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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 to140 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

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

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

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

Strengths of this study were the relatively large dataset, obtained through international 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.

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

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

Conclusion

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

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Table 1. Patient demographics and baseline characteristics

	Unadjusted data			Weighted data[a]		
Characteristic	**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.

[a]Weighted by the propensity score.

[b]For variables expressed in N (%), the weighted N was calculated from the weighted proportions and rounded.

[c]If no death occurred.

[d]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

	Unadjusted				Propensity analysis			
Outcome	**IVIg group**	**Non-IVIg group**	**Effect size[a]**	***P***	**IVIg group[b]**	**Non-IVIg group[b]**	**Effect size[a]**	***P***
	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.

[a]Expressed in estimated mean difference (95% CI) for numerical outcomes or OR (95% CI) for proportions.

[b]For variables expressed in N (%), the weighted N was calculated from the weighted proportions and rounded.

[c]Difference in gestational age at onset of severe anemia between index and previous pregnancy.

[d]If no death occurred.

[e]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.