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

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# Accepted Manuscript



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

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1 **Postponing Early intrauterine Transfusion with Intravenous immunoglobulin**

2 **Treatment; the PETIT study on severe hemolytic disease of the fetus and newborn**

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7

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11

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14

## 1 **Condensation**

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

4

## 5 **Implications and contributions**

6 A. To evaluate whether maternal treatment with intravenous immunoglobulins  
7 defers the development of severe fetal anemia and its consequences in  
8 pregnancies at risk for severe hemolytic disease of the fetus and newborn  
9 (HDFN).

10 B. Intravenous immunoglobulin treatment in mothers pregnant with a fetus at risk  
11 for hemolytic disease appears to have a potential clinically relevant, beneficial  
12 effect on the course and severity of the disease. For example, IVIg was associated  
13 with a reduced risk of hydrops and need for exchange transfusions.

14 C. There is lack of evidence on the effect of IVIg on the course of fetal hemolytic  
15 disease. This multicenter study actualizes the issue, adds to the knowledge,  
16 suggests beneficial effects and stresses the need for a randomized controlled  
17 trial.

18

## 19 **Short title**

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

22

23

24

25

1 **Abstract**

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

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

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

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

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

5  
6 **Key words**

7 Alloimmune fetal hydrops

8 Fetal anemia

9 Intrauterine blood transfusion

10 Intravenous immunoglobulin

11 Perinatal loss

12 Red cell alloimmunization in pregnancy

13



## 1 **Introduction**

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

17  
18 The use of intravenous immunoglobulin(s) (IVIg) may postpone or even replace  
19 invasive intrauterine treatment in fetuses of mothers with severe alloimmunization in  
20 previous pregnancies.<sup>9-11</sup> IVIg may theoretically dilute circulating maternal antibodies  
21 and induce competition at the placenta, reducing transplacental transfer of maternal  
22 antibodies. Furthermore, it might increase antibody turnover and thus lower maternal  
23 alloantibody levels and, after transfer to the fetus, block fetal macrophage function.<sup>12,13</sup>  
24 As a result, IVIg might prevent hemolysis, but cannot treat existing fetal anemia.<sup>14</sup>

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

4

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

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

15

16

## 17 **Material and Methods**

### 18 *Study design, setting and study population*

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

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

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

8  
9 In the participating centers, 10 to 140 women with red cell immunization are seen  
10 annually, receiving 5-60 IUTs that are performed by 1-4 operators. A 20 or 22 Gauge  
11 needle was used for intrauterine intravascular transfusion in all participating centers.  
12 The preferred transfusion access sites were the placental cord insertion and the  
13 intrahepatic part of the umbilical vein.

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

18  
19 We documented patient characteristics, laboratory results, Doppler measurement  
20 results, data on additional treatments, IUT details, delivery details and data on neonatal  
21 outcome (up to three months of age) from all pregnancies. Furthermore, details on IVIg  
22 treatment were collected of all index pregnancies.

23  
24 *Outcome definitions*

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

#### 21 *Ethical considerations*

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

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

4

#### 5 *Statistics*

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

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

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

21

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

25

## 1 **Results**

### 2 *Characteristics of the mothers and their pregnancies*

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

7  
8 After exclusion of four pregnancies because death in the previous pregnancy did not  
9 result from the severity of HDFN, but was most likely caused by lack of timely  
10 diagnostics or treatment options, a total of 52 pregnancies remained; 28 in the non-IVIg  
11 group and 24 in the IVIg group. One mother started IVIg treatment at the time of her  
12 first IUT and this pregnancy was therefore analysed in the non-IVIg group for outcomes  
13 at the time of first transfusion only (gestational age, Hb, hydrops) and excluded for  
14 other outcomes. Table 1 shows the baseline characteristics of all index  
15 pregnancies/fetuses.

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

21  
22 In 51 of 52 index pregnancies an IUT was needed. There was only one index pregnancy  
23 without IUT, of a woman with a previous neonatal death at 36 weeks and 5 days. IVIg  
24 was started from 13 weeks onwards and no signs of fetal anemia were detected. In the  
25 first week of life, the Hb was 9.7 g/dL.

1

2 *Gestational age at onset of severe fetal anemia*

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

4 The gestational age at onset of severe fetal anemia in the pregnancies with IVIg

5 treatment was on average 15 days later (95% confidence interval (CI) 0 to +31 days)

6 than in the mother's previous pregnancy, whereas this was 9 days earlier (CI -20 to +2)

7 in the non-IVIg group. The adjusted estimated mean difference in this 'delta gestational

8 age' between treatment groups was 4 days (CI -10-18,  $P=.564$ ). If IVIg was started

9 before 13 weeks' gestation, anemia occurred 25 days later than in the previous

10 pregnancy (unadjusted data).

11 The gestational age at onset of severe anemia in the individual cases is provided by

12 figure 1. This figure also illustrates that in both groups (those with and without IVIg

13 treatment), the course of disease was very heterogeneous. Although anemia on average

14 developed 9 days earlier in subsequent pregnancies without IVIg treatment, 11 out of

15 28 fetuses in this group had a later onset of fetal anemia than in the previous pregnancy.

16

17 The development of fetal anemia before and after 20 weeks' gestation, in patients

18 treated or not treated with IVIg and in the subcohort of patients treated with early IVIg,

19 is displayed in figure 2. In the subcohort in which immunoglobulin treatment was

20 started before 13 weeks, anemia developed 31% less before 20 weeks' gestation (54%

21 compared to 23%) than in the previous pregnancy. In pregnancies with early onset of

22 anemia (before 20 weeks,  $N=9$ ) IVIg was started at a median of 14 weeks, this was 1223 weeks and 4 days in pregnancies in which anemia developed later ( $N=15$ ). The dose of

24 IgG administered was 0.67 g/kg (range 0.32-0.69) and 0.54 g/kg (range 0.32-0.73)

25 maternal weight respectively, in pregnancies with early and later anemia.

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

3

#### 4 *Other clinical outcomes*

5 Overall survival was 45/51 (88%) and did not differ between treatment groups  
6 (adjusted OR 1.2 (0.1-11.7),  $P=.894$ ; table 2). In the index pregnancies of the IVIg group,  
7 one fetal and one neonatal death occurred, respectively due to a CMV infection at 18  
8 weeks' gestation and to necrotizing enterocolitis. In the non-IVIg group, four index  
9 pregnancies ended in fetal death, following the first IUT performed at gestational ages of  
10 18, 21, 22 and 27 weeks. The cause of fetal death in these cases was either procedure-  
11 related or due to the compromised fetal condition.

12 The decisive MCA Doppler measurement, used to set the indication IUT, was 1.69 MoM  
13 in the IVIg group and 1.84 in the non-IVIg group (adjusted estimated mean difference -  
14 0.3 MoM, CI -0.6 to 0 MoM,  $P=.043$ ). In both treatment groups, patients received 0.8  
15 MCA Doppler measurements per week, or one MCA Doppler measurement every 8 to 9  
16 days (adjusted estimated mean difference 0.1 measurement/week, CI -0.2-0.3,  $P=.537$ ).

17

18 In both treatment groups, three first transfusions were performed intraperitoneally  
19 without intravascular access. For 8 non-IVIg patients the degree of anemia was  
20 measured in hematocrit only. Consequently, a fetal Hb measurement was available for  
21 20/23 IVIg (and IUT) treated and 14/28 non-IVIg pregnancies. At the time of first IUT,  
22 fetuses in both groups had similar Hb levels: 6.4 SD's below the mean for gestational age  
23 in the IVIg group and 7.6 SD's in the non-IVIg group (adjusted estimated mean  
24 difference 0.9 SDs less deviation from the mean in the IVIg group, CI -0.4 to 2.3 SDs,



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

6

### 7 *Sensitivity analyses*

8 The results of the sensitivity analyses are shown in supplemental table 1. The estimated  
9 mean differences of the difference ('delta') in gestational age at onset of anemia in the  
10 index and previous pregnancy between treatment groups were all in favor of IVIg  
11 treatment, although not statistically significant. The largest raw and adjusted effect sizes  
12 were seen in patients with D antibodies only, where fetal anemia occurred on average  
13 18 days later in IVIg treated pregnancies, compared to previous pregnancies, and 14  
14 days earlier in pregnancies from women not treated with IVIg, compared to previous  
15 pregnancies (adjusted estimated mean difference between groups 10 days, 95% CI -6 to  
16 + 26,  $P=.202$ ).

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

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

25

**1 Comment**

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

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

17  
18 Our international collaborative study is the first study in the past two decades on the  
19 effect of IVIg in severe HDFN, comparing fetuses from IVIg treated pregnancies with  
20 non-IVIg treated pregnancies. The only comparable study was published in 1997, when  
21 monitoring of fetal anemia was entirely different, and mainly based on serial  
22 amniocentesis for bilirubin levels.<sup>10</sup> Nevertheless, in an IVIg-treated group of 30  
23 severely alloimmunized pregnancies, they found less hydrops, less fetal death and a  
24 mean delay in the need for intrauterine treatment of 1,5 weeks, compared to 39

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

3  
4 The positive effect of IVIg treatment on the time of onset of severe fetal anemia was not  
5 statistically significant. However, the point estimates of all outcomes point in the same  
6 positive direction, supporting a potential clinically relevant and beneficial effect of IVIg  
7 on the course of HDFN. For example, IVIg was associated with less fetal hydrops and a  
8 lesser need for neonatal exchange transfusions compared to treatment without IVIg.

9 Furthermore, early IVIg treatment appeared to delay the onset of severe anemia with  
10 3.5 weeks compared to previous pregnancies (unadjusted data, supplemental table 1).  
11 We hypothesize that the effect of IVIg on time of onset of fetal anemia may have been  
12 underestimated. Although fetuses in both groups were monitored with equal intervals,  
13 those in the IVIg group might have been monitored more thoroughly, as decisive  
14 Doppler measurements before IUT were lower than compared to pregnancies without  
15 IVIg. This may reflect an earlier suspicion of severe fetal anemia and intervention with  
16 IUT in IVIg treated pregnancies compared to non-IVIg treated pregnancies. A more  
17 expectant management in the IVIg group could have resulted in longer recorded time to  
18 onset of anemia.

19  
20 Mothers in the IVIg group seemed to have a history of more aggressive course of disease  
21 (based on the onset of anemia and the number of previous deaths), however we found  
22 strikingly less hydrops in currently IVIg-treated pregnancies. This is in accordance with  
23 the study of Voto et al.<sup>10</sup> Although in our study, there may be an effect of relatively early  
24 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.<sup>22</sup>

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.<sup>23,24</sup> 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.<sup>25</sup>

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

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

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

11

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

20 Another limitation is that the definition of 'onset of severe fetal anemia' is relatively  
21 broad. Patients are not monitored daily and anemia may be present a few days before  
22 their scheduled appointment. Although this can never be fully circumvented, we did  
23 perform a sensitivity analysis without patients in which this uncertainty was larger than  
24 a few days (for example, fetal death noticed at a scheduled appointment). The result of  
25 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.<sup>27</sup>

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.

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- 24



**Table 1.** Patient demographics and baseline characteristics

Characteristic	Unadjusted data			Weighted data <sup>a</sup>		
	IVIg group	Non-IVIg group	SMD	IVIg group <sup>b</sup>	Non-IVIg group <sup>b</sup>	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 <sup>c</sup>	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						
D	19 (79)	19 (68)	-26	20 (87)	19 (74)	-29
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 <sup>d</sup>	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.

<sup>a</sup>Weighted by the propensity score.

<sup>b</sup>For variables expressed in N (%), the weighted N was calculated from the weighted proportions and rounded.

<sup>c</sup>If no death occurred.

<sup>d</sup>Amount 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 <sup>a</sup>	<i>P</i>	IVIg group <sup>b</sup>	Non-IVIg group <sup>b</sup>	Effect size <sup>a</sup>	<i>P</i>
	N=24	N=28			N=24	N=26		
Delta gestational age, days <sup>c</sup>	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 <sup>d</sup>	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) <sup>e</sup>			0	0		
Intrauterine infection	0	0			0	0		
Emergency cesarean section	1 (5)	0			0 (1.5)	0		
Fetal death	0	4 (15) <sup>e</sup>			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.

<sup>a</sup>Expressed in estimated mean difference (95% CI) for numerical outcomes or OR (95% CI) for proportions.

<sup>b</sup>For variables expressed in N (%), the weighted N was calculated from the weighted proportions and rounded.

<sup>c</sup>Difference in gestational age at onset of severe anemia between index and previous pregnancy.

<sup>d</sup>If no death occurred.

<sup>e</sup>PROM 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.



