# Optimising neonatal management of haemolytic disease of the foetus and newborn

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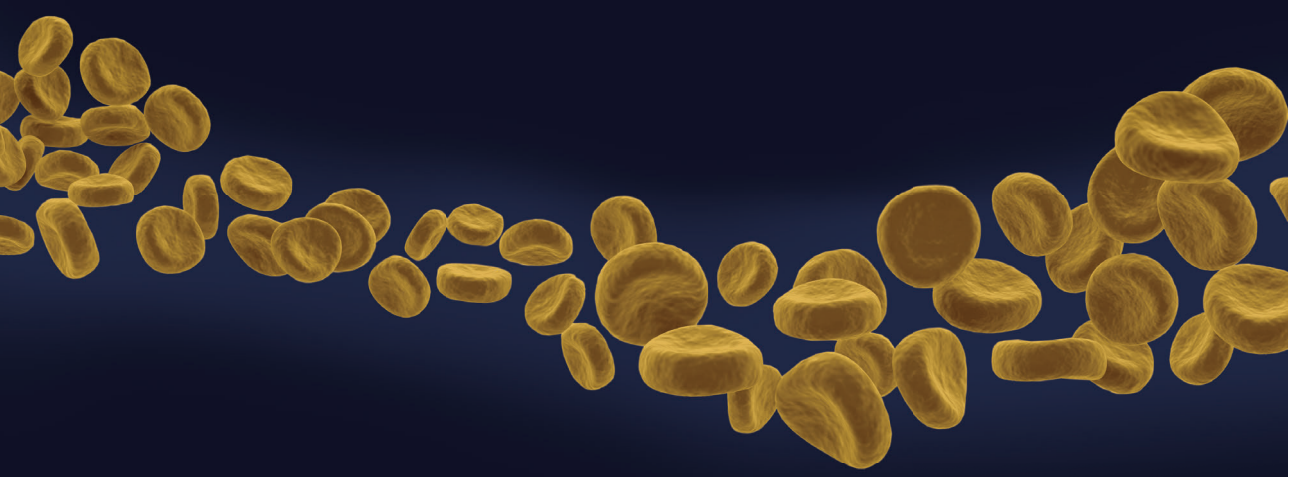
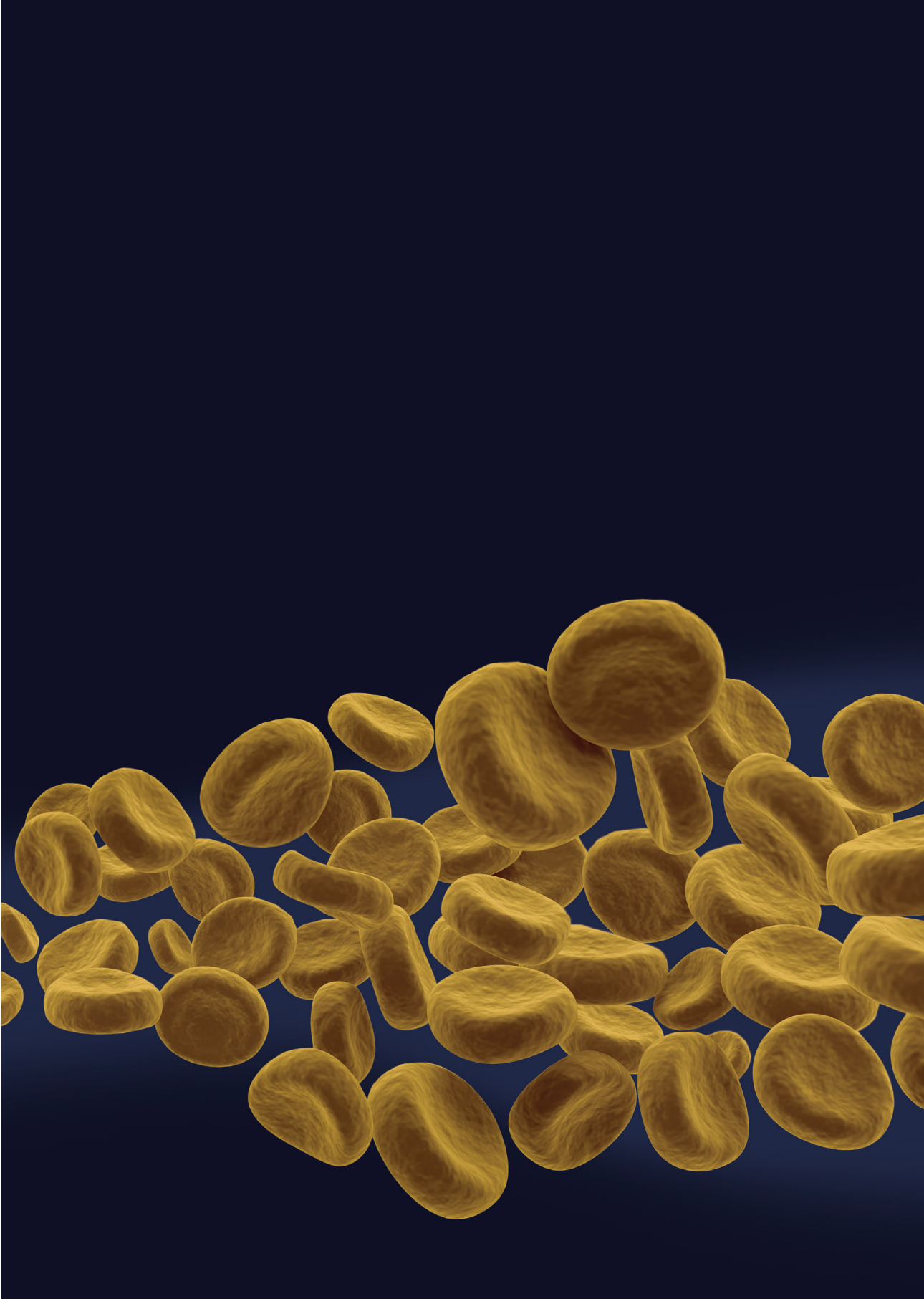
# Part 2 - Predictors of severe disease





# Chapter two

## Are foetal bilirubin levels associated with the need for neonatal exchange transfusions in haemolytic disease of the foetus and newborn?

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# Abstract

## Background

Foetal bilirubin is routinely measured at our centre when taking a pretransfusion blood sample at intrauterine transfusions in haemolytic disease of the foetus and newborn. However, the clinical value of foetal 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

To evaluate the predictive value of foetal bilirubin for exchange transfusions in severe haemolytic disease of the foetus and newborn.

## Study design

A total of 186 infants with Rh alloantibody-mediated haemolytic disease of the foetus and newborn, treated with one or more intrauterine transfusions at the Leiden University Medical Centre between 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 foetal bilirubin level before the last intrauterine transfusion in relation to the need for exchange transfusion.

## Results

In a multivariate logistic regression analysis, the foetal 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 IUTs (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 of 0.43. The corresponding foetal bilirubin level was 5 mg/dL and had a sensitivity of 79% and a specificity of 64%.

## Conclusion

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

# Introduction

Haemolytic disease of the foetus and newborn (HDFN) is a condition in which maternal alloantibodies lead to the destruction of foetal erythrocytes. In the antenatal period, this can lead to severe foetal anaemia, foetal hydrops and ultimately intrauterine demise.[1,2] The mainstay of antenatal treatment in HDFN is intrauterine transfusion (IUT) to correct foetal anaemia. In the postnatal period, HDFN can lead to severe hyperbilirubinaemia and prolonged anaemia. Hyperbilirubinaemia may cause kernicterus or chronic bilirubin encephalopathy, which can lead to permanent brain damage.[1] Hyperbilirubinaemia 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, as well as metabolic derangement.[3.] The rate of ET in infants with severe HDFN treated at our centre was approximately 15% after more restrictive guideline adaptation from the American Academy of Paediatrics (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 individualised postnatal care.

As foetal bilirubin levels are routinely measured at our centre before each IUT procedure and high bilirubin levels after birth are indicators for ET, we hypothesised that high levels of foetal bilirubin before birth could predict the need of ET in the neonatal period. The primary aim of this study was to evaluate whether foetal 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 IUT(s) 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 centre for maternal-foetal therapy in the Netherlands for treatment of severe foetal anaemia caused by red blood cell alloimmunisation. After foetal treatment with IUT, admission of the neonate to the LUMC is highly recommended;

therefore, the study was composed of nearly all live-born infants treated with IUT for HDFN in the Netherlands during the study period. Incidentally, infants were admitted elsewhere do to spontaneous preterm delivery. Furthermore, we included only infants treated with IUT as foetal samples of bilirubin are only taken during this procedure and not in cases without IUT. The cohort was selected from 2006 onwards as ET and phototherapy thresholds were changed in 2005. 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 alloimmunisation 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), birth weight, caesarean delivery, GA at first IUT (weeks), number of IUTs per foetus, type of alloimmunisation, number of ETs per infant, hours after birth until first ET, bilirubin level at birth, maximum bilirubin level after birth, and foetal bilirubin level at all IUTs. Because of the non-invasive nature of this study, the medical ethics committee of our centre 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 foetal 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 foetal 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 cut-off value for foetal bilirubin as a predictor for ET, the Youden index was calculated. The Youden Index is presented as an absolute foetal 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 due to 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.

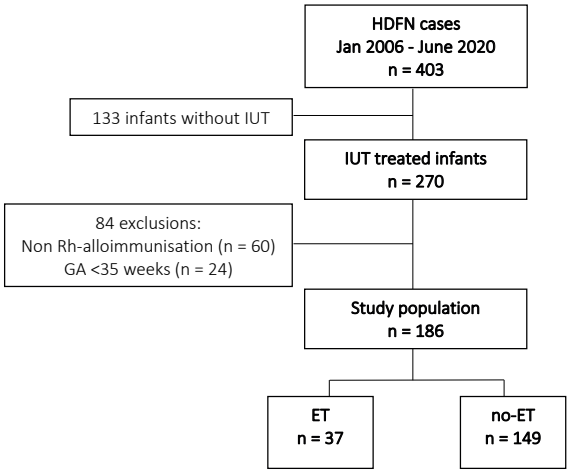


**Figure 1.** Flowchart of the study population
*ET*, exchange transfusion; *GA*, gestational age; *HDFN*, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion.

**Table 1.** Baseline characteristics

| | **Infants treated with IUT(s), n = 186** |
|---|---|
| Male - n (%) | 107 (58) |
| Gestational age at birth (weeks) - median (IQR) | 36 (36-37) |
| Birth weight (g) - mean ± SD | 2869 ± 362 |
| Gestational age at first IUT (weeks) - median (IQR) | 29 (25-32) |
| Number of IUTs per foetus - median (IQR) | 2.0 (1.0-3.3) |
| Type of alloimmunisation[a] | |
| D alloimmunisation - n (%) | 174 (94) |
| C alloimmunisation - n (%) | 1 (1) |
| c alloimmunisation - n (%) | 10 (5) |
| E alloimmunisation - n (%) | 1 (1) |

*IQR*, interquartile range*; IUT*, intrauterine transfusion;w *n*, number; *SD*, standard deviation.
Data are presented as mean ± *SD,* median (*IQR*), or n with percentage (%).
[a] Percentage value does not add up to 100% because of rounding.

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 foetal 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).

T**able 2.** Predictors for exchange transfusions in infants with HDFN treated with intrauterine transfusions

| | **ET (n = 37)** | **No ET (n = 149)** | **Univariable OR (95% CI)** | **Multivariable OR (95% CI)** |
|---|---|---|---|---|
| Foetal bilirubin at the last IUT (OR per 1 mg/dL)[a] | 6.02 (5.18-7.09) | 4.33 (3.27-6.20) | 1.32 (1.10-1.58) | 1.32 (1.09-1.61) |
| Male - n (%) | 26 (70) | 81 (54) | 0.50 (0.23-1.09) | 1.52 (0.66-3.50) |
| Gestational age <37 weeks - n (%) | 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)[a] | 19 (15-24) | 21 (19-26) | 0.97 (0.93-1.01) | 1.00 (0.96-1.03) |
| Number of IUTs (OR per IUT)[a] | 2.0 (1.0-3.0) | 2.0 (2.0-4.0) | 0.65 (0.47-0.90) | 0.63 (0.44-0.91) |
| Bilirubin at birth (OR per 1 mg/dL)[a] | 7.89 (6.52-10.82) | 5.47 (4.09-6.56) | 1.72 (1.41-2.10) | - |

*CI*, confidence interval; *HDFN*, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Data presented as median (*IQR*).

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). Foetal 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 foetal bilirubin was 0.71. The corresponding Youden Index was calculated at 0.43 with a cut-off foetal bilirubin level of 5 mg/dL with a sensitivity of 79% and a specificity of 64% (Figure 2). To visualise the course of foetal bilirubin, a boxplot of the foetal bilirubin level at each IUT was computed (Figure 3). The figure shows a small and statistically insignificant decline in foetal bilirubin over the course of IUTs.

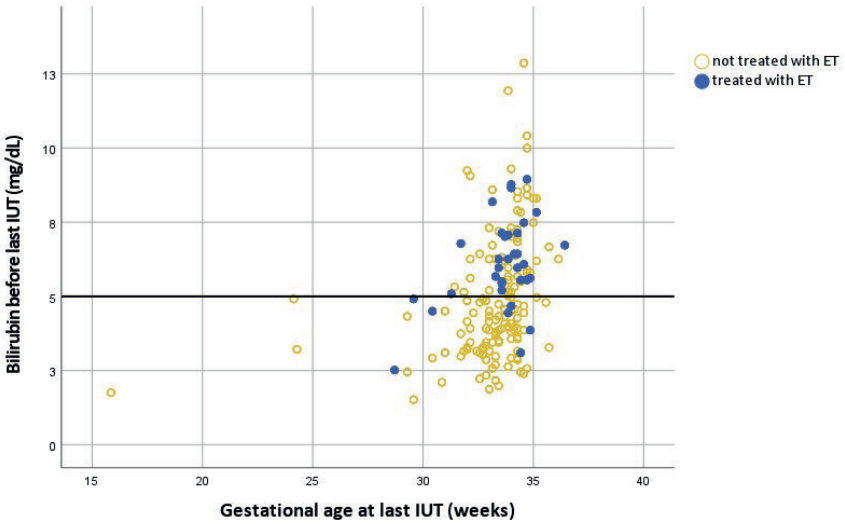


**Figure 2**. Absolute bilirubin levels per foetus before last IUT
*ET*, exchange transfusion; *IUT*, intrauterine transfusion.
The *line* is plotted at the bilirubin value of 5 mg/dL, the value with the highest sensitivity and specificity in predicting ET (the Youden index).

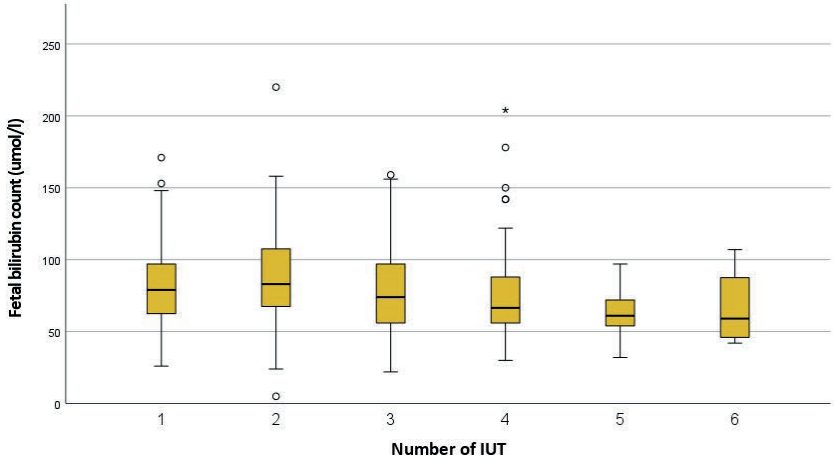


**Figure 3.** Boxplot of foetal bilirubin levels throughout IUT treatment
*IQR*, interquartile range; *IUT*, intrauterine transfusion.
*Boxplots* display median values with IQR, *dots* represent outliers, and *asterisks* represent extreme outliers defined as 3 times the IQR.

# Discussion

## Principal Findings

This study shows that foetal bilirubin measured at the last IUT treatment has a predictive value for the need for ET after birth. A foetal bilirubin cut-off 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 hypothesised to be caused by the replacement of foetal, incompatible blood by compatible donor blood and thus interference with the haemolytic process, causing less bilirubin to accumulate and a longer blood transfusion-free interval after birth.[6]

Here, the rate of ET among infants treated with IUT is 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 centre declined from 67% (200-2005) to 10% (2015-2000).[7]

## Clinical Implications

High foetal bilirubin levels could be viewed as an early, foetal 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 haemolytic process with continued production of foetal 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 foetal bilirubin metabolism in relation to IUT treatment.

The mechanism of bilirubin metabolism in infants is well known,[8] but foetal bilirubin metabolism is less well understood. The foetal liver is poorly capable of conjugating bilirubin and is suggested to have an excretory defect so that even if the foetus 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, due to the gradient in favour of the transport from foetal side to maternal side, foetal unconjugated bilirubin is cleared from foetal 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 foetal bilirubin levels on the foetus or provide foetal bilirubin reference values. If basal ganglia are just as susceptible to bilirubin toxicity during foetal life as in the neonatal period, high levels of foetal 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 centre is the national referral centre for HDFN, we have a uniformly treated cohort, limiting variability in treatment of HDFN. A limitation to this study is 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 was treated with ET at our centre in the same period. As these infants did not receive IUT treatment, no foetal blood sampling was available in this group.

## Conclusions

Our study demonstrated the possibility of using foetal bilirubin levels as a predictor for ET, making it possible to further accommodate adequate and individualised 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 foetal bilirubin, to create personalised risk and treatment profiles for infants affected by severe HDFN.

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