

Postponing Early intrauterine Transfusion with Intravenous immunoglobulin Treatment; the PETIT study on severe hemolytic disease of the fetus and newborn

Kilby, Mark

DOI:

[10.1016/j.ajog.2018.06.007](https://doi.org/10.1016/j.ajog.2018.06.007)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Kilby, M 2018, 'Postponing Early intrauterine Transfusion with Intravenous immunoglobulin Treatment; the PETIT study on severe hemolytic disease of the fetus and newborn', *American journal of obstetrics and gynecology*. <https://doi.org/10.1016/j.ajog.2018.06.007>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

Published in American Journal of Obstetrics and Gynecology on 11/06/2018

DOI: 10.1016/j.ajog.2018.06.007

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Accepted Manuscript



Postponing Early intrauterine Transfusion with Intravenous immunoglobulin Treatment; the PETIT study on severe hemolytic disease of the fetus and newborn

Carolien Zwiers, MD, Johanna G. van der Bom, MD PhD, Inge L. van Kamp, MD PhD, Nan Van Geloven, PhD, Enrico Lopriore, MD PhD, John Smoleniec, MD PhD, Roland Devlieger, MD PhD, Pauline E. Sim, BSc, Marie Anne Ledingham, MD PhD, Eleonor Tiblad, MD PhD, Kenneth J. Moise, Jr., MD PhD, Karl-Philip Gloning, MD PhD, Mark D. Kilby, MD PhD, Timothy G. Overton, MD, Ditte S. Jørgensen, MD, Katrine V. Schou, MD, Bettina Paek, MD, Martin Walker, MD, Emma Parry, MD, Dick Oepkes, MD PhD, Masja de Haas, MD PhD

PII: S0002-9378(18)30494-0

DOI: [10.1016/j.ajog.2018.06.007](https://doi.org/10.1016/j.ajog.2018.06.007)

Reference: YMOB 12235

To appear in: *American Journal of Obstetrics and Gynecology*

Received Date: 16 March 2018

Revised Date: 18 April 2018

Accepted Date: 5 June 2018

Please cite this article as: Zwiers C, van der Bom JG, van Kamp IL, Geloven NV, Lopriore E, Smoleniec J, Devlieger R, Sim PE, Ledingham MA, Tiblad E, Moise Jr. KJ, Gloning K-P, Kilby MD, Overton TG, Jørgensen DS, Schou KV, Paek B, Walker M, Parry E, Oepkes D, de Haas M, Postponing Early intrauterine Transfusion with Intravenous immunoglobulin Treatment; the PETIT study on severe hemolytic disease of the fetus and newborn, *American Journal of Obstetrics and Gynecology* (2018), doi: 10.1016/j.ajog.2018.06.007.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Postponing Early intrauterine Transfusion with Intravenous immunoglobulin**Treatment; the PETIT study on severe hemolytic disease of the fetus and newborn**

Carolien ZWIERS, MD, Leiden, the Netherlands; Department of Obstetrics, Leiden

University Medical Center.

Johanna G. VAN DER BOM, MD PhD, Leiden, the Netherlands; Center for Clinical

Transfusion Research, Sanquin Research; Department of Clinical Epidemiology, Leiden

University Medical Center.

Inge L. VAN KAMP, MD PhD, Leiden, the Netherlands; Department of Obstetrics, Leiden

University Medical Center.

Nan VAN GELOVEN, PhD, Leiden, the Netherlands; Department of Medical Statistics and

Bio-informatics, Leiden University Medical Center.

Enrico LOPRIORE, MD PhD, Leiden, the Netherlands; Department of Pediatrics, Leiden

University Medical Center.

John SMOLENIEC, MD PhD, Liverpool, Australia; Feto-maternal unit, Liverpool Hospital.

Roland DEVLIEGER, MD PhD, Leuven, Belgium; Department of Obstetrics and

Gynecology, University Hospitals KU Leuven.

Pauline E. SIM, BSc, Glasgow, United Kingdom; The Ian Donald Fetal Medicine Unit, The

Queen Elizabeth Hospital.

Marie Anne LEDINGHAM, MD PhD, Glasgow, United Kingdom; The Ian Donald Fetal

Medicine Unit, The Queen Elizabeth Hospital.

Eleonor TIBLAD, MD PhD, Stockholm, Sweden; Center for Fetal Medicine, Karolinska

University Hospital.

Kenneth J. MOISE Jr., MD PhD, Houston, Texas; McGovern Medical School, UT Health; the

Fetal Center, Children's Memorial Hermann Hospital.

Karl-Philip GLONING, MD PhD, München, Germany; Pränatal Medizin München,
Frauenärzte und Humangenetiker.

Mark D. KILBY, MD PhD, Birmingham, United Kingdom; Fetal Medicine Centre,
Birmingham Women's & Children's Foundation Trust; Institute of Metabolism &
Systems Research, College of Medical & Dental Sciences.

Timothy G. OVERTON, MD, Bristol, United Kingdom; Fetal Medicine Unit, St Michael's
Hospital.

Ditte S. JØRGENSEN, MD, Copenhagen, Denmark; Center of Fetal Medicine, Copenhagen
University Hospital Rigshospitalet.

Katrine V. SCHOU, MD, Copenhagen, Denmark; Center of Fetal Medicine, Copenhagen
University Hospital Rigshospitalet.

Bettina PAEK, MD, Kirkland, Washington; Evergreen Fetal Therapy Program, Evergreen
Health Medical Center.

Martin WALKER, MD, Kirkland, Washington; Evergreen Fetal Therapy Program,
Evergreen Health Medical Center.

Emma PARRY, MD, Auckland, New Zealand; Maternal-Fetal Medicine, Auckland District
Health Board.

Dick OEPKES, MD PhD, Leiden, the Netherlands; Department of Obstetrics, Leiden
University Medical Center.

Masja DE HAAS, MD PhD, Leiden and Amsterdam, the Netherlands; Center for Clinical
Transfusion Research, Sanquin Research; Department of Immunohematology and Blood
Transfusion, Leiden University Medical Center; Immunohematology Diagnostic Services,
Sanquin Blood Supply.

Conflict of interest: Authors report no conflict of interest.

Source of funding: This research was supported by a grant from Sanquin Blood Supply (L2181). The design, conduct or publication of the study was not influenced by this financial support.

Paper presented (interim analysis only) at: 16th World Congress in Fetal Medicine, the Fetal Medicine Foundation, Ljubljana, Slovenia, June 25th-29th 2017.

Corresponding author: Carolien Zwiers, Department of Obstetrics, Room K6-034, Leiden University Medical Center, PO box 9600, 2300 RC Leiden, the Netherlands, c.zwiers@lumc.nl, +31612766799, fax number +31715266741.

Abstract word count: 286

Text word count: 3654

Condensation

IVIg treatment seems associated with delayed postpone fetal anemia in alloimmunized pregnancies and with less hydrops and postnatal exchange transfusions.

Implications and contributions

- A. To evaluate whether maternal treatment with intravenous immunoglobulins defers the development of severe fetal anemia and its consequences in pregnancies at risk for severe hemolytic disease of the fetus and newborn (HDFN).
- B. Intravenous immunoglobulin treatment in mothers pregnant with a fetus at risk for hemolytic disease appears to have a potential clinically relevant, beneficial effect on the course and severity of the disease. For example, IVIg was associated with a reduced risk of hydrops and need for exchange transfusions.
- C. There is lack of evidence on the effect of IVIg on the course of fetal hemolytic disease. This multicenter study actualizes the issue, adds to the knowledge, suggests beneficial effects and stresses the need for a randomized controlled trial.

Short title

Prenatal intravenous immunoglobulin seems to improve the course of alloimmunized pregnancies.

Abstract

Background: Intrauterine transfusion for severe alloimmunization in pregnancy performed before 20 weeks' gestation is associated with a higher fetal death rate. Intravenous immunoglobulins may prevent hemolysis and could therefore be a non-invasive alternative for early transfusions.

Objective(s): We evaluated whether maternal treatment with intravenous immunoglobulins defers the development of severe fetal anemia and its consequences in a retrospective cohort to which 12 fetal therapy centers contributed.

Study Design: We included consecutive pregnancies of alloimmunized women with a history of severe hemolytic disease and by propensity analysis compared index pregnancies treated with intravenous immunoglobulins (n=24) with pregnancies managed without intravenous immunoglobulins (n=28),.

Results: In index pregnancies with intravenous immunoglobulin treatment, fetal anemia developed on average 15 days later compared to previous pregnancies (8% less often before 20 weeks' gestation). In pregnancies without intravenous immunoglobulin treatment anemia developed 9 days earlier compared to previous pregnancies (10% more before 20 weeks), an adjusted 4-day between-group difference in favor of the immunoglobulin group (95%CI -10 to 18, $P=.564$). In the subcohort in which immunoglobulin treatment was started before 13 weeks, anemia developed 25 days later and 31% less before 20 weeks' gestation (54% compared to 23%) than in the previous pregnancy. Fetal hydrops occurred in 4% of immunoglobulin-treated pregnancies and in 24% of those without intravenous immunoglobulin treatment (OR 0.03, 95%CI 0 to 0.5, $P=.011$). Exchange transfusions were given to 9% of neonates born from pregnancies with and in 37% without immunoglobulin treatment (OR 0.1, 95%CI 0 to 0.5, $P=.009$).

Conclusion(s): Intravenous immunoglobulin treatment in mothers pregnant with a fetus at risk for hemolytic disease seems to have a potential clinically relevant, beneficial effect on the course and severity of the disease. Confirmation in a multicenter randomized trial is needed.

Key words

Alloimmune fetal hydrops

Fetal anemia

Intrauterine blood transfusion

Intravenous immunoglobulin

Perinatal loss

Red cell alloimmunization in pregnancy

Introduction

Hemolytic disease of the fetus and newborn (HDFN) is caused by maternal alloimmunization against fetal red blood cells. The maternal antibodies can destruct fetal red blood cells and consequently cause fetal anemia, hydrops and perinatal death.^{1,2} Intrauterine blood transfusion (IUT) is currently the only treatment option to prevent fetal death and reduce neurological impairment of these fetuses.³ Although relatively safe in experienced hands, IUT remains an invasive procedure, and complications may occur.⁴ Early transfusions are technically challenging, especially when performed before 22 weeks' gestation, and carry a significantly higher risk of fetal loss compared with procedures performed later in gestation.⁵⁻⁷ In the largest single-center cohort series from the Leiden University Medical Center (LUMC) (the Netherlands), procedure-related fetal death rates after intravascular intrauterine transfusions performed either before or after 20 weeks' gestation were 8.5% and 0.9% per procedure, respectively.⁴ Alternatively, some suggested the use of technically easier intraperitoneal transfusions.^{8,9} To date, no other treatment option for fetuses suffering from severe anemia early in pregnancy has been proven to be effective.

The use of intravenous immunoglobulin(s) (IVIg) may postpone or even replace invasive intrauterine treatment in fetuses of mothers with severe alloimmunization in previous pregnancies.⁹⁻¹¹ IVIg may theoretically dilute circulating maternal antibodies and induce competition at the placenta, reducing transplacental transfer of maternal antibodies. Furthermore, it might increase antibody turnover and thus lower maternal alloantibody levels and, after transfer to the fetus, block fetal macrophage function.^{12,13} As a result, IVIg might prevent hemolysis, but cannot treat existing fetal anemia.¹⁴

Administration of IVIg in pregnancy is considered safe, although side effects may include urticaria, myalgia, chills, headache, nausea or fever.¹⁵ Another disadvantage is that IVIg treatment is relatively expensive (approximately \$6,000/week).¹⁶

A few single-center case series have reported on the possible effects of intravenous immunoglobulins on morbidity and mortality in hemolytic disease of the fetus and newborn.^{9,11,17} In the largest study, from the 1990s, IVIg appeared to lead to a major reduction in fetal mortality from 51% to 20%.¹⁰

Although several fetal therapy centers occasionally use IVIg treatment in pregnancies at risk for recurrence of severe HDFN, there is still much uncertainty about the indications and true effects of IVIg. In this study, we gathered the international experience of treatment with IVIg to evaluate whether (early) administration in high-risk alloimmunized pregnancies is successful in delaying the onset of severe fetal anemia and thus diminishing its clinical consequences.

Material and Methods

Study design, setting and study population

We conducted a retrospective multicenter cohort study. The cohort consisted of pregnancies of women with an earlier pregnancy with severe HDFN ('previous pregnancy'), managed in the first trimester of a new pregnancy between January 2010 and June 2016 ('index pregnancy'). A list of participating centers is provided as supplemental data. Patients from the Leiden University Medical Center (LUMC) were included from 2001 onward, as the antenatal management of HDFN has not changed since the early 2000's in our center.⁴

In all included current ('index') pregnancies women were either treated with IVIg ('IVIg group') or were managed without IVIg ('non-IVIg group'). Severe HDFN was defined as either a previous fetal and neonatal death as a result of HDFN, or the need for IUT prior to 24 weeks' gestation in the previous pregnancy.

All eligible pregnancies of all mothers were included. We excluded pregnancies in which a previous fetal or neonatal death was the result of a lack of diagnostic or therapeutic care, rather than caused by severe HDFN.

In the participating centers, 10 to 140 women with red cell immunization are seen annually, receiving 5-60 IUTs that are performed by 1-4 operators. A 20 or 22 Gauge needle was used for intrauterine intravascular transfusion in all participating centers. The preferred transfusion access sites were the placental cord insertion and the intrahepatic part of the umbilical vein.

Treatment with IVIg was preferably started before 13 completed weeks' gestation. Most cases were treated with Nanogam® or Privigen® IVIg. Alternatively, Gammagard®, Intragam®, Vigam®, Flebogamma® or a combination was used. Most centers dosed IVIg at 1g/kg maternal weight and administered it in weekly doses.

We documented patient characteristics, laboratory results, Doppler measurement results, data on additional treatments, IUT details, delivery details and data on neonatal outcome (up to three months of age) from all pregnancies. Furthermore, details on IVIg treatment were collected of all index pregnancies.

Outcome definitions

We chose the difference in gestational age at onset of severe fetal anemia, requiring IUT, between the index and previous pregnancy ('delta gestational age') as our primary outcome, because the expert opinion is that fetal anemia tends to occur earlier in gestation in subsequent pregnancies of the same alloimmunized mother.¹⁸ As anemia may be present for days before it is diagnosed, the exact onset of severe anemia is impossible to determine. Therefore, we use 'onset of severe anemia' when we mean 'diagnosis of severe anemia' throughout this manuscript. The (diagnosis of) onset of severe anemia was defined as either the day of IUT, the day fetal death was diagnosed, or the day the Doppler peak systolic velocity in the middle cerebral artery (MCA-PSV)¹⁹ was measured above 1.5 MoM, in case fetal death followed at an unknown time point. We elected the need for IUT before 20 weeks as a secondary outcome, because of the clinical relevance of this endpoint due to the associated increased risk for procedure-related complications.⁴ Furthermore, we assessed perinatal survival, fetal hemoglobin (Hb) and Z hemoglobin (ZHb) and the presence of hydrops at time of the first IUT, the occurrence of complications (premature rupture of membranes, emergency cesarean section and fetal or neonatal death), the number of IUTs per pregnancy and the proportion of neonates needing exchange transfusions. ZHb is the deviation of fetal Hb from the mean for gestational age (1 standard deviation corresponds to 1 g/dL deviation).²⁰

Ethical considerations

Depending on the local regulations of the participating centers, this research was approved by the relevant institutional review boards or ethics committees and accordingly, written informed consent was obtained if prescribed. All study data were analyzed anonymously and only the local caregivers knew the identity of their patients.

Therefore, the medical ethics committee of the LUMC approved this research (P15.327/SH/sh) and decided, according to the Medical Research Involving Human Subjects Act (WMO), that written informed consent was not needed from Dutch cases.

Statistics

All primary and secondary outcomes were analyzed in collaboration with our statistician and clinical epidemiologist.

As several women were included with more than one index pregnancy and because pregnancies of the same woman are interrelated, outcomes were compared using generalized estimating equations (GEE). Within the GEE, a binary logistic or linear model was used for comparison of estimated odds or means, respectively.

To adjust for possible confounding by indication, propensity scores were calculated that represent the probability that women would be selected for IVIg treatment by their caregivers in the index pregnancy.²¹ Factors included were gestational age at onset of anemia in the previous pregnancy, pregnancy interval and the number of previous births, type of antibody (D or Kell), maternal BMI and number of IUTs performed in the previous pregnancy. More information on how these factors were included in the propensity score is available as supplemental data. We used inverse probability of treatment weighing (IPTW) based on the generated propensity score in all GEE analyses.

Seven sensitivity analyses were performed for the primary outcome and one for the secondary outcome hydrops. Details on in- and excluded cases and the results of these analyses are available as supplemental data.

Results

Characteristics of the mothers and their pregnancies

A total of 50 pregnancies of women with a severe HDFN were included. Five of these women had more than one pregnancy eligible for inclusion; four women were included with two and one woman with three index pregnancies. One woman was included as a non-IVIg case with her 9th pregnancy. In her 10th pregnancy she received IVIg.

After exclusion of four pregnancies because death in the previous pregnancy did not result from the severity of HDFN, but was most likely caused by lack of timely diagnostics or treatment options, a total of 52 pregnancies remained; 28 in the non-IVIg group and 24 in the IVIg group. One mother started IVIg treatment at the time of her first IUT and this pregnancy was therefore analysed in the non-IVIg group for outcomes at the time of first transfusion only (gestational age, Hb, hydrops) and excluded for other outcomes. Table 1 shows the baseline characteristics of all index pregnancies/fetuses.

In previous pregnancies of the IVIg group, more women had experienced a fetal or neonatal death (63% vs. 44%), anemia occurred 3 weeks earlier (20 vs. 23 weeks) and more IUTs were performed per pregnancy (5 vs. 4), compared to the group that was not treated with IVIg in their current (index) pregnancies. These and other patient characteristics were used to generate propensity scores.

In 51 of 52 index pregnancies an IUT was needed. There was only one index pregnancy without IUT, of a woman with a previous neonatal death at 36 weeks and 5 days. IVIg was started from 13 weeks onwards and no signs of fetal anemia were detected. In the first week of life, the Hb was 9.7 g/dL.

Gestational age at onset of severe fetal anemia

Primary and secondary outcomes are shown as unadjusted and adjusted data in table 2.

The gestational age at onset of severe fetal anemia in the pregnancies with IVIg treatment was on average 15 days later (95% confidence interval (CI) 0 to +31 days) than in the mother's previous pregnancy, whereas this was 9 days earlier (CI -20 to +2) in the non-IVIg group. The adjusted estimated mean difference in this 'delta gestational age' between treatment groups was 4 days (CI -10-18, $P=.564$). If IVIg was started before 13 weeks' gestation, anemia occurred 25 days later than in the previous pregnancy (unadjusted data).

The gestational age at onset of severe anemia in the individual cases is provided by figure 1. This figure also illustrates that in both groups (those with and without IVIg treatment), the course of disease was very heterogeneous. Although anemia on average developed 9 days earlier in subsequent pregnancies without IVIg treatment, 11 out of 28 fetuses in this group had a later onset of fetal anemia than in the previous pregnancy.

The development of fetal anemia before and after 20 weeks' gestation, in patients treated or not treated with IVIg and in the subcohort of patients treated with early IVIg, is displayed in figure 2. In the subcohort in which immunoglobulin treatment was started before 13 weeks, anemia developed 31% less before 20 weeks' gestation (54% compared to 23%) than in the previous pregnancy. In pregnancies with early onset of anemia (before 20 weeks, $N=9$) IVIg was started at a median of 14 weeks, this was 12 weeks and 4 days in pregnancies in which anemia developed later ($N=15$). The dose of IgG administered was 0.67 g/kg (range 0.32-0.69) and 0.54 g/kg (range 0.32-0.73) maternal weight respectively, in pregnancies with early and later anemia.

In five pregnancies, IVIg was continued after the first IUT and the second IUT was performed a median of 18 days later (range 13-26 days).

Other clinical outcomes

Overall survival was 45/51 (88%) and did not differ between treatment groups (adjusted OR 1.2 (0.1-11.7), $P=.894$; table 2). In the index pregnancies of the IVIg group, one fetal and one neonatal death occurred, respectively due to a CMV infection at 18 weeks' gestation and to necrotizing enterocolitis. In the non-IVIg group, four index pregnancies ended in fetal death, following the first IUT performed at gestational ages of 18, 21, 22 and 27 weeks. The cause of fetal death in these cases was either procedure-related or due to the compromised fetal condition.

The decisive MCA Doppler measurement, used to set the indication IUT, was 1.69 MoM in the IVIg group and 1.84 in the non-IVIg group (adjusted estimated mean difference -0.3 MoM, CI -0.6 to 0 MoM, $P=.043$). In both treatment groups, patients received 0.8 MCA Doppler measurements per week, or one MCA Doppler measurement every 8 to 9 days (adjusted estimated mean difference 0.1 measurement/week, CI -0.2-0.3, $P=.537$).

In both treatment groups, three first transfusions were performed intraperitoneally without intravascular access. For 8 non-IVIg patients the degree of anemia was measured in hematocrit only. Consequently, a fetal Hb measurement was available for 20/23 IVIg (and IUT) treated and 14/28 non-IVIg pregnancies. At the time of first IUT, fetuses in both groups had similar Hb levels: 6.4 SD's below the mean for gestational age in the IVIg group and 7.6 SD's in the non-IVIg group (adjusted estimated mean difference 0.9 SDs less deviation from the mean in the IVIg group, CI -0.4 to 2.3 SDs,

$P=.171$).²⁰ Fetuses in the non-IVIg group had signs of hydrops in 24% at the time of first IUT, compared to 4% in IVIg-treated subjects (adjusted OR 0.03, CI 0-0.5, $P=.011$). After birth, 2/22 (9%) IVIg treated neonates with available neonatal data required an exchange transfusion, which was significantly less than the 7/19 (37%) newborns in the non-IVIg group (adjusted OR 0.1, 95% CI 0-0.5, $P=.009$).

Sensitivity analyses

The results of the sensitivity analyses are shown in supplemental table 1. The estimated mean differences of the difference ('delta') in gestational age at onset of anemia in the index and previous pregnancy between treatment groups were all in favor of IVIg treatment, although not statistically significant. The largest raw and adjusted effect sizes were seen in patients with D antibodies only, where fetal anemia occurred on average 18 days later in IVIg treated pregnancies, compared to previous pregnancies, and 14 days earlier in pregnancies from women not treated with IVIg, compared to previous pregnancies (adjusted estimated mean difference between groups 10 days, 95% CI -6 to + 26, $P=.202$).

In eight patients IVIg treatment was combined with plasmapheresis. Additionally, one of these patients received corticosteroids. None of the patients in the non-IVIg group received plasmapheresis or other additional treatments. The sensitivity analysis without pregnancies in which plasmapheresis was performed had a similar result to the original analysis (supplemental table 1).

In patients that were monitored with MCA Doppler in the participating center at least twice before IUT was performed, hydrops was noted in none of the 21 IVIg-treated fetuses and in 4/18 fetuses of the non-IVIg group (weighted $P<.001$).

Comment

In this study, 12 fetal therapy centers from Europe, North-America, Australia and New Zealand collaborated to evaluate the effect of IVIg on the onset of fetal anemia in pregnant women with severe fetal anemia in a previous pregnancy. We found that the onset of severe fetal anemia was later in IVIg treated pregnancies, compared to previous pregnancies. A larger (unadjusted) effect size was seen in the subgroup where IVIg was started ≤ 13 weeks. In the pregnancies not treated with IVIg, severe fetal anemia occurred earlier than in previous pregnancies. The adjusted difference between groups in this 'delta gestational age' was a non-statistically significant 4 days in favor of the IVIg group. The largest effect was observed in pregnancies with HDFN resulting from D immunization. IVIg started at or before 13 weeks appeared to positively influence the number of transfusions before 20 weeks' gestation.

Additionally, we observed that only one fetus of the 24 IVIg-treated pregnancies developed hydrops, whereas 6/28 of the non-IVIg fetuses did. Furthermore, the neonatal exchange transfusion rate was impressively and significantly lower for neonates from the IVIg group compared to those of the non-IVIg group.

Our international collaborative study is the first study in the past two decades on the effect of IVIg in severe HDFN, comparing fetuses from IVIg treated pregnancies with non-IVIg treated pregnancies. The only comparable study was published in 1997, when monitoring of fetal anemia was entirely different, and mainly based on serial amniocentesis for bilirubin levels.¹⁰ Nevertheless, in an IVIg-treated group of 30 severely alloimmunized pregnancies, they found less hydrops, less fetal death and a mean delay in the need for intrauterine treatment of 1,5 weeks, compared to 39

controls. Their conclusion that 'IVIg treated patients seem to have better fetal outcome' is similar to ours.

The positive effect of IVIg treatment on the time of onset of severe fetal anemia was not statistically significant. However, the point estimates of all outcomes point in the same positive direction, supporting a potential clinically relevant and beneficial effect of IVIg on the course of HDFN. For example, IVIg was associated with less fetal hydrops and a lesser need for neonatal exchange transfusions compared to treatment without IVIg.

Furthermore, early IVIg treatment appeared to delay the onset of severe anemia with 3.5 weeks compared to previous pregnancies (unadjusted data, supplemental table 1).

We hypothesize that the effect of IVIg on time of onset of fetal anemia may have been underestimated. Although fetuses in both groups were monitored with equal intervals, those in the IVIg group might have been monitored more thoroughly, as decisive Doppler measurements before IUT were lower than compared to pregnancies without IVIg. This may reflect an earlier suspicion of severe fetal anemia and intervention with IUT in IVIg treated pregnancies compared to non-IVIg treated pregnancies. A more expectant management in the IVIg group could have resulted in longer recorded time to onset of anemia.

Mothers in the IVIg group seemed to have a history of more aggressive course of disease (based on the onset of anemia and the number of previous deaths), however we found strikingly less hydrops in currently IVIg-treated pregnancies. This is in accordance with the study of Voto et al.¹⁰ Although in our study, there may be an effect of relatively early IUT timing in the IVIg group, a real preventive effect of IVIg on the development of fetal

1 hydrops cannot be ruled out and needs further investigation. Preventing fetal hydrops is
2 known to be very beneficial for perinatal survival and long-term outcome.²²

3
4 A new finding was the reduced need for neonatal exchange transfusions after prenatal
5 IVIg treatment. The rate of exchange transfusions in the IVIg group appeared to be
6 significantly lower than in the non-IVIg group and comparable to the expected 15%
7 observed in other (D-only) studies.^{23,24} Apart from the limited sample sizes, a restriction
8 in this finding is the relatively high number of missing data in the non-IVIg group, as
9 chances on missing data are higher in cases where no exchange transfusion is performed.
10 In the scenario that indeed none of these patients with missing data would have
11 received an exchange transfusion, the difference in exchange transfusion rate would still
12 be 6 vs 23% in favor of the IVIg group. If this could be confirmed in a prospective study,
13 it would be highly relevant for the clinical setting. These complex procedures become
14 increasingly rare, with only a few centers able to maintain the necessary skills, and the
15 risks and complications are often underestimated.²⁵

16
17 Lastly, this study is the first to provide valuable insight in the course of disease in
18 subsequent pregnancies with a high risk of HDFN. A surprising finding was the
19 relatively high number of women in the non-IVIg group (11/28) in which the disease
20 did not worsen in the subsequent pregnancy. Although caution is needed in challenging
21 the accepted concept that the disease is more severe in every following pregnancy, we
22 feel that this deserves further study.

23
24 Strengths of this study were the relatively large dataset, obtained through international
25 multicentre collaboration, and the performance of weighted analyses based on the

propensity score, to correct for potential confounders. All centers used practically identical strategies for diagnosis and treatment of fetal anemia. As it is possible that centers that do not offer IVIg treatment may serve a less severely affected patient population, the propensity score analysis may be influenced by this unmeasured 'case mix'²⁶ differences in population between centers. We addressed this by performing a sensitivity analysis including only centers that offer IVIg, which resulted in a similar outcome.

Furthermore, we addressed other potential differences in disease or management by performing additional sensitivity analyses. The effect sizes of these analyses all appeared to be similar to the original analysis (supplemental table 1).

The most important limitation of our study was the heterogeneity of the groups, due to the rarity of severe HDFN, the heterogeneous course of disease and the retrospective nature of the study. Only a prospective, preferably randomized and ideally blinded study could overcome this issue. Together with the still limited sample size, and the rarity of adverse outcomes, absence of statistical significance of observed differences was not unexpected. However, all observed differences point towards a potential clinically relevant benefit of IVIg in this group of alloimmunized pregnancies with very high risk on severe disease.

Another limitation is that the definition of 'onset of severe fetal anemia' is relatively broad. Patients are not monitored daily and anemia may be present a few days before their scheduled appointment. Although this can never be fully circumvented, we did perform a sensitivity analysis without patients in which this uncertainty was larger than a few days (for example, fetal death noticed at a scheduled appointment). The result of this analysis was similar to the original analysis.

1 Finally, despite the propensity analysis, a possible residual confounding effect of the
2 type of antibody remained. This is reflected by the standardized mean difference of this
3 variable being >10% (table 1), which is the proposed threshold below which imbalance
4 seems to be negligible.²⁷

5 Despite the limitations, we feel that gathering the international experience on the use of
6 IVIg in red cell alloimmunization in a conjoint cohort, is an important first step for
7 future research on this subject.

8 9 *Conclusion*

10 In women pregnant with a fetus at high risk for early hemolytic disease, treatment with
11 weekly IVIg seems to positively influence the course and severity of disease. To truly
12 assess the beneficial effects of IVIg, a prospective and preferably randomized controlled
13 trial would be required, and is planned. Due to the rarity of the disease, conducting such
14 a study will be challenging and requires further intensive international collaboration.

15 16 **Acknowledgements**

17 This research was supported by a grant from Sanquin Blood Supply (L2181). The
18 design, conduct or publication of the study was not influenced by this financial support.

References

1. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Haemolytic disease of the fetus and newborn. *Vox Sang* 2015; **109**: 99-113.
2. Abbasi N, Johnson JA, Ryan G. Fetal anemia. *Ultrasound Obstet Gynecol* 2017; **50**: 145-153.
3. van Kamp IL, Klumper FJ, Meerman RH, Oepkes D, Scherjon SA, Kanhai HH. Treatment of fetal anemia due to red-cell alloimmunization with intrauterine transfusions in the Netherlands, 1988-1999. *Acta Obstet Gynecol Scand* 2004; **83**: 731-737.
4. Zwiers C, Lindenburg ITM, Klumper FJ, de Haas M, Oepkes D, Van Kamp IL. Complications of intrauterine intravascular blood transfusion: lessons learned after 1678 procedures. *Ultrasound Obstet Gynecol* 2017; **50**: 180-186.
5. Yinon Y, Visser J, Kelly EN, Windrim R, Amsalem H, Seaward PG, Ryan G. Early intrauterine transfusion in severe red blood cell alloimmunization. *Ultrasound Obstet Gynecol* 2010; **36**: 601-606.
6. Canlorbe G, Mace G, Cortey A, Cynober E, Castaigne V, Larsen M, Mailloux A, Carbonne B. Management of very early fetal anemia resulting from red-cell alloimmunization before 20 weeks of gestation. *Obstet Gynecol* 2011; **118**: 1323-1329.
7. Poissonnier MH, Picone O, Brossard Y, Lepercq J. Intravenous fetal exchange transfusion before 22 weeks of gestation in early and severe red-cell fetomaternal alloimmunization. *Fetal Diagn Ther* 2003; **18**: 467-471.
8. Howe DT, Michailidis GD. Intraperitoneal transfusion in severe, early-onset Rh isoimmunization. *Obstet Gynecol* 2007; **110**: 880-884.
9. Fox C, Martin W, Somerset DA, Thompson PJ, Kilby MD. Early intraperitoneal transfusion and adjuvant maternal immunoglobulin therapy in the treatment of severe red cell alloimmunization prior to fetal intravascular transfusion. *Fetal Diagn Ther* 2008; **23**: 159-163.
10. Voto LS, Mathet ER, Zapaterio JL, Orti J, Lede RL, Margulies M. High-dose gammaglobulin (IVIG) followed by intrauterine transfusions (IUTs): a new alternative for the treatment of severe fetal hemolytic disease. *J Perinat Med* 1997; **25**: 85-88.
11. Connan K, Kornman L, Savoia H, Palma-Dias R, Rowlands S. IVIG - is it the answer? Maternal administration of immunoglobulin for severe fetal red blood cell alloimmunisation during pregnancy: a case series. *Aust N Z J Obstet Gynaecol* 2009; **49**: 612-618.
12. Gelfand EW. Intravenous immune globulin in autoimmune and inflammatory diseases. *N Engl J Med* 2012; **367**: 2015-2025.
13. van den Akker ES, Oepkes D. Fetal and neonatal alloimmune thrombocytopenia. *Best Pract Res Clin Obstet Gynaecol* 2008; **22**: 3-14.
14. Margulies M, Voto LS, Mathet E, Margulies M. High-dose intravenous IgG for the treatment of severe rhesus alloimmunization. *Vox Sang* 1991; **61**: 181-189.
15. Clark AL. Clinical uses of intravenous immunoglobulin in pregnancy. *Clin Obstet Gynecol* 1999; **42**: 368-380.
16. Thung SF, Grobman WA. The cost effectiveness of empiric intravenous immunoglobulin for the antepartum treatment of fetal and neonatal alloimmune thrombocytopenia. *Am J Obstet Gynecol* 2005; **193**: 1094-1099.
17. Kriplani A, Malhotra Singh B, Mandal K. Fetal intravenous immunoglobulin therapy in rhesus hemolytic disease. *Gynecol Obstet Invest* 2007; **63**: 176-180.
18. Moise KJ, Jr. Management of rhesus alloimmunization in pregnancy. *Obstet Gynecol* 2002; **100**: 600-611.
19. Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ, Jr., Dorman KF, Ludomirsky A, Gonzalez R, Gomez R, Oz U, Detti L, Copel JA, Bahado-Singh R, Berry S, Martinez-Poyer J, Blackwell SC. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. *N Engl J Med* 2000; **342**: 9-14.

- 1 20. Nicolaides KH, Soothill PW, Clewell WH, Rodeck CH, Mibashan RS, Campbell S. Fetal
2 haemoglobin measurement in the assessment of red cell isoimmunisation. *Lancet* 1988; **1**:
3 1073-1075.
- 4 21. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of
5 Confounding in Observational Studies. *Multivariate Behav Res* 2011; **46**: 399-424.
- 6 22. Lindenburg IT, Smits-Wintjens VE, van Klink JM, Verduin E, van Kamp IL, Walther FJ,
7 Schonewille H, Doxiadis, II, Kanhai HH, van Lith JM, van Zwet EW, Oepkes D, Brand A,
8 Lopriore E. Long-term neurodevelopmental outcome after intrauterine transfusion for
9 hemolytic disease of the fetus/newborn: the LOTUS study. *Am J Obstet Gynecol* 2012; **206**:
10 141.e141-148.
- 11 23. Santos MC, Sa C, Gomes SC, Jr., Camacho LA, Moreira ME. The efficacy of the use of
12 intravenous human immunoglobulin in Brazilian newborns with rhesus hemolytic disease: a
13 randomized double-blind trial. *Transfusion* 2013; **53**: 777-782.
- 14 24. Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, te Pas AB, Kramer CM, Oepkes D,
15 Brand A, Lopriore E. Intravenous immunoglobulin in neonates with rhesus hemolytic disease:
16 a randomized controlled trial. *Pediatrics* 2011; **127**: 680-686.
- 17 25. Steiner LA, Bizzarro MJ, Ehrenkranz RA, Gallagher PG. A decline in the frequency of neonatal
18 exchange transfusions and its effect on exchange-related morbidity and mortality. *Pediatrics*
19 2007; **120**: 27-32.
- 20 26. Orchard C. Comparing healthcare outcomes. *Bmj* 1994; **308**: 1493-1496.
- 21 27. Austin PC, Stuart EA. Moving towards best practice when using inverse probability of
22 treatment weighting (IPTW) using the propensity score to estimate causal treatment effects
23 in observational studies. *Stat Med* 2015; **34**: 3661-3679.

Table 1. Patient demographics and baseline characteristics

Characteristic	Unadjusted data			Weighted data ^a		
	IVIg group	Non-IVIg group	SMD	IVIg group ^b	Non-IVIg group ^b	SMD
	N=24	N=28		N=24	N=25	
Previous pregnancy						
Fetal or neonatal death	15 (63)	12 (44)	37	8 (32)	9 (36)	-7
Gestational age at onset of anemia, weeks+days	20+0 [16-36]	23+0 [17-31]	-66	24+0 [16-36]	21+0 [17-31]	4
IUTs before 20 weeks, n(%)	11 (46)	3 (11)	85	5 (19)	4 (14)	15
Number of IUTs ^c	5 [0-7]	4 [3-7]	-10	4 [0-7]	5 [4-7]	3
Index pregnancy						
Years between pregnancies	2 [0-6]	3 [0-9]	-35	3 [0-6]	2 [0-9]	-4
Gender of child			38			117
Boy	12 (50)	15 (68)		6 (24)	16 (74)	
Girl	12 (50)	7 (32)		18 (76)	6 (26)	
Antibody against			-26			-29
D	19 (79)	19 (68)		20 (87)	19 (74)	
Kell	5 (21)	9 (32)		3 (14)	6 (26)	
Number of previous births	3 [1-9]	3 [1-8]	-7	3 [1-9]	2 [1-8]	1
Maternal age at first IUT, years	33 [24-41]	32 [24-43]	10	37 [24-41]	29 [24-43]	129
Maternal body mass index	24 [20-41]	28 [22-38]	-72	24 [20-41]	26 [22-38]	-11
Laboratory predictor of disease						
Highest titer in pregnancy	1024 [256-16000]	512 [32-8000]	32	256 [256-16000]	1000 [32-8000]	17
Highest Quantitation in pregnancy (IU/mL)	47 [13-305]	70 [22-381]	-19	39 [13-305]	75 [23-381]	49
IVIg dose, g/kg maternal weight ^d	0.63 [0.32-0.73]	-	-	-	-	-
Number of days between IVIg treatments	7 [7-28]	-	-	-	-	-
Treated with plasmapheresis	8 (33.3)	0	82	4 (15)	0	46

Data presented in median [range] or N (%). Abbreviation: *N*, number of index pregnancies; *SMD*, Standardized Mean Difference; *IUT*, Intrauterine transfusion; *IVIg*, intravenous immunoglobulins.

^aWeighted by the propensity score.

^bFor variables expressed in N (%), the weighted N was calculated from the weighted proportions and rounded.

^cIf no death occurred.

^dAmount of IgG1 and IgG3 administrated, calculated as IVIg dose in g/kg maternal weight * percentage IgG in substrate * percentage IgG1 and IgG3.

Table 2. Primary and secondary outcomes

Outcome	Unadjusted				Propensity analysis			
	IVIg group	Non-IVIg group	Effect size ^a	<i>P</i>	IVIg group ^b	Non-IVIg group ^b	Effect size ^a	<i>P</i>
	N=24	N=28			N=24	N=26		
Delta gestational age, days ^c	15 (0-31)	-9 (-19-1)	24 (6-43)	.011	0 (-11-11)	-4 (-13-5)	4 (-10-18)	.564
Gestational age at onset of anemia, weeks+days	22+6 (21-25)	22+0 (21-23)	5 (-12-22)	.560	22+3 (21-24)	21+5 (20-23)	5 (-9-19)	.475
IUTs before 20 weeks, n(%)	9 (38)	6 (21)	0.5 (0.1-1.5)	.207	6 (24)	6 (22)	1.1 (0.1-8.9)	.908
Survival, n (%)	22 (92)	23 (85)	1.9 (0.3-11.4)	.477	22 (92)	23 (89)	1.2 (0.1-11.7)	.894
Fetal hemoglobin at first IUT, g/dL	5.8 (4.7-7.0)	4.6 (3.3-5.9)	1.2 (-0.5-3.0)	.151	5.4 (4.6-6.2)	4.4 (3.0-5.7)	1.1 (-0.5-2.6)	.184
Hydrops at time of first IUT, n (%)	1 (4)	6 (24)	0.1 (0-1.4)	.092	1 (3)	12 (46)	0.03 (0-0.5)	.011
Number of IUTs ^d	5 (4-6)	5 (4-5)	0.1 (-1.3-1.6)	.859	4 (4-5)	5 (4-6)	-0.4 (-1.5-0.8)	.505
Complication after first IUT, n (%)			0.3 (0-2.6)	.261			0.2 (0-2.1)	.159
PROM or preterm delivery	0	1 (3.7) ^e			0	0		
Intrauterine infection	0	0			0	0		
Emergency cesarean section	1 (5)	0			0 (1.5)	0		
Fetal death	0	4 (15) ^e			0	3 (12)		
Need for exchange transfusion, n (%)	2/22 (9)	7/19 (37)	0.2 (0-0.96)	.045	1 (6)	9 (47)	0.1 (0-0.5)	.009

Data presented in estimated mean (95% CI) or N (%). Abbreviation: *N*, number of index pregnancies; *PROM*, premature rupture of membranes.

^aExpressed in estimated mean difference (95% CI) for numerical outcomes or OR (95% CI) for proportions.

^bFor variables expressed in N (%), the weighted N was calculated from the weighted proportions and rounded.

^cDifference in gestational age at onset of severe anemia between index and previous pregnancy.

^dIf no death occurred.

^ePROM led to fetal death.

Figure legends

Figure 1. Gestational age at onset of severe anemia in previous and index pregnancy.

Red lines reflect earlier onset of severe anemia in the index pregnancy compared to previous pregnancies, blue lines reflect later anemia. The black, bold lines reflect the weighted estimated means. (A) Group treated with intravenous immunoglobulins. (B) Reference patients without intravenous immunoglobulin treatment in index pregnancy. (C) Subgroup treated with intravenous immunoglobulins started before 13 weeks' gestation.

Figure 2. Onset of fetal anemia before and after 20 weeks' gestation in previous and index pregnancies.

Red bars reflect onset of severe anemia before 20 weeks' gestations, blue bars reflect anemia after 20 weeks' gestation.



