as odds ratios (ORs) with 95% confidence intervals (95% CIs). A receiver operating characteristic (ROC) curve was made, and the result is presented as the area under the curve (AUC). To find the cutoff value for fetal bilirubin as a predictor for ET, the Youden index was calculated. The Youden index is presented as an absolute fetal bilirubin level with corresponding sensitivity and specificity. Statistical analyses were performed using IBM SPSS Statistics (version 26.0; SPSS Inc, Chicago, IL).

Results

During the study period, 403 infants were born with HDFN and admitted to the LUMC, of which 207 were treated with IUT during the study period and thus were eligible for this study. A total of 84 infants were excluded, as HDFN was caused by a non-Rh antigen in 60 infants and an additional 24 infants were excluded because of a GA of <35 weeks. The derivation of the study population is shown in Figure 1. The baseline characteristics of the study group are presented in Table 1.

FIGURE 1
Flowchart of the study population



ET, exchange transfusion; *GA*, gestational age; *HDFN*, hemolytic disease of the fetus and newborn; *IUT*, intrauterine transfusion.

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TABLE 1
Baseline characteristics

Characteristic	Infants treated with IUT (N=186)
Male,	107 (58)
Gestational age at birth (wk)	36.0 (36.0–37.0)
Birthweight (g)	2869±362
Gestational age at first IUT (wk)	29 (25–32)
Number of IUTs per fetus	2.0 (1.0–3.25)
Type of alloimmunization[a]	
D alloimmunization	174 (94)
C alloimmunization	1 (1)
c alloimmunization	10 (5)
E alloimmunization	1 (1)

Data are presented as mean±standard deviation, median (interquartile range), or number (percentage).

IUT, intrauterine transfusion.

[a] Percentage value does not add up to 100% because of rounding.*Ree. Fetal bilirubin in severe hemolytic disease of the fetus and newborn in relation to exchange transfusion. Am J Obstet Gynecol MFM 2021.*

Here, 37 infants (20%) received an ET after birth. In the ET group, the median number of ET per infant was 1 with a median of 24 hours after birth until the first ET. The maximum bilirubin level after birth reached an average of 19 mg/dL.

Univariate analysis of potential predictors for ET was performed (Table 2). The level of fetal bilirubin at the last IUT was positively associated with an increased need for ET after birth (unadjusted OR, 1.32; 95% CI, 1.10–1.58 per 1 mg/dL). A higher number of IUTs received was associated with a reduced need for ET (OR, 0.65; 95% CI, 0.47–0.90 per IUT).

To assess the independent association of these risk factors on the need for ET after birth, the factors were entered in a multivariate logistic regression model (Table 2). Fetal bilirubin at last IUT (OR, 1.32; 95% CI, 1.09–1.61 per 1 mg/dL) and the number of IUTs (OR, 0.63; 95% CI, 0.44–0.91 per IUT) were still independently associated with the need for ET.

An ROC curve was plotted and the AUC for fetal bilirubin was 0.71. The corresponding Youden index was calculated at 0.43 with a cutoff fetal bilirubin level of 5 mg/dL with a sensitivity of 79% and a specificity of 64% (Figure 2). To visualize the course of fetal bilirubin, a boxplot of the fetal bilirubin level at each IUT was computed (Figure 3). Figure 3 shows a small and statistically insignificant decline in fetal bilirubin over the course of IUTs.

Discussion

Principal findings

This study shows that fetal bilirubin measured at the last IUT treatment has a predictive value for the need for ET after birth. A fetal bilirubin cutoff value of 5 mg/dL at the last IUT has the highest sensitivity and specificity in terms of accurately predicting ET treatment after birth. An additional finding in this study was that a higher number of IUTs decreases the need for ET. This finding confirmed previous reports from our study group.[6] The cumulative protective effect of IUTs on ET treatment was hypothesized to be caused by the replacement of fetal, incompatible blood by compatible donor blood and thus interference with the hemolytic process, causing less bilirubin to accumulate and

TABLE 2
Predictors for exchange transfusions in infants with hemolytic disease of the fetus and newborn treated with intrauterine transfusions

Characteristic	ET (n=37)	No ET (n=149)	Univariable OR (95% CI)	Multivariable OR (95% CI)
Fetal bilirubin at the last IUT (OR per 1 mg/dL)	6.02 (5.18–7.09)	4.33 (3.27–6.20)	1.32 (1.10–1.58)[a]	1.32 (1.09–1.61)[a]
Male	26 (70)	81 (54)	0.50 (0.23–1.09)	1.52 (0.66–3.50)
Gestational age of <37 wk	23 (62)	86 (58)	0.83 (0.40–1.74)	0.95 (0.41–2.19)
Number of days between birth and last IUT (OR per day)	19.00 (15.00–24.00)	21.00 (19.00–26.00)	0.97 (0.93–1.01)	1.00 (0.96–1.03)
Number of IUTs (OR per IUT)	2.00 (1.00–3.00)	2.00 (2.00–4.00)	0.65 (0.47–0.90)[a]	0.63 (0.44–0.91)[a]
Bilirubin at birth (OR per 1 mg/dL)	7.89 (6.52–10.82)	5.47 (4.09–6.56)	1.72 (1.41–2.10)[a]	—

Data are presented as number (percentage) or mean (interquartile range), unless otherwise indicated.

CI, confidence interval; *ET*, exchange transfusion; *IUT*, intrauterine transfusion; *OR*, odds ratio.

[a] Values are ORs with statistical significance.

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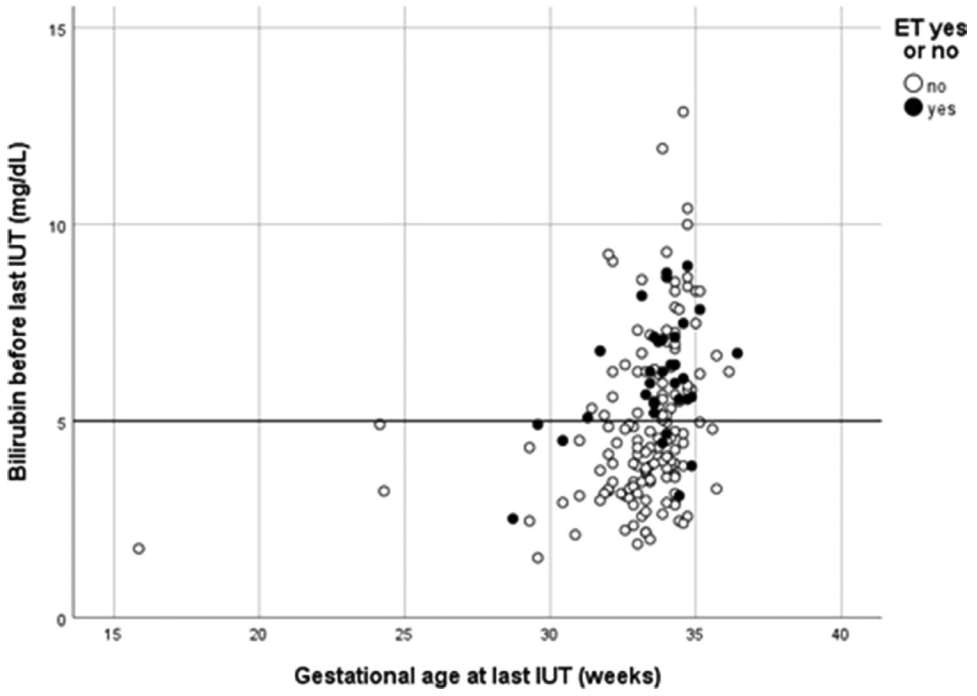
FIGURE 2
Absolute bilirubin levels per fetus before last IUT



The *line* is plotted at the bilirubin value of 5 mg/dL, the value with the highest sensitivity and specificity in predicting ET (the Youden index).

ET, exchange transfusion; *IUT*, intrauterine transfusion.

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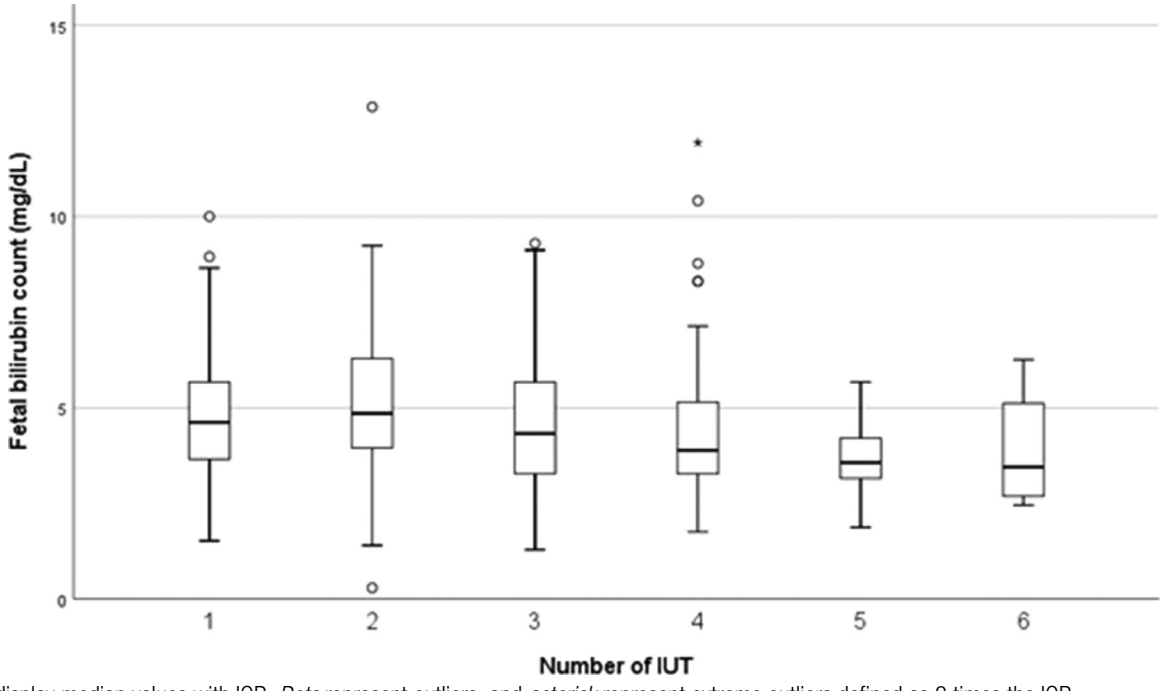
FIGURE 3
Boxplot of fetal bilirubin levels throughout IUT treatment



Boxplots display median values with IQR. *Dots* represent outliers, and *asterisk* represent extreme outliers defined as 3 times the IQR.

IQR, interquartile range; *IUT*, intrauterine transfusion.

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a longer blood transfusion-free interval after birth.[6]

Here, the rate of ET among infants treated with IUT was 20%; the rate was in line with a general international ET decline over the past 20 years. Among all infants with HDFN, the rate of ET at our center declined from 67% (200−2005) to 10% (2015−2000).[7]

Clinical implications

High fetal bilirubin levels could be viewed as an early, fetal predictor for the need for ET. Combined with other risk factors, it could potentially be used to determine a personal risk profile for infants affected by severe HDFN to predict more accurately the neonatal course and plan neonatal care and follow-up accordingly.

We speculated that higher bilirubin levels in the ET group could indicate a stronger hemolytic process with continued production of fetal cells, explaining the correlation with ET treatment after birth. Other possible explanations could be related to impaired placenta function or perhaps saturation of maternal bilirubin excretion capacity.

Research implications

Above all, the findings of this study add to the pathophysiological understanding of HDFN and fetal bilirubin metabolism in relation to IUT treatment.

The mechanism of bilirubin metabolism in infants is well known,[8] but fetal bilirubin metabolism is less well understood. The fetal liver is poorly capable of conjugating bilirubin and is suggested to have an excretory defect so that even if the fetus could conjugate bilirubin, it could not excrete it.[9]

Animal studies in guinea pigs and monkeys show that unconjugated bilirubin can diffuse freely over the placenta and, because of the gradient in favor of the transport from fetal side to maternal side, fetal unconjugated bilirubin is cleared from fetal circulation and transported to the maternal circulation where it is conjugated and excreted.[9,10] In addition, in vitro experiments provided evidence for the existence of carrier-mediated systems for transport of unconjugated bilirubin across the plasma membranes of human placental trophoblast in addition to transport by diffusion.[11,12] None of these studies mention the (possibly harmful) effect of high fetal bilirubin levels on the fetus or provide fetal bilirubin reference values. If basal ganglia are just as susceptible to bilirubin toxicity during fetal life as in the neonatal period, high levels of fetal bilirubin could lead to increased risk of brain injury and impaired neurodevelopmental outcome. More research is needed to address this hypothesis.

Strengths and limitations

One of the strengths of our study was that because our center is the national referral center for HDFN, we have a uniformly treated cohort, limiting variability in treatment of HDFN. A limitation to this study was the relatively small sample size; however, it is the largest homogenous cohort to date to address this subject. Finally, we could not include infants with HDFN without IUTs in this study, despite 15% of these infants being treated with ET at our center in the same period. As these infants did not receive IUT treatment, no fetal blood sampling was available in this group.

Conclusions

Our study demonstrated the possibility of using fetal bilirubin levels as a predictor for ET, making it possible to further accommodate adequate and individualized neonatal care. To translate this study to the clinical setting, more research is necessary, including prospective validation. Studies should focus on computing a model that incorporates multiple risk factors, such as fetal bilirubin, to create personalized risk and treatment profiles for infants affected by severe HDFN. ■

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