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## Neonatal management and outcome in red cell alloimmunization

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# 8

## **Top-up transfusions in neonates with Rhesus hemolytic disease in relation to exchange transfusions**

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## Abstract

**Objective:** To study the effect of a restrictive guideline for exchange transfusion (ET) on the number of top-up transfusions in neonates with Rhesus hemolytic disease.

**Study design:** Retrospective study of all (near)-term neonates with Rhesus hemolytic disease admitted to our center between 2000 and 2008. In December 2005, policy changed from using liberal ET criteria to more restrictive ET criteria. We recorded the number of ETs and the number of top-up transfusions in the group of neonates before (group I, n =156) and after (group II, n =27) the guideline change.

**Results:** The percentage of neonates requiring an ET decreased from 66% (103/156) in group I to 26% (7/27) in group II ( $p < 0.01$ ). The percentage of neonates receiving a top-up transfusion increased from 68% (105/154) in group I to 81% (22/27) in group II ( $p = 0.25$ ). The median number of top-up transfusions increased from 1 (interquartile range 0-2) in group I to 2 (interquartile range 1-3) in group II ( $p = 0.01$ ).

**Conclusion:** In this study, restrictive ET criteria in neonates with Rhesus hemolytic disease lead to a reduction of the rate of ET but an increase in the number of top-up transfusions for neonatal anemia.

## Introduction

The mainstay of antenatal treatment for hemolytic disease of the fetus and newborn due to Rhesus alloimmunization (HDFN) is intrauterine transfusions (IUT) to treat severe fetal anemia. The mainstay of postnatal treatment for hemolytic disease of the newborn (HDN) secondary to Rhesus alloimmunization is (1) intensive phototherapy and exchange transfusion (ET) to treat hyperbilirubinemia and prevent kernicterus, and (2) top-up transfusions to treat neonatal anemia. Neonatal anemia secondary to Rhesus alloimmunization can be divided into two types: "hyporegenerative anemia" characterized by depressed erythropoiesis and "late anemia of hemolytic disease" caused by persisting hemolysis by remaining antibodies.<sup>1</sup> Both causes of anemia contribute to the necessity of top-up transfusions during the first months of life. The percentage of infants with HDN secondary to Rhesus alloimmunization requiring top-up transfusions for neonatal anemia varies from 27 to 83%.<sup>2,3</sup>

Several risk factors for neonatal anemia secondary to Rhesus alloimmunization have been reported, including IUT (due to suppression of fetal erythropoiesis)<sup>2</sup> and severity of HDN.<sup>1,4,5</sup> Use of ET during the neonatal period has been reported to protect against neonatal anemia.<sup>3</sup> In addition to removing excess bilirubin, ET also removes antibody-coated erythrocytes and maternal antibodies, hence reducing the risk for continuing hemolysis and neonatal anemia.<sup>3,6</sup> However, the protective role of ET for neonatal anemia has only been demonstrated in one small study.<sup>3</sup>

In December 2005, we implemented a new guideline for the management of neonatal hyperbilirubinemia and HDN secondary to Rhesus alloimmunization based on the guidelines from the American Academy of Pediatrics (AAP).<sup>7</sup> The new guideline for ET was more restrictive than the previous ones and led to a more than 50% decrease in the rate of ET. Restrictive ET criteria may hypothetically lead to an increase in top-up transfusions ascribed to ongoing hemolysis due to antibody coated cells not being removed from the circulation. Whether the reduction in ET indeed resulted in an increased rate of top-up transfusions was not clear.

The aim of this study was to evaluate the effect of this new guideline on the number of top-up transfusions and determine if neonates with hemolytic disease of the newborn secondary to Rhesus alloimmunization treated with ET are less likely to develop neonatal anemia.

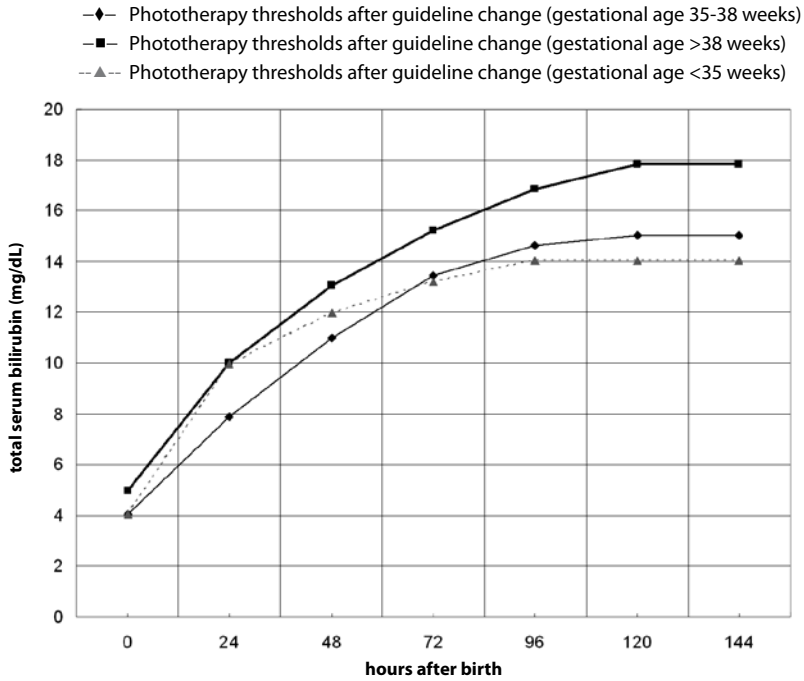
## Materials and Methods

All neonates with a gestational age  $\geq 35$  weeks with HDN secondary to Rhesus D, C, c or E antibodies admitted between January 2000 and November 2008 to the neonatal division of the Leiden University Medical Center (LUMC) were included in this retrospective observational study. LUMC is the national referral center for the management and intrauterine treatment of red cell alloimmunization in the Netherlands. Neonates with Kell, Jka or Cw red cell alloimmunization, and neonates receiving transfusions for unrelated pathology were excluded from this study. We also excluded neonates participating in an ongoing randomized double-blind placebo-controlled trial for the use of immunoglobulin in RHD, which started in August 2006 at our institution (the LIVIN study: <http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=832>)

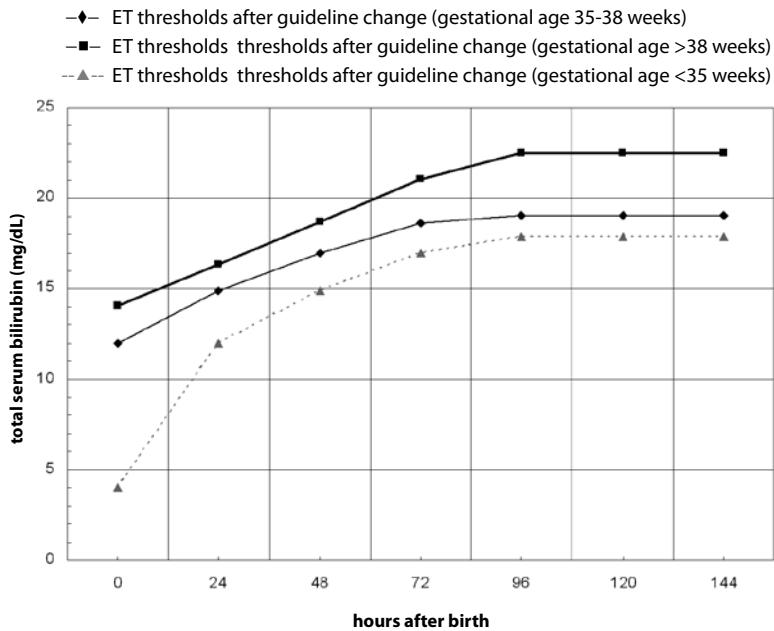
In December 2005, we implemented a new guideline for the management of neonatal hyperbilirubinemia and HDN secondary to Rhesus alloimmunization based on the guidelines from the American Academy of Pediatrics (AAP).<sup>7</sup> The differences in phototherapy thresholds before and after introduction of the new guideline are shown in Figure 1. The guideline for phototherapy administration at our neonatal division has previously been described.<sup>2</sup> The bilirubin thresholds for ET in the new guideline were higher than in the previous one. The differences in ET thresholds of total serum bilirubin before and after guideline change are shown in Figure 2. The criteria for ET before December 2005 included a total serum bilirubin level at birth  $> 3.5$  mg/dL (measured in neonatal blood at birth or in a few cases in umbilical cord blood),<sup>2</sup> (early criterium) and/or a total serum bilirubin level above ET thresholds (rise of bilirubin value  $> 0.5$  mg/dL/hr despite intensive phototherapy).<sup>2</sup> In neonates not treated with IUT, a hemoglobin level at birth of  $< 12.9$  g/dL was also considered as an early criterium for ET. <sup>2</sup> Bilirubin levels were measured in all neonates every 2 to 3 hours during the first few days after birth.

Early criteria for ET were abandoned after the guideline change. The criteria for ET after December 2005 were: (1) total serum bilirubin above (new) ET thresholds<sup>7</sup> (Fig 2) and/or (2) rise of bilirubin  $> 0.5$  mg/dL/hr despite intensive phototherapy, and/or (3) clinical symptoms of acute bilirubin encephalopathy regardless of bilirubin level. ET was performed with double-volume transfusion (160 mL/kg) using irradiated and leukocyte-depleted compatible erythrocytes.

After initial discharge from the LUMC, top-up transfusions were performed when hemoglobin levels were  $< 8.0$  g/dL or  $< 9.6$  g/dL if clinical symptoms of neonatal anemia (lethargy, feeding problems, need for oxygen or failure to thrive) were present. The volume used for top-up transfusions during the study period was 15 ml/kg bodyweight. The volume and criteria for top-up transfusion were the same in both groups. Neonatal anemia, often referred to



**Figure 1** Phototherapy thresholds before and after guideline change in neonates with Rh hemolytic disease



**Figure 2** ET thresholds before and after guideline change in neonates with Rh hemolytic disease

as "late anemia" in previous studies<sup>3,5,8,9</sup> was defined as a hemoglobin level below thresholds requiring a top-up transfusion during the first three months of life. Folic acid 50 mcg/day was administered orally during the first three months of life to all neonates with RHD. According to our management guidelines, neonates with RHD were not treated with erythropoietin.

We recorded the following obstetric and neonatal data: fetal hemoglobin at first IUT and number of IUTs, gestational age at birth, birth weight, hemoglobin level and reticulocyte count at birth, bilirubin level at birth, maximum bilirubin level during admission, duration of phototherapy (days), number of ETs required, number of top-up red blood cell transfusions received during the first 3 months of life and hemoglobin levels prior to the top-up transfusion. Obstetric data were gathered using a dedicated database for Rhesus complicated pregnancies in which data are prospectively collected. Neonatal data were collected using medical files. Data on top-up transfusions and hemoglobin levels prior to the top-up transfusion in infants who received follow-up outside the LUMC were gathered through correspondence with the local pediatrician or blood transfusion department.

Primary outcome was the number of top-up transfusions in the group of neonates admitted before (group I) and after (group II) the new guideline implementation in December 2005. Data are reported as means and standard deviations (SD) or as medians and interquartile range (IQR), as appropriate. Statistical analysis was performed using Student-t-test and Mann-Whitney test for continuous variables. Chi square and Fisher's exact test were used for categorical variables, as appropriate. To assess the relationship between the number of ETs and the number of top-up transfusions, Spearman correlations were calculated. A p-value <0.05 was considered statistically significant. Statistical analysis was executed with SPSS 16.0 (SPSS Inc, Chicago, IL).

## Results

During the study period 270 infants with hemolytic disease delivered  $\geq 35$  weeks' gestation were admitted to our neonatal division. Fifty neonates were excluded because of participation in a randomized double-blind placebo-controlled trial for the use of intravenous immunoglobulin. Thirty-six neonates were excluded due to hemolytic disease caused by Kell (n=34), Jka (n=1) or Cw (n=1). One neonate requiring major cardio-thoracic surgery for congenital heart disease was also excluded. A total of 183 patients were included in this study, of whom 156 in the group before (group I) and 27 in the group after (group II) the implementation of the new guideline in December 2005. Baseline characteristics in both groups are summarized in Table 1.

**Table 1** Baseline characteristics in neonates with Rhesus hemolytic disease in group I and group II

	<b>Group I</b> (n = 156)	<b>Group II</b> (n = 27)	<b>p-value</b>
Neonates treated with IUT – n (%)	99 (63)	15 (56)	0.52
Number of IUTs per neonate <sup>a</sup>	2 (0 - 3)	1 (0 - 2)	0.29
Gestational age at first IUT – weeks <sup>a</sup>	30 (24 – 33)	31 (23 - 32)	0.70
Hemoglobin level at first IUT – g/dL <sup>a</sup>	6.6 (5.1 – 7.7)	5.8 (5.0 – 7.4)	0.70
Gestational age at birth – weeks <sup>a</sup>	37 (36 - 37)	37 (36 - 37)	0.89
Birth weight – grams <sup>b</sup>	3020 ± 445	2940 ± 396	0.40
Male – n (%)	87 (56)	21 (78)	0.04
Neonates with Rhesus D – n (%)	140 (90)	21 (78)	0.10
Neonates with Rhesus c – n (%)	13 (8)	4 (15)	0.29
Neonates with Rhesus C – n (%)	2 (1)	0 (0)	1.00
Neonates with Rhesus E – n (%)	1 (1)	2 (7)	0.06

<sup>a</sup>Value given as median (IQR)

<sup>b</sup>Value given as mean ± SD

### The use of IUT, phototherapy and ET

Detailed information on the use of IUT is presented in Table 1 and on the use of phototherapy and ET in group I and group II is presented in Table 2. Serial IUTs were performed in 99 (63%) infants in group I and 15 (56%) in group II ( $p = 0.52$ ). At least one ET was required in 66% (103/156) of the patients in group I compared to 26% (7/27) of the patients in group II ( $p < 0.01$ ). The rate of ET performed on day 1 dropped from 96% (94/103) in group I to 57% (4/7) in group II ( $p < 0.01$ ). The median number of ETs was 1 in group I (IQR 0-1, range 0-5) and 0 in group II (IQR 0-1, range 0-2) ( $p < 0.01$ ). No significant relationship was found between the number of IUTs and the number of ETs (Spearman correlation coefficient = -0.009;  $p = 0.90$ ).

### The use of top-up transfusions

Complete information on the number of top-up red cell transfusions was obtained for 98% (180/183) of neonates. Detailed information on the use of top-up transfusions in group I and group II is presented in Table 3. The percentage of neonates in group I and group II receiving a top-up transfusion was 68% (105/154) and 81% (22/27), respectively ( $p = 0.25$ ). The median number of top-up transfusions per infant in group I and II was 1 (IQR 0-2, range 0-6) and 2 (IQR 1-3, range 0-5) ( $p = 0.01$ ).



**Table 2** Neonatal outcome in group I and group II

	<b>Group I</b> (n = 156)	<b>Group II</b> (n = 27)	<b>p-value</b>
Hemoglobin level at birth - g/dL <sup>a</sup>	11.6 ± 2.58	12.6 ± 3.40	0.25
Bilirubin level at birth - mg/dL <sup>a</sup>	6.0 ± 2.70	6.2 ± 2.85	0.89
Reticulocyte count at birth - % <sup>o</sup> b,c	21 (3 - 61)	47.5 (3 - 106)	0.19
Maximum bilirubin - mg/dL <sup>b</sup>	13.6 (10.3 - 16.8)	15.4 (13.2 - 18.0)	0.03
Neonates treated with phototherapy - n (%)	150 (96)	27 (100)	0.59
Phototherapy - days <sup>a</sup>	4.1 ± 1.88	4.7 ± 1.59	0.11
Neonates treated with ET - n (%)	103 (66)	7 (26)	< 0.01
Number of ETs per neonate <sup>b</sup>	1 (0 - 1)	0 (0 - 1)	< 0.01
ETs performed on day 1 - n (%)	94 (96)	4 (57)	< 0.01
Duration of admission at our center - days <sup>a</sup>	6.0 ± 3.3	6.3 ± 3.9	0.47

<sup>a</sup>Value given as mean ± SD

<sup>b</sup>Value given as median (IQR)

<sup>c</sup>Reticulocyte count at birth was assessed in 75 and 20 patients of group I and II, respectively

**Table 3** Top-up transfusions in neonates with Rhesus hemolytic disease in group I and group II

	<b>Group I</b> (n = 156)	<b>Group II</b> (n = 27)	<b>p-value</b>
Neonates receiving top-up transfusions - n (%)	105/154 (68)	22/27 (81)	0.25
Number of top-up transfusions per infant <sup>a</sup>	1 (0-2) <sup>c</sup>	2 (1-3)	0.01
Neonates receiving:			
1 top-up transfusion - n (%)	41 (39)	3 (14)	0.08
2 top-up transfusions - n (%)	37 (36)	9 (41)	0.31
3 top-up transfusions - n (%)	11 (11)	6 (27)	0.03
4 top-up transfusions - n (%)	11 (11)	3 (14)	0.44
5 top-up transfusions - n (%)	2 (2)	1 (4)	0.39
6 top-up transfusions - n (%)	2 (2)	0 (0)	1.00
Days after birth until first top-up transfusion <sup>a</sup>	19.5 (1 - 34.8) <sup>c</sup>	10 (2.5 - 23.0) <sup>d</sup>	0.23
Hemoglobin level at first top-up transfusion - g/dL <sup>b</sup>	8.3 ± 1.5 <sup>e</sup>	8.2 ± 1.3 <sup>f</sup>	0.72

<sup>a</sup> Value given as median (IQR)

<sup>b</sup> Value given as mean ± SD

<sup>c</sup> Assessed in 153/156 neonates

<sup>d</sup> Assessed in 17/22 neonates

<sup>e</sup> Assessed in 95/105 neonates

<sup>f</sup> Assessed in 16/22 neonates

Combining group I and II, we found a negative correlation between the number of ETs and the number of top-up blood transfusions (Spearman correlation coefficient  $-0.183$ ;  $p = 0.01$ ). This correlation between a lower number of ETs and a higher number of top-up transfusions was present mainly in the subgroup of neonates treated with IUT ( $n = 114$ ) (Spearman correlation coefficient  $= -0.340$ ;  $p < 0.01$ ). In the subgroup of neonates without IUT ( $n = 69$ ), the correlation between the number of ETs and the number of top-up blood transfusions was not significant (Spearman correlation coefficient  $= 0.011$ ;  $p = 0.93$ ). Of the 110 neonates that received an ET 73 neonates (66%) were treated with IUT.

## Comment

This study shows that after implementation of a more restrictive guideline for ET in neonates with RHD, the rate of ETs decreased considerably from 66% to 26%. The reduction in ET rate was associated with a significant increase in the number of top-up transfusions for neonatal anemia. Neonates in group II required twice more top-up transfusions than neonates in group I. Since the criteria for top-up transfusions remained unchanged before and after the ET guideline changes, our findings suggest that neonatal anemia in neonates with RHD is more likely to occur if restrictive ET criteria are used.

Our findings are in accordance with a previous study by al-Alaiyan et al. in a small group of 36 (near-) term neonates with RHD treated with IUT.<sup>3</sup> Al-Alaiyan et al. reported a similar association between ET rate and top-up transfusions.<sup>3</sup> The risk of neonatal anemia in neonates treated with and without ET was 36% and 92%, respectively ( $p = 0.04$ ).<sup>3</sup>

The reduced rate of top-up transfusions in infants treated with ET can be attributed to the removal of antibodies and IgG coated erythrocytes during ET, hence reducing the hemolytic process and the risk of neonatal anemia.<sup>3,6</sup> However, the effect of ET on the antibody titer on the short term is limited. Since the antibodies are distributed in the intravascular as well as the extravascular fluid, ET reduces the antibody titer only by about 25 percent.<sup>4,10</sup> The beneficial effect of ET lies in replacing nearly all of the Rhesus positive red cells by immunologically compatible cells, hence surviving cells in the blood stream.<sup>4</sup>

In contrast with previous studies<sup>2,8</sup> we found a significant negative correlation between the number of ETs and top-up transfusions. This negative correlation was found only in neonates treated with IUT, but was not found in neonates without IUT treatment. Since fetuses requiring IUT treatment are more severely affected by RHD than fetuses without IUT treatment, we speculate that the beneficial effect of ET in reducing the number of top-up transfusions occurs

primarily in severely affected neonates with high titer antibodies. Washing out antibodies and replacing Rhesus positive cells through ET treatment may be particularly more effective in severely affected neonates.

The pathogenesis of neonatal or late anemia in RHD is not completely clarified and can be due to either depressed erythropoiesis (“hyporegenerative anemia”) or persisting reduction in half-life of the Rhesus positive erythrocytes caused by remaining antibodies (“late anemia of hemolytic disease”) if age-appropriate or elevated reticulocytes are present.<sup>11,12</sup> “Hyporegenerative anemia” occurs in particular after IUT due to suppression of the erythropoiesis.<sup>2,9</sup> An alternative explanation for failing compensatory reticulocytosis is destruction of bone marrow precursors and reticulocytes by antibodies.<sup>4,13</sup> Other contributing factors to neonatal anemia are reduced survival of transfused red blood cells,<sup>5</sup> natural decline of the hemoglobin level toward the physiological nadir, and the increasing intravascular volume of the growing neonate.<sup>1</sup> Finally, erythropoietin deficiency can be a possible contributing factor to neonatal anemia.<sup>12</sup> Treatment with erythropoietin has been suggested to reduce the number of top-up transfusions, but the evidence to recommend routine use of erythropoietin is very limited.<sup>14</sup>

The data in this study should be interpreted with care due to the retrospective design of the study which may have led to a selection bias and influenced by changing transfusion attitudes over time. Although both groups were similar in terms of severity of fetal anemia, need of IUT, and hemoglobin levels at birth, it is conceivable that neonates in group II were more severely affected than neonates in group I, hence requiring more top-up transfusions.<sup>2</sup> Importantly, the percentage of male infants in group II was higher than in group I. Male infants have a higher prevalence of HDN secondary to Rhesus alloimmunization,<sup>15</sup> a higher neonatal death rate from kernicterus<sup>16</sup> and appear to be more severely affected than females in terms of need for IUT, development of hydrops fetalis and perinatal mortality.<sup>17</sup> A larger study is required to determine if this sex difference in baseline characteristic may have influenced our results.

In conclusion, this study shows that the number of ETs in neonates with HDN secondary to Rhesus alloimmunization decreased significantly after the introduction of restrictive ET criteria. Reduction of ET rate resulted in a doubling of the number of top-up transfusions. Restrictive ET criteria in HDN secondary to Rhesus alloimmunization during the neonatal period may thus lead to an increased rate of neonatal anemia. Nevertheless, the risk of adverse events associated with ETs (in particular catheter-related complications) is high (7%)<sup>18</sup> compared to the transfusion-related risks of blood transfusion in general (< 0.04%).<sup>19</sup> A reduction in ETs, despite an increase in top-up transfusions may therefore be more beneficial for neonates with HDN secondary to Rhesus alloimmunization, although the long-term effects on neurodevelopmental outcome requires longer follow-up.

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