Immunoglobulins in Neonates with Rhesus Hemolytic Disease of the Fetus and Newborn: Long-Term Outcome in a Randomized Trial

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Key Words
Rhesus hemolytic disease of the fetus and newborn · Intravenous immunoglobulin

Abstract
Objective: Prophylactic intravenous immunoglobulin (IVIg) does neither reduce the need for exchange transfusion nor the rates of other adverse neonatal outcomes in neonates with rhesus hemolytic disease of the fetus and newborn (rhesus HDFN) according to our randomized controlled trial analysis. Our objective was to assess the long-term neurodevelopmental outcome in the children included in the trial and treated with either IVIg or placebo. Methods: All families of the children included in the trial were asked to participate in this follow-up study. The long-term neurodevelopmental outcome in children at least 2 years of age was assessed using standardized tests. The primary outcome was the incidence of neurodevelopmental impairment defined as at least one of the following: cerebral palsy, severe cognitive and/or motor developmental delay (with a test score of less than –2 SD), bilateral deafness or blindness. Results: Sixty-six of the 80 children (82.5%) who had been recruited to the initial randomized controlled trial participated in the follow-up study. The children were assessed at a median age of 4 years (range 2–7). The median cognitive score was 96 (range 68–118) in the IVIg group and 97 (range 66–118) in the placebo group (p = 0.79). There was no difference in the rate of neurodevelopmental impairment between the IVIg and the placebo group [3% (1/34) vs. 3% (1/32); p = 1.00]. Conclusions: The long-term neurodevelopmental outcome in children treated with IVIg was not different from that in children treated with placebo. Standardized long-term follow-up studies with large enough case series and sufficient power are needed to replicate these findings.

Introduction
Rhesus hemolytic disease of the fetus and newborn (rhesus HDFN) results from maternal red cell alloimmunization against red blood cell antigens for which the mother and fetus are incompatible. Rhesus HDFN can lead to perinatal death if fetal anemia is left untreated and can cause severe hyperbilirubinemia in neonatal survivors [1].

Conventional postnatal treatment consists mainly of intensive phototherapy, exchange transfusions (ETs) and top-up red blood cell transfusion. ET is necessary in rhe-
sus HDFN when intensive postnatal treatment fails and serum bilirubin levels approach the threshold for ET. However, ET is an invasive procedure and is associated with several adverse events [2].

Treatment with intravenous immunoglobulin (IVIg) combined with phototherapy has been suggested as an alternative therapy to reduce the need for ET. In a recent randomized double-blind placebo-controlled trial we found that prophylactic treatment with IVIg did not reduce the need for ETs in children with rhesus HDFN [3]. The effect of IVIg in children with rhesus HDFN on the long-term neurodevelopmental outcome has not yet been studied.

The primary aim of this study was to evaluate the long-term neurodevelopmental outcome in all children with rhesus HDFN included in the randomized controlled trial and treated with either IVIg or placebo. Our secondary aim was to assess the presence of allergies and the susceptibility to ear, nose and throat infections. In a follow-up study on children after antenatal treatment for fetal/neonatal alloimmune thrombocytopenia (FNAIT), Radder et al. [4] found significantly less ear, nose and throat problems in those exposed to IVIg treatment compared to children not exposed to maternal IVIg treatment.

Patients and Methods

This follow-up study was performed at the Leiden University Medical Center, The Netherlands, a national referral center for the management, intrauterine and perinatal treatment of rhesus HDFN. Parents of all children with rhesus HDFN included in the randomized controlled trial and treated with IVIg (n = 41) or placebo (n = 39) between 2006 and 2010 were asked to participate in this follow-up study. The trial was initiated to investigate whether prophylactic use of IVIg reduces the need for ETs in neonates with rhesus HDFN [3]. After stratification for treatment with intrauterine transfusion (IUT), neonates were randomized for IVIg or placebo. Informed consent was obtained from all families. The long-term neurodevelopmental outcome in 20 of these children was previously reported in a large follow-up study after IUT (the LOTUS study) [1]. All families were asked to visit our outpatient department for neurodevelopmental examination. Families who were unable to travel to our outpatient department were visited at home.

Our primary study outcome was a composite outcome termed ‘neurodevelopmental impairment’ (NDI) defined as at least one of the following: cerebral palsy, severe cognitive delay (with a test score of less than –2 SD), severe motor delay (with a test score of less than –2 SD), bilateral deafness requiring hearing amplification and/or bilateral blindness. Secondary outcome measures in the current study were the presence of allergies and the susceptibility to ear, nose and throat infections. Parents were questioned about the presence of recurrent ear, nose and throat infections, hospitalization and required surgery.

Cognitive development in children aged 2–3 years was assessed according to the Dutch version of the Bayley Scales of Infant and Toddler Development, 2nd edition (BSID-II) [5]. BSID-II provides a mental development index and a psychomotor development index. Children between 3 and 7 years of age were examined using the Dutch version of the Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI-III). WPPSI-III provides a full-scale Intelligence Quotient (IQ) score and subsection scores for verbal IQ and performance IQ [6]. BSID and WPPSI scores follow a normal distribution curve, with a mean score of 100 and a standard deviation (SD) of 15. A score of 70–84 indicates mild delay (less than –1 SD), and a score of <70 indicates severe delay (less than –2 SD). Parents of the children were still blinded at the time of assessment and will be informed after the completion of this follow-up study. The socioeconomic status of the parents was determined according to the Dutch Sociaal en Cultureel Planbureau and was registered as high, average or low [7].

Statistical Analysis

Categorical variables were compared using the χ² test or Fisher’s exact test, as appropriate. Student’s t test and the Mann-Whitney test were used for continuous variables. A p value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, Ill., USA).

Results

All families of the 80 children included in the trial were approached to participate in this follow-up study. Fourteen children (18%) could not be assessed since the parents declined consent or due to loss of contact information, of whom 7/41 were in the IVIg group and 7/39 in the placebo group. No significant differences in baseline characteristics and socioeconomic status were found between the included and the lost-to-follow-up group. Complete follow-up data were obtained from 66 (83%) children by a visit at our outpatient department (n = 57) or a visit at home (n = 9). A flow diagram of the study participants is shown in figure 1. The baseline characteristics of the IVIg and placebo groups were similar (table 1). Detailed information on the long-term neurodevelopmental outcome of the children included for follow-up in both groups, overall and after stratification for treatment with or without IUT, is presented in table 2. The median age at follow-up assessment was 4.0 years (range 2–7) in the IVIg group and 4.1 years (range 2–7) in the placebo group. The incidence of NDI in children treated with IVIg was 3% (1/34) compared to 3% (1/32) in the placebo group (p = 1.00). NDI in the 2 children was due to severe cognitive delay (with a cognitive score of 66 and 68). The median cognitive score in children in the IVIg group was 96 (range 68–118) compared to 97 (range 66–118) in the placebo group (p = 0.79). Mild cognitive
delay (less than –1 SD) was detected in 18% (6/34) and 16% (5/32) in the IVIg group and placebo group, respectively (p = 0.83). None of the children had cerebral palsy, bilateral blindness or deafness. Similar results were obtained for the subgroups of children after stratification for treatment with or without IUT.

No differences in the occurrence of allergies and ear, nose and throat infections between the IVIg and placebo groups were detected. The incidence of allergies in children treated with IVIg was 12 (4/34), compared to 19 (6/32) in those treated with placebo (p = 0.51). Recurrent ear, nose and throat infections were present in 7/34 (21%) children treated with IVIg versus 9/32 (28%) children treated with placebo (p = 0.48).

**Discussion**

This is the first and largest randomized controlled study to date on long-term neurodevelopmental outcome in children with rhesus HDFN treated postnatally with IVIg or placebo. We were able to follow up 83% children
who participated in the initial randomized controlled tri-
and found no difference in long-term neurodevelop-
mental outcome between children treated with either
IVIg or placebo. The incidence of NDI was 3% in children
treated with IVIg compared to 3% in children who re-
ceived placebo. After stratification for treatment with or
without IUT, similar results were obtained. Analysis from
our initial randomized controlled trial showed that IVIg
had no beneficial effect on the short-term outcome. In
addition, this study suggests that IVIg does not have a
beneficial effect on the long-term neurodevelopmental
outcome.

The incidence of severe developmental delay in both
groups (3 and 3%) seems in line with the incidence of se-
vere delay in the general population (2.3%) [8]. None of
the children had cerebral palsy, bilateral blindness or
defaunax. The incidence of NDI found in this study was
slightly lower compared to a large follow-up study per-
formed recently by our study group on 291 children with
rhesus HDFN (the LOTUS study). In this large cohort,
the incidence of NDI was 5%. However, all children in-
cluded in the LOTUS study were treated with IUT for
fetal anemia, and in 26% of cases, fetal hydrops was pres-
ent. As shown in the LOTUS study, fetal hydrops is an
important risk factor for adverse neurodevelopmental
outcome. In the cohort of children included in the cur-
rent study, only 4 children had mild fetal hydrops, and
none of them developed severe developmental delay.

Our findings also suggest that treatment with IVIg in
the neonatal period does not seem to have harmful effects
on the long-term outcome, which is in accordance with
previous studies. In a randomized controlled trial of 82
infected neonates treated with IVIg prophylaxis in addi-
tion to antibiotics, von Muralt and Sidiroponoulos [9] found
no evidence of harmful effects on neurodevelopmental
outcome. In another study, Ahmed and Gurcan [10] used
IVIg therapy during pregnancy in 8 patients with pem-
phigus vulgaris. No developmental or behavioral abnor-
malities were detected in children born after a pregnancy
with IVIg, with a mean follow-up period of 7 years. These studies, including ours, may however be too
small to reliably determine the effect of IVIg on the long-
term outcome.

The secondary objective of this study was to evaluate
the incidence of allergies and ear, nose and throat infec-
tions in both groups. The immune system is known to be
involved in the regulation of IgE-mediated immediate-
hypersensitivity reactions and allergies [11]. In our study,
a comparison between both groups revealed no associa-
tion between IVIg and allergies and ear, nose and throat
infections. Not much is known on the association be-
 tween IVIg in the perinatal period and the development
of allergies and ear, nose and throat problems at a later
age. In a previous study on 48 children after antenatal
treatment for FNAIT, Radder et al. [4] found significantly
less ear, nose and throat problems in those exposed to
IVIg treatment compared to children not exposed to ma-
ternal IVIg treatment. However, in our group, IVIg was
given only once compared to repeated weekly adminis-
tration of IVIg in the FNAIT group.

The most important limitation of our study is the rela-
tively incomplete follow-up. We were not able to examine
14 children (18%) due to the loss of contact address or the
parents’ decline to participate. However, a comparison of
baseline characteristics between the study and the lost-to-
follow-up group showed no significant differences, as-
suming little bias. Lastly, our randomized controlled
study was designed to detect a difference in the short-
term outcome (namely the use of ET in the neonatal pe-
riod) and was not designed to detect a difference in the
long-term neurodevelopmental outcome. Our conclu-
sions may thus be limited by the relatively small sample
size and power.

Conclusion

We found no differences in long-term neurodevelop-
mental outcome in children with rhesus HDFN treated
with IVIg compared to placebo. Standardized long-term
follow-up studies with large enough case series and suf-
ficient power are needed to replicate these findings.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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