# Neonatal management and outcome in red cell alloimmunization

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# Intravenous immunoglobulin in neonates with Rhesus hemolytic disease: a randomized controlled trial

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*Pediatrics 2011; 127:680-686*

## Abstract

**Background:** Despite limited data, international guidelines recommend the use of intravenous immunoglobulin (IVIg) in neonates with Rhesus hemolytic disease.

**Objective:** To test whether prophylactic use of IVIg reduces the need for exchange transfusions in neonates with Rhesus hemolytic disease.

**Design and setting:** We performed a randomized double-blind placebo-controlled trial in neonates with Rhesus hemolytic disease. After stratification for treatment with intrauterine transfusion, neonates were randomized for IVIg (0.75 g/kg) or placebo (glucose 5%). Primary outcome was the rate of exchange transfusions. Secondary outcomes were duration of phototherapy, maximum bilirubin levels and the need of top-up red cell transfusions.

**Results:** Eighty infants were included, of whom 53 (66%) were treated with intrauterine transfusion(s). There was no difference in the rate of exchange transfusions between the IVIg and placebo groups (17% (7/41) versus 15% (6/39), p=1.00) and in number of exchange transfusions per patient (median (range) 0 (0-2) versus 0 (0-2), p=0.90), nor in duration of phototherapy (4.7 (1.8) versus 5.1 (2.1) days, p=0.34), maximum bilirubin levels (14.8 (4.7) versus 14.1 (4.9) mg/dL, p=0.52) and proportion of neonates requiring top-up red cell transfusions (83% (34/41) versus 87% (34/39), p=0.76).

**Conclusion:** Prophylactic IVIg does not reduce the need for exchange transfusion nor the rates of other adverse neonatal outcomes. Our findings do not support the use of IVIg in neonates with Rhesus hemolytic disease.

## Introduction

Rhesus hemolytic disease of the neonate (HDN) may lead to excessive hyperbilirubinemia and permanent brain damage due to kernicterus. Traditional neonatal treatment of Rhesus HDN consists of intensive phototherapy and exchange transfusion (ET). Phototherapy lowers bilirubin through photo-oxidation, whereas ET removes bilirubin and hemolytic antibodies, and corrects anemia.[1] However, ET is a high-risk invasive procedure associated with a significant rate of adverse effects. Although the mortality rate associated with ET is nowadays reported to be less than 0.3% in term infants, the morbidity rates can reach 24% and includes catheter-related complications, sepsis, thrombocytopenia and hypocalcemia.[1-7]

Neonatal treatment with intravenous immunoglobulin (IVIg) has been suggested as an alternative therapy for ET in Rhesus HDN.[8] In many Western countries, including the Netherlands, IVIg is widely used.[9] A few small randomized controlled trials (RCT) reported that IVIg combined with phototherapy reduces serum bilirubin levels and the need for ET in neonates with Rhesus HDN compared to phototherapy alone.[10-13] In these studies, treatment with IVIg reduced the duration of phototherapy and length of hospitalization, but increased the need for top-up red cell transfusions.

Recommendations for the routine use of IVIg are controversial due to various methodological limitations of the studies. A Cochrane review suggested in 2002 that the results of further trials of higher quality should be awaited.[14] The American Academy of Pediatrics (AAP) recommended in 2004 the use of IVIg (0.5–1 g/kg) in Rhesus HDN in case of failure of phototherapy, based on the same limited data.[8] Given these conflicting recommendations, a well-designed RCT for the use of IVIg in Rhesus HDN was urgently needed. We hypothesized that IVIg reduces the need for ET and we designed a RCT to address this question.

## Materials and Methods

We performed a prospective randomized single-center double-blind placebo-controlled trial (http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=832). The Leiden University Medical Center (LUMC) is the national referral center for the management and intrauterine treatment of red cell alloimmunization in the Netherlands. All neonates of 35 or more weeks of gestation with Rhesus HDN, born between 2006 and 2010 and admitted to the neonatal nursery of the LUMC were eligible. Rhesus HDN was defined as (1) Maternal Antibody Dependent Cellular Cytotoxicity-test (ADCC) > 50%, a validated functional test predicting severe hemolysis and comparable with a titer of > 1:64[15] and (2) positive direct antiglobulin test

caused by anti-Rhesus D (Rh D) or c antibodies in the fetus/neonate of a Rh D or c negative mother. We excluded all neonates (1) with perinatal asphyxia (defined as an Apgar score at 5 minutes less than 3 and/or umbilical cord arterial pH less than 7.0), (2) with hemolytic disease other than Rh D or c and (3) with Rhesus HDN presenting > 4 hours after birth.

Written informed parental consent was obtained before birth. After stratification into two groups (with and without IUT), infants were assigned at birth to the IVIg treatment group (IVIg-group) or placebo control group (placebo-group) through pharmacy-controlled randomization. Method of treatment allocation was computer-generated randomization sequence, with randomization code kept by the chief pharmacist. The block size for randomization was 4 in the IUT-group and 2 in the group without IUT (because of the expected smaller proportions of infants in the non-IUT-group). The hospital pharmacy provided identical coded drug boxes and infusion solutions were delivered in sequentially-numbered identical vials containing either IVIg or placebo. To prevent discrepancy between two children of the same family, in case of twins the same vial was used for both children. Clinicians, nurses and parents were blinded to the randomization and allocation.

In the IVIg-group, patients received conventional intensive phototherapy plus prophylactic IVIg as a single dose of 0.75 g/kg (administered in approximately 5-6 hours) starting within the first 4 hours after birth. In the placebo-group, patients received conventional intensive phototherapy plus an equal amount of glucose 5% intravenous infusion.
The IVIg product used in this trial (Nanogam®, Sanquin Amsterdam, The Netherlands) is treated with solvent-detergent to inactivate enveloped viruses and subjected to filtration through a 15 nanometer filter to remove non-enveloped viruses, including Parvo B19. Nanogam® contains more than 95% monomeric IgG and no aggregates.

All infants with Rhesus HDN admitted to our neonatal nursery receive intensive phototherapy directly after birth using white light with an intensity of 12-20 µW/cm/nm given by air shield and Ohmeda lamps, in combination with a bilirubin-blanket providing blue light 30 µW/cm/nm. During phototherapy, extra fluids (10 ml/kg) are administered. Phototherapy and ET were performed according to the latest AAP guidelines.[8] The criteria for ET were: (1) total serum bilirubin above (higher) ET thresholds 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 criteria were not based on fixed bilirubin thresholds, but were derived from the nomograms of AAP and varied according to postnatal age (hours/days) of the neonate. ET was performed with double-volume transfusion (160 mL/kg) using irradiated and leukocyte-depleted compatible erythrocytes.

We recorded the following obstetric and neonatal data: fetal hemoglobin (Hb) concentration and gestational age at first IUT, number of IUTs, gestational age at birth, birth weight, Hb concentration, reticulocyte count and bilirubin level from cord blood at birth, maximum bilirubin level during admission, duration of phototherapy and admission (days), number of ETs required, number of top-up red blood cell transfusions received during the first 3 months of life and Hb levels prior to top-up transfusion. Hb levels were measured routinely every week up to three months of age. After discharge from our center, top-up transfusions were performed in referring hospitals when Hb levels were < 8.0 g/dL, or < 9.6 g/dL in the presence of clinical symptoms of anemia (such as lethargy, feeding problems, need for oxygen or failure to thrive). Folic acid (50 mcg/day) was administered orally during the first three months of life to all neonates. Data on the number of top-up transfusions and Hb levels in infants managed (after discharge) outside our center were collected through correspondence with the local pediatrician or blood transfusion department.

Primary outcome was the rate of ET and the number of ETs per infant. Secondary outcomes were duration of phototherapy and hospital stay, maximum serum bilirubin levels and the need of top-up red cell transfusions in the first three months of life.

**Statistical analysis**

Based on the available literature, we calculated that a minimum of 40 infants in each study arm was required to demonstrate a 5-fold reduction in need of ET between the placebo-group and the IVIg-group (30% versus 6%) with a significance of 0.05 and a power of 80%, by two-tailed analysis. The expected rate (30%) of ET in the placebo-arm was derived from the recorded incidence on ET at our department in 2005-2006. The expected rate (6%) of ET in the IVIg-group was calculated from the reported data in the literature (Gottstein and Cooke)[11]. According to the meta-analysis from Gottstein and Cooke, the use of IVIg in neonates with Rhesus HDN could lead to a 5-fold reduction in the incidence of ET (relative risk (RR) 0.21, 95% confidence intervals (CI) 0.10 to 0.45).

Data are reported as means and standard deviations (SD) or as median and ranges, 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. A p-value <0.05 was considered statistically significant. Statistical analysis was executed with SPSS 17.0 (SPSS Inc, Chicago, IL, USA).

## Results

A total of 121 neonates with Rhesus hemolytic disease were born in the study period, of whom 41 (34%) were excluded (Figure 1). We enrolled 80 patients in the study, 41 patients in the IVIg-group and 39 in the placebo-group. One pair of twins was included in the IVIg-group. Both children received IVIg from the same vial according to the protocol. During infusion of the study medication no potential side-effects such as hypotension, tachycardia or allergic reactions were reported. The baseline characteristics of the two treatment groups were similar (Table 1).

**Neonatal outcome: phototherapy and ET**

All neonates were treated with intensive phototherapy directly after birth. The mean number (SD) of days of phototherapy in neonates in the IVIg-group and placebo group was 4.7 (1.8) and 5.1 (2.1), respectively (p=0.34). At least one ET was required in 17% (7/41) of the neonates in the IVIg-group compared to 15% (6/39) in the placebo-group (p=1.00). The median number of ETs in the IVIg-group and placebo-group was 0 (range 0-2) and 0 (range 0-2), respectively (p=0.90). Median time from birth to (first) ET was 44 hours (range 9-60) in the IVIg-group and 31 hours (range 22-66) in the placebo-group. IVIg or placebo was administered within the first 4 hours after birth.

Maximum mean bilirubin levels during admission were similar in both groups (14.8 ± 4.7 versus 14.1 ± 4.9 mg/dL, respectively (p=0.52). Similar results for the primary and secondary outcomes were observed for the sub-groups of neonates after stratification for treatment with or without IUT. Detailed information on neonatal treatment and outcome in both groups, overall and after stratification for IUT, is presented in Table 2.

One included patient developed a Bacillus cereus sepsis with brain abscesses a few days after an ET performed through an umbilical venous catheter. Because of this serious adverse event the randomization code for the patient was opened and showed that the infant had received IVIg. Sterility tests on the used IVIg batches were subsequently performed and found to be sterile. In addition, cultures of all donor blood products used for the IUTs and ET were examined and found to be sterile. Therefore the cause of infection remained unclear and may have been related to the umbilical venous catheterization and ET. Detailed information on this exceptional case can be found in a case report.[16]

**Top-up transfusions**

The percentage of neonates requiring a top-up transfusion in the IVIg-group and placebo-group was 83% (34/41) and 87% (34/39), respectively (p=0.76). The median number of top-up

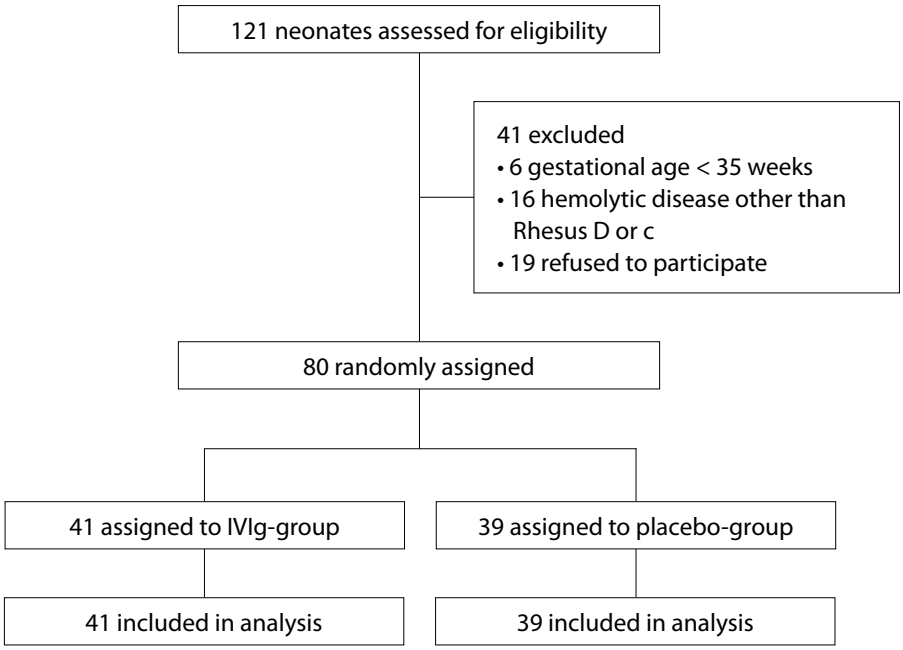


**Figure 1** Flow diagram of study participants

**Table 1** Baseline characteristics of the included patients

| | **IVIg-group** (n=41) | **placebo-group** (n=39) | **p-value** |
|---|---|---|---|
| Gestational age at birth-weeks [a] | 36.7 ± 1.0 | 36.5 ± 0.6 | 0.23 |
| Birth weight-grams [a] | 2994 ± 485 | 2953 ± 424 | 0.68 |
| Male-n (%) | 29 (71) | 25 (64) | 0.64 |
| Neonates treated with IUT-n (%) | 27 (66) | 26 (67) | 0.99 |
| Number of IUTs per neonate [b] | 1 (0-4) | 1 (0-6) | 0.47 |
| Gestational age at first IUT-weeks [a] | 29 ± 4 | 28 ± 6 | 0.44 |
| Hemoglobin level at first IUT-g/dL [a] | 6.9 ± 2.2 | 6.5 ± 2.3 | 0.44 |
| Rhesus D immunization-n (%) | 36 (88) | 35 (90) | 0.59 |
| Hemoglobin level at birth-g/dL [a] | 12.2 ± 2.9 | 11.9 ± 2.6 | 0.52 |
| Reticulocyte count at birth-‰ [a] | 64 ± 51 | 52 ± 57 | 0.31 |
| Bilirubin level at birth-mg/dL [a] | 7.0 ± 3.9 | 5.7 ± 2.3 | 0.07 |

[a] Value given as mean ± SD
[b] Value given as median (range)

**Table 2** Neonatal outcome in the IVIg-group and placebo-group, and according to stratification for IUT

| | **Total group (n=80)** | | | **IUT group (n=53)** | | | **no-IUT group (n=27)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **IVIg** (n=41) | **placebo** (n=39) | **p-value** | **IVIg** (n=27) | **placebo** (n=26) | **p-value** | **IVIg** (n=14) | **placebo** (n=13) | **p-value** |
| Neonates with ET-n (%) | 7 (17) | 6 (15) | 0.99 | 7 (26) | 4 (15) | 0.50 | 0 (0) | 2 (15) | 0.22 |
| Number of ETs per neonate [b] | 0 (0-2) | 0 (0-2) | 0.90 | 0 (0-2) | 0 (0-2) | 0.54 | 0 (0-0) | 0 (0-1) | 0.14 |
| Maximum bilirubin-mg/dL [a] | 14.8 ± 4.7 | 14.1 ± 4.9 | 0.52 | 14.9 ± 5.2 | 12.6 ± 4.8 | 0.11 | 14.7 ± 3.8 | 17.0 ± 3.7 | 0.11 |
| Phototherapy-days [a] | 4.7 ± 1.8 | 5.1 ± 2.1 | 0.34 | 4.4 ± 1.6 | 4.5 ± 2.0 | 0.74 | 5.3 ± 1.9 | 6.2 ± 2.0 | 0.23 |
| Hospitalization-days [a] | 7 ± 4 | 7 ± 3 | 0.37 | 6 ± 4 | 7 ± 3 | 0.58 | 7 ± 4 | 8 ± 3 | 0.45 |

[a] Value given as mean ± SD
[a] Value given as median (range)

**Table 3** Top-up transfusions in neonates with Rh D or c hemolytic disease treated with or without IVIg

| | Total group (n=80) | | | IUT group (n=53) | | | no-IUT group (n=27) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **IVIg** (n=41) | **placebo** (n=39) | **p-value** | **IVIg** (n=27) | **placebo** (n=26) | **p-value** | **IVIg** (n=14) | **placebo** (n=13) | **p-value** |
| Neonates requiring top-up transfusions–n (%) | 34 (83) | 34 (87) | 0.76 | 23 (85) | 24 (92) | 0.67 | 11 (78) | 10 (77) | 0.99 |
| Number of top-up transfusions per neonate [b] | 2 (0-6) | 2 (0-6) | 0.93 | 2 (0-6) | 2 (0-6) | 0.71 | 2 (0-5) | 1 (0-5) | 0.70 |
| 1 top-up transfusion–n (%) | 8 (20) | 10 (26) | 0.51 | 6 (22) | 5 (19) | 0.79 | 2 (14) | 5 (38) | 0.21 |
| 2 top-up transfusions–n (%) | 12 (29) | 8 (21) | 0.37 | 7 (26) | 7 (27) | 0.93 | 5 (36) | 1 (8) | 0.16 |
| 3 top-up transfusions–n (%) | 6 (15) | 9 (23) | 0.33 | 4 (15) | 7 (26) | 0.28 | 2 (14) | 2 (15) | 0.99 |
| 4 top-up transfusions–n (%) | 4 (10) | 4 (10) | 0.99 | 3 (11) | 3 (12) | 0.99 | 1 (7) | 1 (8) | 0.99 |
| 5 top-up transfusions–n (%) | 2 (5) | 2 (5) | 0.99 | 1 (4) | 1 (4) | 0.99 | 1 (7) | 1 (8) | 0.99 |
| 6 top-up transfusions–n (%) | 2 (5) | 1 (3) | 0.99 | 2 (7) | 1 (4) | 0.99 | 0 (0) | 0 (0) | 0.99 |
| Days after birth until first top-up transfusion [a] | 12 ± 12 | 16 ± 15 | 0.24 | 12 ± 12 | 16 ± 16 | 0.33 | 13 ± 11 | 17 ± 15 | 0.50 |
| Hb level at first top-up transfusion–g/dL [a] | 8.4 ± 1.3 | 8.1 ± 1.4 | 0.38 | 8.5 ± 1.3 | 8.1 ± 1.5 | 0.25 | 8.0 ± 1.1 | 8.1 ± 1.0 | 0.77 |

[a] Value given as mean ± SD
[a] Value given as median (range)

transfusions per neonate in the IVIg-group and placebo-group was 2 (range 0-6) and 2 (range 0-6), respectively (p=0.93). Mean hemoglobin level at first top-up transfusion and median number of days until first top-up transfusion were similar in both groups. Detailed information on the use of top-up transfusions in the IVIg-group and the placebo-group is presented in Table 3.

## Comment

In this RCT we have shown that prophylactic treatment with IVIg in neonates with Rhesus hemolytic disease did not reduce the need for ET nor the rates of other adverse neonatal outcomes. Our results do not support the recommendation to give IVIg in Rhesus hemolytic disease, as stated in recent AAP guidelines[8]. Our study adds to the Cochrane analysis that there is no evidence to recommend routine use of IVIg.[14]

In the past, several studies have suggested a positive effect of IVIg in reducing the rate of hemolysis in Rhesus hemolytic disease.[10,12,13,17-19] Although the exact mechanism of action of IVIg remains unclear, IVIg has been reported to block Fc-receptors on macrophages, resulting in a decreased removal of anti-Rh antibody coated erythrocytes from the circulation. IVIg might increase IgG catabolism, resulting in a shorter half-life of antibodies (including anti-Rh antibodies). A third hypothesis is the presence of anti-idiotypic antibodies in IVIg neutralizing anti-Rh antibodies.[12,20-22]

Our results are in contrast with the most recent recommendations of the AAP to use 0.5-1.0 g/kg IVIg in Rhesus hemolytic disease in case of failure of phototherapy.[8] These guidelines were published in 2004 and based on a limited number of small RCTs. Several important methodological limitations hampered the interpretation of these studies, including sub-optimal study-designs and the wide range of inclusion criteria.[18,19] The Cochrane Collaboration performed a review on three studies, in which a total of 189 infants were included.[10,12-14] Rubo et al.[10] included 32 infants with Rhesus hemolytic disease in a multicenter RCT. No details on IUT and gestational age were given. Several years later Dagoglu et al.[13] included 29 preterm and 12 term infants in a RCT. Cut-off for prematurity and criteria for top-up red cell transfusions were not defined. In 1999 Alpay et al.[12] enrolled 116 infants, predominantly with ABO incompatibility (n=93), but also neonates with Rhesus hemolytic disease (n=16) and both Rh and ABO incompatibility (n=7) were included. However, results were not given for each group separately. None of the studies described detailed phototherapy guidelines and none of them used a placebo in the control-group or described any method of blinding the intervention after allocation concealment. According to the Cochrane review, none of the trials fulfilled

criteria for high quality study. Our study is the first well-designed randomized double-blind placebo-controlled trial on this topic.

In 4 other studies, infants with ABO incompatibility were included.[12,17,18,23] In general, compared to Rhesus immunization, ABO incompatibility causes less severe hemolysis and therefore less neonatal morbidity.[24] For that reason, we included only neonates with Rhesus disease. These important methodological differences between our study and the previous ones may explain the discordant results.

Several other explanations can be envisaged to explain the lack of effect of IVIg in our study. A possible explanation could be the treatment with intensive and prophylactic phototherapy starting immediately after birth, thereby reducing the risk of severe hyperbilirubinemia. In addition, the majority of infants included in our study were treated with IUT. By IUT, Rhesus incompatible erythrocytes of the fetus are replaced by Rhesus compatible cells of the donor. Dependent on the interval between the last IUT and delivery, these donor cells are still present after birth, resulting in less or more delayed hemolysis.[25, 26] However, several groups including ours have shown that even after IUT, neonates with Rhesus hemolytic disease still often require ET.[27,28] In our study, IVIg was neither effective in the IUT group nor in the group without IUT. However, the number of patients included in the subgroup without IUT (n=27) may be too small to draw firm conclusions. Recently, a research group from Brazil finalized a similar RCT on IVIg for neonates with Rhesus hemolytic disease and, in accordance with our results, found no difference between both groups on the rate of ET. Importantly, in their study the vast majority of patients (n=80) had no prior treatment with IUT (ClinicalTrials.gov NCT00288600).[29] Therefore, both our RCT and the RCT from the Brazilian research group failed to show any effect of IVIg in Rhesus hemolytic disease infants, irrespective of whether or not the infants were treated with IUT. Care should be taken when interpreting our results, particularly the subgroup analyses, due to the relatively limited number of patients. In addition, caution should be used before applying the results of this study to all Rhesus isoimmunized infants. There may be a subset of Rhesus isoimmunized infants with (inappropriate) delayed start of intensive phototherapy, for whom IVIg might be effective. More studies are needed to study the effect of IVIg in this specific subset of infants.

Although IVIg is considered to be an extremely safe product, adverse events can not be totally eliminated. Rare but serious side effects such as transfusion transmitted diseases, anaphylaxis, hypersensitivity, thrombosis, pulmonary emboli and renal failure have been reported.[21,22] Recently, Figueras-Aloy et al[30] reported a higher incidence of necrotizing enterocolitis (NEC) in near-term infants with Rhesus hemolytic disease treated with IVIg compared to a control group managed without IVIg. The authors correctly suggest that their results must be inter-

preted with care given the retrospective nature of the study. Whether occurrence of NEC was related to the administration of IVIg or to the fact that infants receiving IVIg were more ill than the control group is not clear. Nevertheless, since potential (but rare) adverse effects associated with the use of IVIg can not be ruled out, the authors call for more caution when using IVIg in neonates with Rhesus hemolytic disease. IVIg is a blood product prepared by separating the gamma-globulin fraction from the plasma pooled from multiple donors. The manufacturing of IVIg, including fractionation and filtration of viruses is an extremely intensive and expensive process. Therefore, the use of IVIg for indications that are not confirmed by well-designed RCTs should be restricted.[22]

## Conclusion

Prophylactic treatment with IVIg (in a dosage of 0.75 g/kg) did not reduce the need for ET nor the rates of other adverse neonatal outcomes. Our findings do not support the current recommendations of the AAP to use IVIg in neonates with Rhesus hemolytic disease. In view of the absence of beneficial effects, the use of IVIg for this indication should be discouraged.

## Acknowledgement

Nanogam was provided by Sanquin Blood Supply Foundation (Amsterdam, The Netherlands).

## References

1. Smits-Wintjens VE, Walther FJ, Lopriore E. Rhesus haemolytic disease of the newborn: Postnatal management, associated morbidity and long-term outcome. *Semin Fetal Neonatal Med.* 2008;13:265-271.
2. Keenan WJ, Novak KK, Sutherland JM, Bryla DA, Fetterly KL. Morbidity and mortality associated with exchange transfusion. *Pediatrics.* 1985;75:417-421.
3. Patra K, Storfer-Isser A, Siner B, Moore J, Hack M. Adverse events associated with neonatal exchange transfusion in the 1990s. *J Pediatr.* 2004;144:626-631.
4. Jackson JC. Adverse events associated with exchange transfusion in healthy and ill newborns. *Pediatrics.* 1997;99:E7.
5. Steiner LA, Bizzarro MJ, Ehrenkranz RA, Gallagher PG. A decline in the frequency of neonatal exchange transfusions and its effect on exchange-related morbidity and mortality. *Pediatrics.* 2007;120:27-32.
6. Thayyil S, Milligan DW. Single versus double volume exchange transfusion in jaundiced newborn infants. *Cochrane Database Syst Rev.* 2006;CD004592.
7. Hovi L, Siimes MA. Exchange transfusion with fresh heparinized blood is a safe procedure. Experiences from 1 069 newborns. *Acta Paediatr Scand.* 1985;74:360-365.
8. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics.* 2004;114:297-316.

9. New HV, Stanworth SJ, Engelfriet CP et al. Neonatal transfusions. *Vox Sang.* 2009;96:62-85.
10. Rubo J, Albrecht K, Lasch P et al. High-dose intravenous immune globulin therapy for hyperbilirubinemia caused by Rh hemolytic disease. *J Pediatr.* 1992;121:93-97.
11. Gottstein R, Cooke RW. Systematic review of intravenous immunoglobulin in haemolytic disease of the newborn. *Arch Dis Child Fetal Neonatal Ed.* 2003;88:F6-10.
12. Alpay F, Sarici SU, Okutan V, Erdem G, Ozcan O, Gokcay E. High-dose intravenous immunoglobulin therapy in neonatal immune haemolytic jaundice. *Acta Paediatr.* 1999;88:216-219.
13. Dagoglu T, Ovali F, Samanci N, Bengisu E. High-dose intravenous immunoglobulin therapy for rhesus haemolytic disease. *J Int Med Res.* 1995;23:264-271.
14. Alcock GS, Liley H. Immunoglobulin infusion for isoimmune haemolytic jaundice in neonates. *Cochrane Database Syst Rev.* 2002;CD003313.
15. Oepkes D, Van Kamp IL, Simon MJ, Mesman J, Overbeeke MA, Kanhai HH. Clinical value of an antibody-dependent cell-mediated cytotoxicity assay in the management of Rh D alloimmunization. *Am J Obstet Gynecol.* 2001;184:1015-1020.
16. Smits-Wintjens VE, Steggerda SJ, Oepkes D, Van Kamp IL, Kramer CM, Walther FJ et al. Bacillus cereus cerebral abscesses in a term neonate with rhesus hemolytic disease treated with exchange transfusion. *J Pediatr Inf Dis*. 2010;5:277-280.
17. Nasseri F, Mamouri GA, Babaei H. Intravenous immunoglobulin in ABO and Rh hemolytic diseases of newborn. *Saudi Med J.* 2006;27:1827-1830.
18. Tanyer G, Siklar Z, Dallar Y, Yildirmak Y, Tiras U. Multiple dose IVIg treatment in neonatal immune hemolytic jaundice. *J Trop Pediatr.* 2001;47:50-53.
19. Voto LS, Sexer H, Ferreiro G et al. Neonatal administration of high-dose intravenous immunoglobulin in rhesus hemolytic disease. *J Perinat Med.* 1995;23:443-451.
20. Kriplani A, Malhotra SB, Mandal K. Fetal intravenous immunoglobulin therapy in rhesus hemolytic disease. *Gynecol Obstet Invest.* 2007;63:176-180.
21. Walsh S, Molloy EJ. Towards evidence based medicine for paediatricians. Is intravenous immunoglobulin superior to exchange transfusion in the management of hyperbilirubinaemia in term neonates? *Arch Dis Child.* 2009;94:739-741.
22. Kumar A, Teuber SS, Gershwin ME. Intravenous immunoglobulin: striving for appropriate use. *Int Arch Allergy Immunol.* 2006;140:185-198.
23. Miqdad AM, Abdelbasit OB, Shaheed MM, Seidahmed MZ, Abomelha AM, Arcala OP. Intravenous immunoglobulin G (IVIg) therapy for significant hyperbilirubinemia in ABO hemolytic disease of the newborn. *J Matern Fetal Neonatal Med.* 2004;16:163-166.
24. Murray NA, Roberts IA. Haemolytic disease of the newborn. *Arch Dis Child Fetal Neonatal Ed.* 2007;92:F83-F88.
25. Oepkes D, Adama van SP. Intrauterine fetal transfusions in the management of fetal anemia and fetal thrombocytopenia. *Semin Fetal Neonatal Med.* 2007;12:432-438.
26. Egberts J, Van Kamp IL, Kanhai HH, Meerman RH, Giordano PC, Gravenhorst JB. The disappearance of fetal and donor red blood cells in alloimmunised pregnancies: a reappraisal. *Br J Obstet Gynaecol.* 1997;104:818-824.
27. De Boer I, Zeestraten EC, Lopriore E, Van K, I, Kanhai HH, Walther FJ. Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. *Am J Obstet Gynecol.* 2008;198:54. e1-54. e4.
28. Rath ME, Smits-Wintjens VE, Lindenburg I et al. Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. *Vox Sang.* 2010;99(1):65-70.
29. Santos MC, Sa, Gomes, Camacho, Moreira. High-dose intravenous immunoglobulin therapy for hyperbilirubinemia due Rh hemolytic disease: a randomized clinical trial. *Pediatric Academic Societies-annual meeting-Vancouver 2010.* 2010;143.
30. Figueras-Aloy J, Rodriguez-Miguelez JM, Iriondo-Sanz M, Salvia-Roiges MD, Botet-Mussons F, Carbonell-Estrany X. Intravenous immunoglobulin and necrotizing enterocolitis in newborns with hemolytic disease. *Pediatrics.* 2010;125:139-144.