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## School performance and behavioral functioning in children after intrauterine transfusions for hemolytic disease of the fetus and newborn

I.M.C. Ree<sup>a,b,\*</sup>, R.M. van 't Oever<sup>b</sup>, L. Jansen<sup>c</sup>, E. Lopriore<sup>b</sup>, M. de Haas<sup>a,d,e</sup>, J.M.M. van Klink<sup>c</sup>

<sup>a</sup> Center for Clinical Transfusion Research, Sanquin Research, Leiden, the Netherlands

<sup>b</sup> Department of Pediatrics, Division of Neonatology, Leiden University Medical Center, Leiden, the Netherlands

<sup>c</sup> Department of Medical Psychology, Leiden University Medical Center, the Netherlands

<sup>d</sup> Department of Hematology, Leiden University Medical Center, Leiden, the Netherlands

<sup>e</sup> Department of Immunohematology Diagnostics, Sanquin, Amsterdam, the Netherlands

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### ABSTRACT

**Aim:** To investigate the school performance and behavioral difficulties in children with hemolytic disease of the fetus and newborn (HDFN) treated with intrauterine transfusion (IUT) compared to Dutch norm data.

**Study design:** Cross-sectional cohort study.

**Subjects:** Children who received one or multiple IUTs for severe Rh- or K (Kell)-mediated HDFN between January 2008 and January 2015 at the LUMC.

**Outcome measures:** School performance reports were assessed as well as behavioral difficulties as assessed with the Dutch child behavioral checklist (CBCL) by parents and caregivers and the Teacher Report Form (TRF) completed by teachers.

**Results:** A response rate of 56% (70 children, aged 5–12 years) was obtained. Grade repetition occurred in 13 cases (19%), 16 children (23%) received some form of additional help, most often support by a speech therapist ( $n = 8$ ), but also support for dyslexia ( $n = 4$ ), physical therapy ( $n = 2$ ) and social-emotional support ( $n = 2$ ). None of the children in our study group attended special-needs education. School performance levels for reading comprehension, spelling and mathematics according to the Dutch National Pupil Monitoring System were similar for the study population and Dutch norm data. The incidence of behavioral problems as reported by parents was similar to the Dutch norm data, teachers reported less behavioral difficulties in the study group.

**Conclusion:** This study shows favorable and reassuring school development in children treated with IUT in an experienced fetal-therapy center. A normal distribution in school and behavioral development is to be expected for children with HDFN treated with IUTs.

### 1. Introduction

Hemolytic disease of the fetus and newborn (HDFN) is a condition in which maternal erythrocyte alloantibodies lead to the destruction of incompatible fetal erythroid cells. This hemolytic process can lead to severe anemia, fetal hydrops and ultimately intrauterine demise. The mainstay of antenatal treatment in HDFN is intrauterine transfusion (IUT) to correct fetal anemia. After birth, infants affected by HDFN are prone to severe hyperbilirubinemia and prolonged anemia and are treated with intensive phototherapy, exchange transfusion in case of treatment failure of phototherapy, and red blood cell transfusions [1,2].

HDFN is nowadays a rare condition due to, in particular, the

introduction of RhD immunoprophylaxis and treatment with IUT. After introduction of RhIg in 1968 and its administration during pregnancy, the RhD immunization rate dropped from 16% to 0.3% [3]. Successful IUT was first described in 1963 and has developed into a safe procedure with only 0.6% procedure-related complications and high survival rates (97%) when performed regularly in an experienced center [4].

The focus in HDFN switched from mere survival to long-term effects on development and impairment after severe HDFN and intrauterine treatment. As described, children affected by HDFN are exposed to severe fetal anemia and hyperbilirubinemia after birth, making them hypothetically prone to fetal hypoxic injury to the developing brain and varying degrees of bilirubin neurotoxicity.

**Abbreviations:** ET, exchange transfusion; HDFN, hemolytic disease of the fetus and newborn; IUT, intrauterine transfusion.

\* Corresponding author at: Department of Pediatrics, Leiden University Medical Center, J6-S, Albinusdreef 2, 2333 ZA Leiden, the Netherlands.

**E-mail address:** [i.m.c.ree@lumc.nl](mailto:i.m.c.ree@lumc.nl) (I.M.C. Ree).

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A few studies with small patient numbers have reported over the years on long-term neurodevelopmental outcome after IUT, showing overall a favorable outcome. In general, these studies focussed on survival rate and gross motor function and most often included more severely affected fetuses and cases of fetal hydrops [5–11]. No follow-up studies were conducted in the last 15 years, apart from an earlier large cohort study in our center, aimed to determine the incidence and risk factors for adverse neurodevelopmental outcome after IUT treatment between 1988 and 2008. It was found that the incidence of neurodevelopmental impairment (NDI) was 4.8% (14/291) [12].

Since this study, no long-term follow-up of these children has been performed by our (and other) centers despite continuous advancements in fetal and neonatal care of infants affected by HDFN and near elimination of severe hydrops. While severe NDI was then comparable to Dutch norm data, it is unclear if less outspoken behavioral, learning and physical difficulties occur in this group and how this potentially translates to a school environment and academic development.

The main purpose of this study is to investigate the current state of school performance and behavioral difficulties of this population by questionnaires and school results. This study will provide clinicians and parents with updated insights in long-term development of children with HDFN that were treated with IUTs, specified to school performances in comparison with Dutch norm data.

## 2. Methods

### 2.1. Study population

This is a cross-sectional cohort study, including all children who received one or multiple IUT for severe Rh- or K-mediated HDFN between January 2008 and January 2015 at the LUMC. Children born before 2008 were studied before [12]. The LUMC is the national referral center for fetal therapy of severe HDFN in the Netherlands. Exclusion criteria were severe congenital anomalies unrelated to intrauterine anemia or treatment. Due to the non-invasive nature of this study, a waiver of consent was granted by the medical ethics committee of our center.

### 2.2. Procedures

The study interventions are standardized questionnaires for parents or caregivers ('child behavioral checklist', CBCL) and teachers ('teachers report form', TRF). Initial contact with parents or caregivers was made by phone, after which invitation letters to participate in the study were sent by mail. If parents or caregivers consented, they received the questionnaires by mail and were asked to give the TRF and an accompanying letter requiring school performance scores to their child's teacher. After two and four weeks parents or caregivers were reminded to respond, once by phone and once by mail. If we were unable to make initial contact with parents or caregivers by phone, an invitation letter was sent by mail and contact information was checked with the child's general practitioner registered in the medical file.

Further relevant demographic and perinatal data was collected for descriptive purposes from the medical files of the children in a computerized database with permission of parents or caregivers. Data included are: type of alloimmunization, gestational age at IUT, number of IUTs, gestational age at birth, gender, birthweight, bilirubin levels at birth and neonatal outcome including hydrops at birth and number of exchange transfusions because of severe hyperbilirubinemia.

The main study parameter was the overall school performance score of children treated before birth with IUT(s) for HDFN compared to Dutch norm scores. Secondary endpoints were the prevalence of behavioral difficulties as assessed with the CBCL and TRF.

### 2.3. Questionnaires

#### 2.3.1. CBCL and TRF

The CBCL and TRF are questionnaires assessing behavioral competency and behavioral problems in children within the past six months.

The CBCL for ages 6 to 18 and TRF for ages 6 to 18 consist of 120 items describing the behavior of school-aged children. Parents and teachers answered the items similar to the three-point Likert scale on the CBCL for ages 1½ to 5. Based on their answers, eight syndrome scales were calculated: (1) anxious/depressed behavior, (2) withdrawn/depressed behavior, (3) somatic complaints, (4) social problems, (5) thought problems, (6) attention problems, (7) rule-breaking behavior and (8) aggressive behavior.

All syndrome scales together form a total problem scale, which can be divided into the subscale internalizing problem behavior (anxious/depressed behavior, withdrawn/depressed behavior and somatic complaints) and the subscale externalizing problem behavior (rule-breaking behavior and aggressive behavior) [13,14].

Standardized t-scores were obtained, where higher scores indicate higher levels of problem behavior. For the syndrome scale, t-scores <65 were considered normal, t-scores between 65 and 70 borderline clinical and t-scores >70 clinical behavioral problems. For the total problem, internalizing and externalizing scale, t-scores <60 were considered normal, t-scores between 60 and 75 borderline clinical and t-scores >65 clinical behavioral problems. Our study population was compared to Dutch norm data, the norm clinical score is 10% in these scales [13,14].

#### 2.3.2. School performance scores

Part of the CBCL and TRF include questions about the attendance of regular education, special-needs education and grade repetition. The results of the study population were compared to Dutch norm data as obtained from the Dutch Central Bureau for Statistics (CBS) on enrollment in special-needs primary education (CBS 2019–2020) and the grade repeat rate (CBS 2019–2020) [15,16]. Data on school performance as reported in the Dutch National Pupil Monitoring System was additionally obtained for reading comprehension (the understanding and interpretation of written language), spelling and arithmetic/mathematics. This system consists of tests taken at specific moments during the school year. The results can be translated into ability scores to compare an individual child to their age matched peers. The ability scores are divided into 5 levels, A to E with A being the top 25% highest scoring children and E being the 10% lowest performing children [17].

#### 2.3.3. Demographic data

In addition to the questionnaires, parents were asked to provide demographic data on their highest level of education and current form of employment. The level of education was classified in 'low', 'intermediate' or 'high'. Low education was defined as primary and secondary school education, intermediate education as intermediate vocational school education and high education as higher vocational school and university education. In 2019, 40% of the Dutch population aged 25–64 years had completed either higher vocational school or university [18].

### 2.4. Statistics

Descriptive results were presented as number of cases and percentages or as mean  $\pm$  standard deviation or median (interquartile range) depending on the distribution of the data. Binomial tests for proportions were conducted to compare the percentage of children in special-needs education, the grade repetition rate and the percentages of children in each level of school performance to their Dutch peers.

All statistical analyses were performed using IBM SPSS Statistics 26.0 (Chicago, Illinois, USA).

### 3. Results

A total of 125 live-born children were treated with IUTs in the study period and were approached to participate in the LOTUS II study. There were no children with severe congenital anomalies. Written consent was obtained from the parents or caregivers of 70 children (56%), the parents or caregivers of 8 children did not consent in participating (6%). Contact was established with parents or caregivers of 16 children (13%), but they never returned the questionnaires. No contact could be made with parents or caregivers, or the primary care physician of 31 children (25%), these cases were classified as loss to follow up.

When written consent was obtained, a complete response with both the CBCL form as the TRF form and school results was received for 57 children. Incomplete responses occurred in 13 cases. The study population is presented in a flowchart, Fig. 1.

#### 3.1. Perinatal outcome

The baseline perinatal characteristics of the children included ( $n = 70$ ) and lost to follow up ( $n = 31$ ) are presented in Table 1. Of the included children, the majority was male (64%) and the median gestational age at birth was 36 weeks (IQR 36–37 weeks). The median number of IUTs per child was 2 (IQR 2–4 IUTs), one child presented with hydrops at birth. In more than half of the cases (66%) RhD was the causative type of alloimmunization. A quarter of children was treated with one or more ETs (26%). 45% of mothers reported educational levels classified as 'high' and 48% of fathers.

#### 3.2. Grade progression

In this study population, grade repetition occurred in 13 cases (19%). Dutch primary school has eight grades, ranging from 1 (4-year olds) to 8 (12-year olds). Groups 1 and 2 are similar to kindergarten, where from group 3 on, children learn to read, write and learn arithmetic/mathematics. Three children repeated grade 1, three repeated group 2, five children repeated group 3, one child repeated group 4 and one child repeated group 6. This is significantly higher than the Dutch norm of 10.3% [16] ( $p = .020$ ). 16 children (23%) received some form of additional help, most often support by a speech therapist ( $n = 8$ ), but also support for dyslexia ( $n = 4$ ), physical therapy ( $n = 2$ ) and social-emotional support ( $n = 2$ ). None of the children in our study group attended special-needs primary education, as compared to 2.6% of the Dutch norm population [15] ( $p = .176$ ).

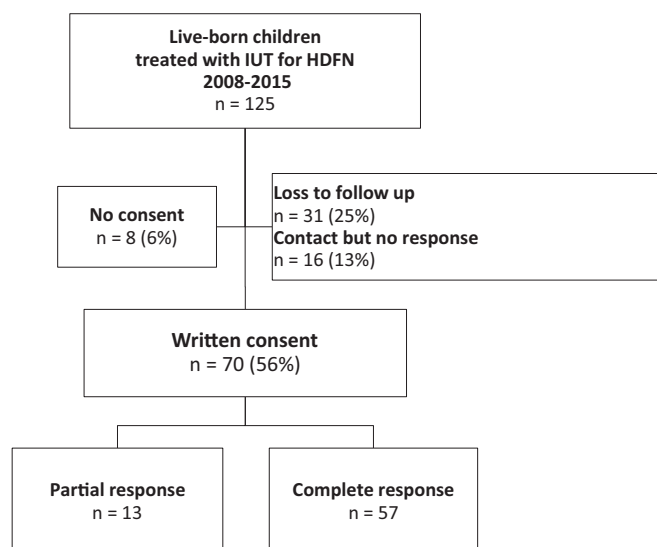


Fig. 1. Flow chart of study population.

Table 1  
Baseline characteristics.

	Analysed group ( $n = 70$ )	Loss to follow up ( $n = 31$ )
Type of alloimmunization - $n$ (%) <sup>a</sup>		
Rh D	46 (66)	27 (87)
K	19 (27)	4 (13)
Rh c/C	4 (6)	0 (0)
Rh E	1 (1)	0 (0)
Gestational age at first IUT - weeks <sup>a</sup>	30 (25–32)	26 (23–32)
Number of IUT(s) per fetus <sup>b</sup>	2 (2–4)	3 (2–4)
Hemoglobin at first IUT - g/dL <sup>b</sup>	5 (3–6)	7 (5–8)
Hydrops present at birth - $n$ (%)	1 (1)	0 (0)
Gestational age at birth - weeks <sup>b</sup>	36 (36–37)	36 (35–37)
Birthweight - grams <sup>b</sup>	2999 (2720–3265)	2710 (2508–2940)
Male gender - $n$ (%)	45 (64)	20 (65)
Neonates treated with ET - $n$ (%)	18 (26)	6 (19)
Maternal education - $n$ (%) <sup>c</sup>		
Low	6 (10)	-
Intermediate	29 (45)	-
High	29 (45)	-
Paternal education - $n$ (%) <sup>c</sup>		
Low	5 (8)	-
Intermediate	28 (44)	-
High	31 (48)	-

IUT, intrauterine transfusion; ET, exchange transfusion.

<sup>a</sup> Rounds up to more than 100% due to rounding.

<sup>b</sup> Data presented as median (interquartile range).

<sup>c</sup> 6 missing values due to incomplete response.

#### 3.3. Academic performance

The different school performance levels for reading comprehension, spelling and mathematics according to the Dutch National Pupil Monitoring System were compared with the study population (Table 2). The scores of the study population are distributed similarly from level A (highest) to E (lowest) as the Dutch norm data for reading comprehension and spelling. Compared to the Dutch norm data, the study population more often scored in the range of level A in mathematics (44% vs 25%,  $p = .002$ ). A  $p$ -value  $< .003$  was considered significant after Bonferroni correction.

Table 2  
Academic performance.

	Study population	Dutch norm population	$p$ -Value
Reading comprehension ( $n = 49$ )			
A	12 (25%)	25%	.544
B	13 (27%)	25%	.456
C	10 (20%)	25%	.288
D	9 (18%)	15%	.310
E	5 (10%)	10%	.550
Spelling ( $n = 53$ )			
A	17 (32%)	25%	.151
B	9 (17%)	25%	.114
C	11 (21%)	25%	.296
D	11 (21%)	15%	.162
E	5 (9%)	10%	.561
Mathematics ( $n = 55$ )			
A	24 (44%)	25%	<b>.002</b>
B	14 (26%)	25%	.521
C	8 (15%)	25%	.045
D	4 (7%)	15%	.070
E	5 (9%)	10%	.524

A  $p$ -value  $< .003$  was considered significant after Bonferroni correction. Statistically significant values were printed in bold.

### 3.4. Behavioral outcome

A total of 63 (90%) parents that gave written consent for participation filled out the CBCL forms and 56 (80%) teachers completed the TRF forms. Teachers and parents reported a diagnosis of dyslexia for four children, there was no specific mentioning of attention deficit hyperactivity disorder (ADHD). When asked directly whether the student (for teachers) or child (for parents) has a physical or mental disability, all teachers and parents answered with 'no'.

There were no significant differences between the scores of the CBCL forms and the Dutch norm data. From the TRF forms, the clinical score of Externalizing problems 1 (2%) and Total problem scale 0 (0%) were both significantly lower than the Dutch norm ( $p$ -value of .020 and .003, respectively). The separate scores per syndrome scale are presented in Table 3.

## 4. Discussion

In this study we aimed to assess long-term outcomes of HDFN treated with IUTs, specified to school performance scores as compared to Dutch norm data. We found that no children in the study population attend special-needs education. Twice as many children in the study group repeated a grade compared to Dutch normative data, although it must be noted that 6 of the 13 children that repeated a grade, did so already at the young age of 4 or 5 years. A quarter of children received a (mild) form of additional support in school. When comparing school performance levels for reading comprehension and spelling, the study population had a similar distribution in scores compared to Dutch norm data. In mathematics, the study population performed even better compared to norm data. While the incidence of behavioral problems as reported by parents was similar to the Dutch norm data, teachers reported less behavioral difficulties in the study group. We found no trend in remarkable behavior or problems as reported by parents and teachers.

When conducting this study, we hypothesized that children born with severe HDFN show more difficulty in their educational development. The severe fetal anemia that these children were exposed to, may have caused fetal hypoxic injury to the developing brain similar to the negative effect of hypoxia during birth. The latter is known to have a negative effect on verbal and cognitive performance [19]. In addition, these children were exposed to often severe hyperbilirubinemia after birth, and may have experienced varying degrees of bilirubin neurotoxicity. Nonetheless, the children of this study population have scores similar to age-related peers.

The response rate in this study was 56%, which is not uncommon for questionnaire studies [20]. Several attempts were made to contact parents or caregivers, including the verification of contact information with the child's registered general practitioner, but still resulted in a 25% loss to follow up rate. This 25% is deemed 'at random' and the children of this group show similar baseline characteristics as the study population. A nonresponse bias could be expected from the parents that were contacted but did not return the questionnaires or did not consent with participation, with for example less parents responding with children experiencing difficulty in (school) development. However, all these parents were contacted by phone by the same researchers and based on these personal interactions, there was no reason to suspect such influence. From the parents that returned the CBCL forms, over half of them (45% of mothers, 48% of fathers) completed higher education, which is higher in comparison with the Dutch adult population (40% in 2019) [18]. Response rates are known to be strongly and positively correlated with higher cognitive test scores at every age [20]. As the level of parental education is a major contributor of child cognitive development [21,22], our data could be skewed by the relatively high response rate among higher educated parents.

Based on our data from a homogenous treated and large cohort, we positively conclude that severe HDFN treated with IUT(s) is not associated with negative effects on children's academic and behavioral

**Table 3**

Behavioral outcome; CBCL and TRF scores 6–18 years.

CBCL	Study population (n = 63)	Dutch norm population	p-Value
Internalizing problems, clinical score - n (%)	8 (13%)	10%	.293
Syndrome scales T-scores (IQR)			
- Anxious/depressed	50 (50–57)		
- Withdrawn/depressed	52 (50–60)		
- Somatic complaints	53 (50–52)		
Externalizing problems, clinical score - n (%)	4 (6%)	10%	.232
Syndrome scales T-scores (IQR)			
- Rule-breaking behavior	50 (50–52)		
- Aggressive behavior	50 (50–52)		
Total problems, clinical score - n (%)	3 (5%)	10%	.113
TRF	Study population n = 56	Dutch norm population	p-Value
Internalizing problems, clinical score - n (%)	8 (14%)	10%	.193
Externalizing problems, clinical score - n (%)	1 (2%)	10%	<b>.020</b>
Total problems, clinical score - n (%)	0 (0%)	10%	<b>.003</b>

CBCL, child behavioral checklist; TRF, teachers report form. Statistically significant values were printed in bold.

development. The conclusion is limited by the nature of our study, as survey studies are prone to various forms of bias such as response and nonresponse bias. Parents may for example be prone to give more positive evaluations of the behavior and school performance of their children, giving rise to response bias, although we did not find major discrepancies between questionnaires and school results as reported by parents, teachers or objective school result forms.

This study gives more insight in the long-term development of children with HDFN treated with IUTs and overall shows favorable and reassuring school development in children treated in an experienced fetal-therapy center. In general, a normal distribution in school and behavioral development is to be expected for live-born children with this condition.

### CRedit authorship contribution statement

IMC Ree: conceptualization, validation, formal analysis, investigation, writing

RM van 't Oever: methodology, formal analysis, investigation, writing

L Jansen: conceptualization, methodology, critical review

E Lopriore: conceptualization, project administration, critical review

M de Haas: methodology, project administration, critical review

JMM van Klink: conceptualization, methodology, validation, supervision, critical review

### Declaration of competing interest

The authors report no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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