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TITLE PAGE

1) Title of the article

Effect of intravenous immunoglobulins to postpone the gestational age of first intrauterine transfusion in very severe red blood cell alloimmunization: A case-control study

2) RUNNING HEAD

Antenatal immunoglobulins to postpone IUT

3) AUTHORS

Emeline Maisonneuve ^{1,2}, Anaïs Dugas ¹, Stéphanie Friszer ^{1,2}, Cécile Toly-Ndour ³, Laura Cariot ¹, Ferdinand Dhombres ^{1,4}, Anne Cortey ^{1,2}, Agnès Mailloux ³, Bruno Carbonne ⁵, Jean-Marie Jouannic ^{1,2,4}

4) DEPARTMENTS AND INSTITUTIONS

¹ Fetal Medicine department, Armand-Trousseau Hospital, 26, avenue du Dr Arnold netter, 75012 Paris, France

² Clinical Unit of CNRHP: Centre National de Référence en Hémobiologie Périnatale, Armand-Trousseau Hospital, 26, avenue du Dr Arnold netter, 75012 Paris, France

³ Biological Unit of CNRHP: Centre National de Référence en Hémobiologie Périnatale, Saint-Antoine Hospital, 184 rue du Faubourg Saint-Antoine, Paris, France

⁴ Sorbonne Université, Paris, France

⁵ Obstetrics and Gynecology Department, Princesse Grace Hospital, 1, avenue Pasteur, 98000 Monaco

5) CORRESPONDING AUTHOR

Emeline Maisonneuve, MD

Fetal Medicine, Armand-Trousseau Hospital

26, avenue du Dr Arnold Netter

75012 PARIS

FRANCE

+33 7 60 72 25 71, fax: +33 1 44 73 63 82

emelinem@yahoo.com

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Effect of intravenous immunoglobulins to postpone the gestational age of first intrauterine transfusion in very severe red blood cell alloimmunization: A case-control study

ABSTRACT (243 words)

BACKGROUND

Early intrauterine transfusion (IUT) is associated with a higher risk of fetal loss. Our objective was to evaluate the efficiency of intravenous immunoglobulins (IVIg) to postpone the gestational age at first IUT beyond 20 weeks of gestation (WG) compared to the previous pregnancy in case of very severe red blood cell (RBC) alloimmunization.

STUDY DESIGN AND METHODS

Very severe RBC alloimmunization was defined by a high titer of antibodies and a previous pregnancy complicated by a first IUT before 24 WG and/or perinatal death directly related to alloimmunization.

We performed a single-center case-control study. Cases and controls were patients respectively treated with weekly IVIg infusions started before 13 WG, and without.

RESULTS

Twenty cases and 21 controls were included. Gestational age (GA) at first IUT was postponed after 20 WG in 18/20 (90%) of patients treated with IVIg and in 15/21 (71%) in the control group ($p=0.24$). Compared to the previous pregnancy, the GA at first IUT was postponed by a median of 22 [+11; +49] days in the IVIg group and occurred in average 2 days earlier [-17 ; +12] in the non-treated group ($p=0.02$). There was no difference between number of IUT and need for exchange-transfusion. IVIg treatment was associated with a significant decrease of antibodies' quantitation.

CONCLUSION

In our series, IVIG tends to differ first IUT beyond 20 WG and have a significant effect in postponing the gestational age of the first IUT in patients with very severe RBC alloimmunization.

KEY WORDS

Very severe RBC alloimmunization

Fetal anemia

Intrauterine transfusion

Intravenous Immunoglobulins

Hemolytic Disease of the Fetus and Newborn

TEXT (2926 words / 3500)

INTRODUCTION

Intrauterine transfusion (IUT) is currently the only treatment option for fetal anemia complicating severe red blood cell (RBC) alloimmunization in pregnancy. However, IUT is associated with procedure-related complications, such as fetal distress leading to premature delivery or perinatal death. The rate of fetal complications is estimated to be 2.7% per procedure and 7.8% per fetus. [1] Despite improved ultrasonographic resolution, the puncture of umbilical vessels before 20-22 weeks of gestation can be technically challenging and a 9 to 10 fold increased risk of fetal loss per IUT has been reported when IUT is performed before 20 weeks of gestation (WG). [2-4] The study of Zwiers *et al.* is the largest during the last two decades assessing the effect of Intravenous Immunoglobulins (IVIG) on the possibility to postpone the first IUT after 20 weeks. [5] They compared 24 pregnancies treated with IVIG to 28 pregnancies without treatment. They found an adjusted 4-day between-group difference in favour of the IVIG group, but it was not significant. In addition, a metaanalysis has concluded that no information is currently available from randomized trials to indicate whether the antenatal use of IVIG is effective in the management of severe fetal RBC alloimmunization. [6] Since 2012, our center offers IVIG to patients with very severe alloimmunization, i.e. with a very high level of red cells antibodies at the beginning of pregnancy and a history of IUT before 24 WG during the previous pregnancy or perinatal death directly related to alloimmunization. [7]

Our primary objective was to assess if IVIG could postpone the gestational age (GA) of first IUT beyond 20 WG as compared with the previous pregnancy without IVIG

administration, or possibly allow to avoid IUT until 36 WG. Our secondary objectives were to compare the number of days gained before first IUT, perinatal outcomes (live birth rates after 28 WG and 32 WG), the number of fetal and total transfusions, and the evolution of antibodies' quantitation between patients treated with and without IVIG respectively.

MATERIALS AND METHODS

Patients

The perinatal data of patients with severe RBC alloimmunization managed between 2005 and 2020 in our French referral center (CNRHP: *Centre National de Référence en Hémiobiologie Périnatale*) were retrospectively reviewed. The inclusion criteria were patients who delivered between January 2005 and December 2020 in our center, presenting very severe RBC alloimmunization. Very severe alloimmunization was defined as anti-D (RH1) antibody level above 15 IU/mL or anti-c (RH4) above 20 IU/ml or anti-Kell (KEL1) antibody titer ≥ 256 and at least one of the following: previous pregnancy marked by IUT performed before 24 WG; intrauterine fetal death (IUFD) or neonatal death directly related to alloimmunization. [5, 8] The exclusion criteria were: chromosomal anomalies or congenital malformations, multiple pregnancies and inappropriate follow-up at previous pregnancy.

Management of patients with very severe RBC alloimmunization in our center

Maternal-fetal incompatibility was assessed respectively by non-invasive fetal *RHD*, *KEL1* or *RHc* genotyping in case of anti-D (RH1) (Jacques Boy, France), anti-Kell (KEL1) or anti-c (in-house CNRHP test) alloimmunization. [9, 10] Antibodies' titration was performed by a standardized tube procedure, in saline medium and with the

indirect antiglobulin test using an anti-IgG reagent, RH:1,2,3,4,5 (DCCeEe positive) RBC for anti-D and anti-c antibodies and KEL:1,2 (heterozygous K positive) RBC for anti-Kell antibody. In addition to titration by indirect antiglobulin test, anti-D and anti-c quantitation by continuous-flow hemagglutination was performed on Astoria White Horse Scientific or AMS Alliance Evolution III autoanalyzer, using RH:1,2,3,4,5 (DCCeEe positive) RBC to assess for a reactivation of maternal immunization with more accuracy. [11] Maternal blood sampling was collected every 2 weeks, just before starting weekly immunoglobulin infusion.

Since 2012, IVIG have been offered in our center to patients with the aim to postpone or avoid the first IUT in cases with very severe RBC alloimmunization. IVIG were started from 11 WG with a regimen of 1g/kg weekly. Our protocol for IVIG includes an infusion with an initial flow ≤ 1 mL/kg/hour during 30 minutes. If the medication is well tolerated, the flow is increased gradually with a maximum of 4 mL/kg/hour. The IVIG used in this study were: TEGELINE (50mg/mL) from 2012 to 2015, and CLAIRYG (50mg/mL) from 2015 to 2020. In case of body mass index over 30 kg/m², the therapeutic dose of IVIG for immunomodulation was reduced from 20% to avoid renal complication related to increase of oncotic pressure and blood viscosity. [12] IVIG were continued throughout the pregnancy, until the first IUT. If there was no sign of fetal anemia during pregnancy, IVIG were continued until delivery. The patients (cases and controls) did not receive other treatment, such as plasmapheresis or corticosteroids.

Middle Cerebral Artery - Peak Systolic Velocity (MCA-PSV) measurements were performed using Voluson E8 or Voluson 730 Expert (General Electrics, Zipf, Austria) or Applio (Toshiba Medical Systems, Tokyo, Japan). Each MCA Doppler measurement was interpreted as multiples of median (MoM) for gestational age. [13,

14] The decision to perform fetal blood sampling, immediately followed by IUT in most cases, was made on the observation of a sharp increase in MCA-PSV above 1.5 MoM, confirmed by a second ultrasound examination within 1-2 days. For the second IUT, we used the MCA-PSV values in association with predictable decrease of haemoglobin concentration, and performed the MCA-PSV weekly. Then, for the following IUTs, we used only the predictable fall of haemoglobin and performed MCA-PSV measurements before and after IUT as previously reported. [14] In our center, IUTs were performed from 17 WG if moderate to severe fetal anemia was suspected. [15] Severely anemic fetuses were transfused with adult donor packed red blood cells, cross matched with the mother, with a hematocrit between 70 and 80%. During both periods, the patients were monitored equally often and thoroughly and there was no changes in policy and procedures related to decisions to proceed with a IUT. Delivery was scheduled at 37 WG, by induction of labour or elective caesarean section, according to obstetrical conditions.

Data collection

For each patient, data of previous and index pregnancies were collected: GA at first IUT, evolution of antibodies quantitation, number of IUTs and transfusions after birth, IUFD after IUT, rates of live births after 28 WG, and 32 WG, hemoglobin on fetal blood sampling at first IUT and on umbilical cord at birth, rates of exchange-transfusion, side effects of IVIG. The decrease in antibodies' quantitation (between quantitation at first trimester and quantitation at first IUT or at the end of pregnancy if no IUT had occurred) was calculated for anti-D (RH1) and c (RH4) alloimmunizations.

Study design and statistical analysis

We conducted a single center case-control study. The group of cases consisted of patients treated with weekly IVIG infusions for very severe RBC alloimmunization and who delivered between 2012 and 2020. Our control group consisted of patients not treated by IVIG who delivered between 2005 and 2012 and one patient followed in 2019 who declined IVIG treatment.

We compared the number of pregnancies in which an IUT was performed beyond 20 weeks. In addition, we calculated for each patient the difference between GA at index and previous pregnancies (in days). If a patient had 2 pregnancies treated with IVIG, these two pregnancies with IVIG were compared with the control pregnancy without IVIG. Other secondary outcomes were also compared between cases and controls: live births beyond 28 WG, beyond 32 WG, and the number of total transfusions (*in utero* and postnatal transfusions), and need for exchange transfusion. One of the secondary outcomes was a composite outcome including: postponing the GA at first IUT of more than two weeks as compared with the previous pregnancy without IVIG, and a live birth after 28 WG or no need for IUT until 36 WG.

Bivariate analyses of significant effects were based on Chi-square test and Fisher's exact test to compare categorical variables; Fisher Exact Test was used when the assumptions of the χ^2 test were violated. The associations between IVIG treatment and the outcomes were studied by bivariate analyses that used generalized estimated equations to take the non-independence of observations for pregnancies from the same patient into account (3 patients had 2 pregnancies in the control group and 4 patients had 2 pregnancies in the cases group). Continuous variables were compared with Wilcoxon Rank Sum tests. *P values* were used to estimate the strength of association with each parameter. Statistical significance was set at $p < 0.05$. Statistical analyses were performed with R 3.5.1 statistical software.

Ethics

Subjects gave their informed consent and the study protocol was approved by the national committee in obstetrics and gynecology research (CEROG 2019-OBS-1205).

Results

Among the 24 patients treated with IVIG for RBC alloimmunization, 20 patients fulfilled the inclusion criteria. All these pregnancies were D, K or c maternal-fetal incompatible. We excluded 4 patients from the analysis : one patient had a history of IUFD with signs of fetal anemia and hydrops at 38 WG related to severe anti-D alloimmunization, but her previous follow-up was inappropriate (no weekly Doppler examination) ; one patient started IVIG at 11 WG and had a miscarriage at 13 WG ; one intrauterine fetal death occurred at 16 WG, without sign of fetal anemia at ultrasound and without etiology found after thorough evaluation including placental and fetal pathology, extensive biological and bacteriological tests and Kleihauer-Betke test ; one patient had a first IUT at 21 WG (hemoglobin increased from 5.2 to 11.2 g/dL) and then she asked for a termination of pregnancy for Down syndrome diagnosed on fetal blood sampling. The 20 patients treated with IVIG were compared with 21 controls (Figure 1). All the baseline obstetrical and immuno-hematological characteristics were comparable in both groups (Table 1). The fathers were the same in pregnancies in both groups. Only antibodies' quantitation at the beginning of the index pregnancy tended to be higher in the IVIG group: 100 [72 - 128] IU/mL and 48 [26 - 70] in the control group ($p=0.09$).

Among all the patients presenting with very severe RBC alloimmunization, 33/41 pregnancies resulted in a live birth (80%). Concerning the primary outcome,

gestational age (GA) at first IUT was postponed after 20 WG in 18/20 (90%) of patients treated with IVIG and in 15/21 (71%) in the control group ($p=0.24$). GA at first IUT was postponed of more than 14 days resulting in a live birth after 28 WG in 63% (10/16) of patients treated with IVIG and in 18% (2/11) of the control group ($p=0.03$) (Table 2). Similarly, the GA at first IUT was postponed by a median of 22 [+11; +49] days in the IVIG group while it occurred in average 2 days earlier [-17 ; +12] in the non-treated group ($p=0.02$) (Figure 2 and table 3). There was no statistical difference between rates of live births after 28 WG (80% with IVIG vs 81% without) and 32 WG (75% with IVIG vs 76% without). The antibodies' quantitation was significantly decreased in pregnancies treated with IVIG (-43% with IVIG vs +35% without, $p < 0.01$). Apart from three patients (15%) presenting with moderate headache after IVIG first infusion, no severe side effects were observed.

Among the 20 patients treated, two patients had no evidence of fetal anemia during the entire pregnancy with IVIG, whereas their fetuses had presented with very severe HDFN in previous pregnancies. The first patient with anti-D (RH1) alloimmunization had 4 IUTs during the previous pregnancy (23, 25, 28 and 32 WG) and the child required 2 postnatal transfusions. For the index pregnancy, no IUTs were needed. Induction of labor was decided at 36 WG for preeclampsia. Her child required only one postnatal transfusion at day 26. The second patient presented with anti-c (RH4) alloimmunization with a history of intrauterine fetal death with hydrops at 32 weeks during the first pregnancy. At her second pregnancy, an IUT was performed at 21 WG for severe fetal anemia (hemoglobin at 5.0 g/dL without hydrops) and it was complicated by IUFD the following day. The index pregnancy treated with IVIG was uneventful. Labor was induced at 37 WG and her child required one postnatal transfusion at day 14.

Discussion

In this case-control study, we found that IVIG treatment significantly delayed the onset of severe fetal anemia and the need for IUT in patients with very severe RBC alloimmunization. In addition, two fetuses of patients treated with IVIG did not require any IUT during the index pregnancies, whereas they had required very early IUT during a previous pregnancy.

In spite of this delay, we did not observe significant difference in the proportion of first IUT after 20 WG in 18/20 (90%) of patients treated with IVIG as compared to 15/21 (71%) in the control group ($p=0.24$).

The strength of our study relies on our single center homogenous management of patients with very severe alloimmunization in which we perform approximately 70 IUT per year and 40% of the IUT in France. [16] Moreover, the protocol of IVIG infusions was exactly the same for all patients with an early homogenous GA at onset of 11-13 WG and duration of treatment until first IUT or delivery if no IUT was needed. The main limitation of the study is the small number of patients, since this is a very rare condition. Nevertheless, although the results of our study involve few patients, they are of utmost importance since we included only very severe HDFN cases with a high rate of poor outcome. Indeed, an additional 2 weeks offers a significant improvement in the technical conditions of IUT performance, with a larger umbilical cord and increased fetal cardiac tolerance.

The use of IVIG in severe RBC alloimmunization was introduced three decades ago following the publication of small case-series. [17-19] These studies found a

beneficial effect on reducing the severity of fetal hemolytic disease, but IVIG were started during the second or third trimester, with heterogeneous regimen. Moreover, these studies were performed before the use of MCA-PSV Doppler and thus do not correspond to the current standard of care. [13] Our results are comparable with those of the multicenter retrospective study conducted by Zwiers *et al.* when taking into account only patients treated with IVIG started before 13 WG. We used the same definition of very severe alloimmunization and criteria for IVIG treatment, in particular the history of first IUT before 24 WG. [5] The main limitation of their study is the heterogeneity of the IVIG-group, with IVIG infusions started at different GA, variable IVIG dose and frequency regimen, and sometimes a combination with plasmapheresis. They considered the "onset of fetal anemia" as primary outcome, with no clear definition of this outcome. In our study, we preferred to compare the GA of first IUT, because this outcome is more accurate and pragmatic. Indeed, this primary outcome proved to be reliable in our study since hemoglobin levels at first IUT were similar in both groups.

In their study, Zwiers *et al.* showed a significant effect of IVIG to postpone the GA of first IUT only when they considered the subcohort in which immunoglobulin treatment was started before 13 weeks. We believe that it is important to start IVIG at 11-13 WG and to continue weekly infusions until the first IUT, because IVIG have multiple actions in order to reduce the immune conflict. They reduce the production of maternal antibodies, shorten their half-life, act on their transplacental transfer and saturate the reticuloendothelial system in order to curb the destruction of the fetus' red cells. [20] We monitored patients' antibodies every 2 weeks and noticed that their concentrations decreased gradually under IVIG, which is in favour of an effect of IVIG on maternal antibodies, which could reduce the transplacental transfer of maternal

antibodies. In the two cases with no IUT during index pregnancy, antibodies quantitation dropped by more than half with IVIG.

We chose the composite outcome "postponing GA of first IUT of more than two weeks resulting in a live birth after 28 WG" as a secondary outcome, because considering only GA of first IUT could be considered as a surrogate marker. [4] On the one hand, the pathophysiological effect of this non-invasive treatment has to be demonstrated in a significant offset of the first IUT. On the other hand, survival without morbidity is the most meaningful outcome and is what is useful for patients and physicians.

Yinon *et al.* calculated the number of patients needed to demonstrate a positive effect of IVIG in survival. They found that, in order to show a 50% reduction in perinatal mortality from 20 to 10% with a power of 80%, 438 patients should be recruited, which remains challenging even in a multicentre study. Therefore, management of early severe RBC alloimmunization will continue to be based on small case series. [3] Even if they were pooled in a meta-analysis, all data remain retrospective. However, given the very elevated cost of IVIG and its human plasmatic origin, a well-designed prospective study seems necessary to assess the effect of IVIG in postponing the GA of first IUT as well as increasing perinatal survival. Adverse effects may occur with IVIG, such as headache, migraines, flushing or nausea. Besides, a novel strategy to inhibit transplacental alloantibody transfer with maternal M281 monoclonal antibody therapy is under evaluation but has not yet been tested on pregnant women. [21]

CONCLUSION

Among patients with very severe RBC alloimmunization, we found that IVIG tend to differ first IUT beyond 20 WG and have a significant effect in postponing the gestational age of the first IUT of more than 2 weeks and resulting in a live birth after 28 WG. Moreover, in some rare cases, IVIG treatment allows to avoid IUT completely during pregnancy. Monitoring antibodies quantitation may be a predictor of treatment efficiency.

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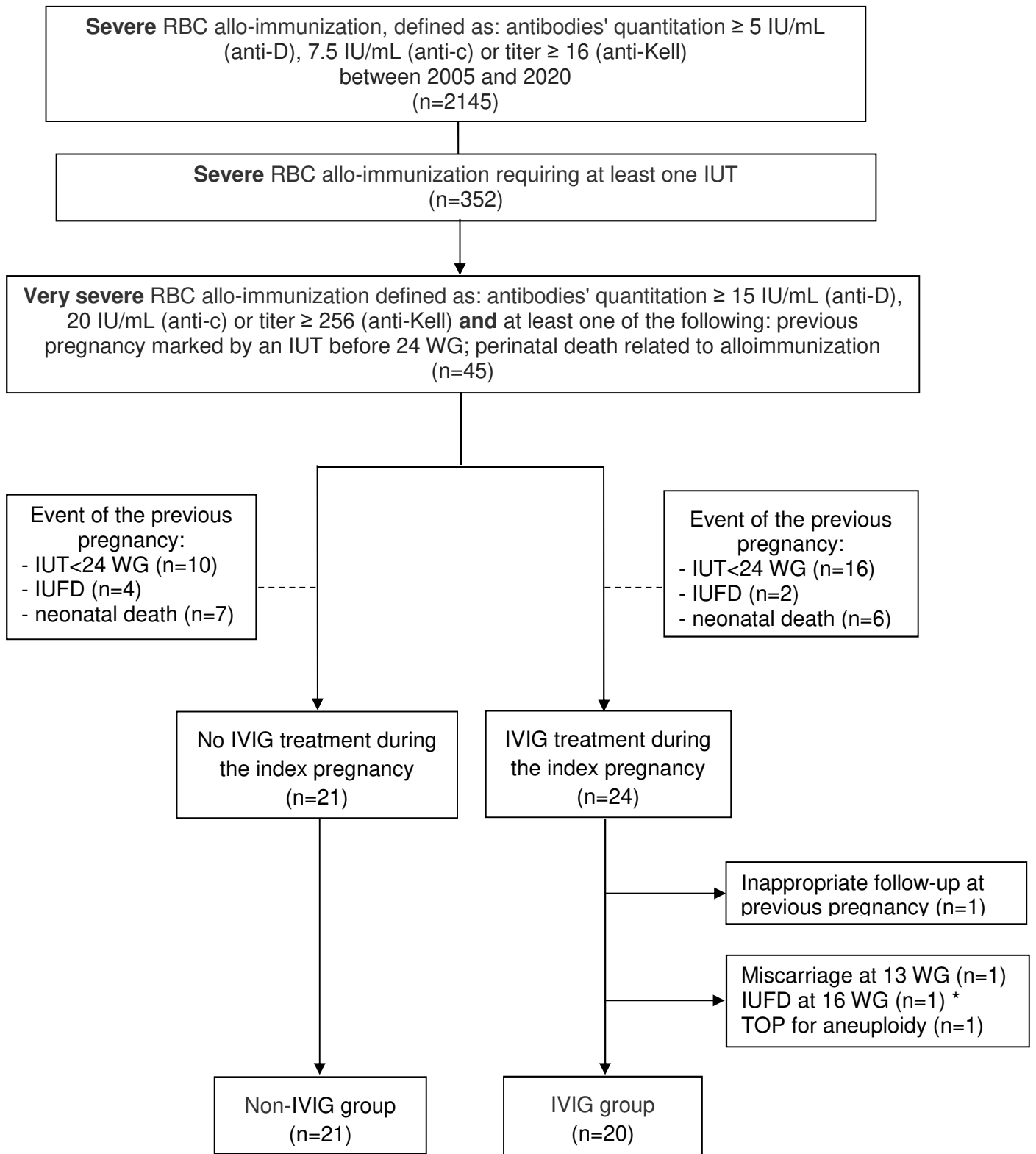


Figure 1: Flowchart of the study

Legend:

IUFD: intrauterine fetal death; IUT: intrauterine transfusion; IVIG: Intravenous immunoglobulins; RBC: red blood cells; TOP: termination of pregnancy; WG: weeks of gestation

* without signs of fetal anemia at ultrasound and without etiology found after thorough evaluation including placental and fetal pathology.

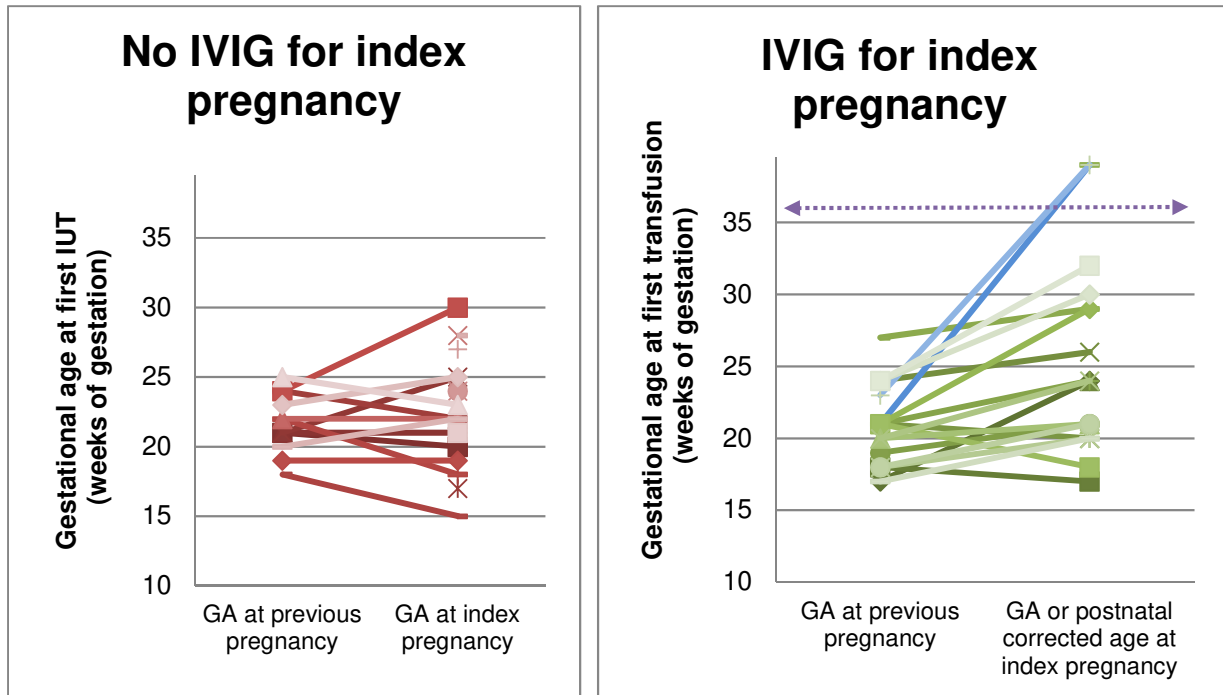


Figure 2: Gestational age at first intrauterine transfusion in previous and index pregnancies in patients according to the intravenous immunoglobulins (IVIG) treatment at index pregnancy

Legend:

GA: Gestational age, IVIG: intravenous immunoglobulins

The blue lines show the two patients for whom no intrauterine transfusion was needed.

The dotted line ($\leftarrow \cdots \rightarrow$) indicates the birth for the index pregnancies without intrauterine transfusion, but with postnatal transfusion.

	Non-IVIG group N=21	IVIG group N=20	p	
Maternal and obstetric characteristics				
Maternal age at index pregnancy	34 [30-38]	36 [32-40]	0.79	
History of personal RBC transfusion	0/21 (0%)	2/20 (10%)	0.23	
Gestivity	4 [3-5]	5 [3-7]	0.76	
Parity	2 [1-3]	2 [1-3]	0.93	
GA at the time of first IUT at previous pregnancy	21 WG ^{5/7} [20 WG ^{3/7} - 23 WG ^{0/7}]	21 WG ^{0/7} [19 WG ^{2/7} - 22 WG ^{5/7}]	0.18	
Hemoglobin at first IUT (g/dL)	5.0 [2.8-7.2]	4.2 [3.8-5.0]	0.59	
MCA-PSV at first IUT (MoM)	1.89 [1.62-2.16]	2.0 [1.8-2.2]	0.92	
GA at the time of first event at previous pregnancy (IUFD or IUT)	22 WG ^{0/7} [20 WG ^{2/7} - 23 WG ^{5/7}]	21 WG ^{0/7} [19 WG ^{2/7} - 22 WG ^{5/7}]	0.45	
History of perinatal death	15/20 (75%)	17/20 (85%)	0.69	
Time between pregnancies (years)	2.0 [1.25-2.75]	1.25 [0.75-1.75]	0.18	
Immuno-hematologic characteristics				
Antibodies' type	Anti-D	15 (71%)	16 (80%)	0.84
	Anti-Kell	5 (24%)	3 (15%)	
	Anti-c	1 (5%)	1 (5%)	
Titer at the beginning of pregnancy *	>256	>256	0.19	
Quantitation at the beginning of pregnancy (IU/mL) **	48 [26-70]	100 [72-128]	0.09	

Table 1: Baseline characteristics of the 2 groups treated or not with intravenous immunoglobulins (IVIG)

GA: gestational age; IUFD: intrauterine fetal death; IQR: interquartile range; MoM : Multiples of Median; RBC: red blood cells; WG : weeks of gestation. Data are expressed in median [Interquartile Range]. Antibodies quantitations are expressed in IU/mL.

* Titrations were performed for all types of antibodies

** Quantitations were performed only for anti-D (RH1) and anti-c (RH4).

	Non-IVIG group (n=21)	IVIG group (n=20)	P
Timing of IUT at index pregnancy			
First IUT \geq 20 WG	15/21 (71%)	18/20 (90%)	0.24
Postponing first transfusion \geq 2 weeks compared to the previous pregnancy	2/11 (18%)	11/16 (69%)	0.02
GA at index pregnancy - GA at previous pregnancy (days)	-2 [-17 ; +12]	+ 22 [+11 ; +49]	0.02
Postponing first transfusion \geq 2 weeks compared to previous pregnancy AND live birth \geq 28 WG	2/11 (18%)	10/16 (63%)	0.03
GA at first IUT when needed*	22 WG ^{3/7} [19 WG ^{6/7} - 25 WG ^{1/7}]	22 WG ^{5/7} [20 WG ^{1/7} - 25 WG ^{2/7}]	0.51
GA at first transfusion (in utero or postnatal) **	22 WG ^{3/7} [19 WG ^{6/7} - 25 WG ^{1/7}]	24 WG ^{0/7} [19 WG ^{4/7} - 28 WG ^{3/7}]	0.11
Number of IUT during pregnancy except pregnancy with IUFD	5 [4-6]	4 [3-5]	0.46
IUFD related to IUT	4/21 (19%)	4/20 (20%)	1
Neonatal outcomes			
GA at delivery (WG)	36.0 [35.0-37.0]	35.0 [34.0-36.0]	0.80
Live birth \geq 28 WG	17/21 (81%)	16/20 (80%)	1
Live birth \geq 32 WG	16/21 (76%)	15/20 (75%)	1
Intraventricular hemorrhage III-IV and/or periventricular leukomalacia	0 (0%)	0 (0%)	1
Bronchopulmonar dysplasia	0 (0%)	0 (0%)	1
Necrotizing enterocolitis	0 (0%)	0 (0%)	1
Live birth after 32 WG without morbidity ***	16/21 (76%)	15/20 (75%)	1
Hemolytic disease			
MCA-PSV Doppler before first IUT (MoM)	1.75 [1.55-1.85]	1.74 [1.64-1.84]	0.65
Hydrops at time of first IUT	3/16 (38%)	3/19 (16%)	0.25
Hemoglobin at first IUT	4.8 [3.3-6.3]	4.45 [1.7-7.2]	0.48
Bilirubin at first IUT (μ mol/L)	59 [39-79]	73 [63-83]	0.74

DAT at first IUT †	4 [3-4]	4 [3-4]	1
Hemoglobin at birth (g/dL)	10.7 [9.8-11.6]	12.0 [10.4-13.6]	0.52
Bilirubin at birth (µmol/L)	80 [56-104]	71 [44-96]	0.47
Exchange transfusion ††	3/15 (20%)	1/16 (6%)	0.33
Number of postnatal transfusions	2 [2-2]	2 [1-3]	0.58
Number of total transfusions (in utero and postnatal)	6 [5-7]	6 [4-8]	0.91
Biologic parameters			
Evolution of antibodies' quantitation (median)	+35% [+19 ; +336]	- 43% [-51 ; -37]	0.0008
Reactivation of immunization †††	6/12 (21%)	2/16 (7%)	0.04
Side effects of IVIG ‡	-	3/20 (15%)	-

Table 2: Comparison of the severity of the hemolytic disease of the fetus and newborn and outcomes in both groups

GA: gestational age ; IUFD: intrauterine fetal death ; IUT: intrauterine transfusion ; IQR : interquartile range ; MCA-PSV : Middle Cerebral Artery - Peak Systolic Velocity ; WG weeks of gestation ; IVIG : intravenous immunoglobulins

Data are expressed in median [Interquartile Range].

* without considering the 2 patients whose fetus did not receive intrauterine transfusion during the index pregnancy treated with IVIG.

** considering the 2 patients whose fetus did not receive intrauterine transfusion during the index pregnancy treated with IVIG, but whose children received neonatal transfusion

*** Morbidity related to preterm birth include: intraventricular hemorrhage III-IV and/or periventricular leukomalacia, bronchopulmonar dysplasia and necrotizing enterocolitis.

† DAT: Direct Antiglobulin Test (Coombs) with intensity between 0 and 4+

†† Exchange transfusion consists in removal of the patient blood (full of bilirubin) and replacing it by donor blood (a mix of packed red blood cell and fresh frozen plasma) up to 2 blood mass. It is indicated when bilirubinemia reaches threshold defined by gestational and post-natal age in hours provided by the American Academy of Pediatrics.

††† Reactivation of alloimmunization is an increase of the antibodies quantitation higher than 30% between two subsequent measurements.

‡ Three patients presented headache after IVIG first infusion only.

The associations between IVIG treatment and the outcomes were studied by bivariate analyses that used generalized estimated equations to take the non-independence of observations for pregnancies from the same patient into account.

		Previous pregnancy		Index pregnancy		
Patient	Anti-bodies	Event	Gestational age at event	Number of IgIV infusions	Gestational age at first IUT	Hemoglobin (g/dL)
1	D	Neonatal death	NA	No	21 WG + 2	8.7
2	D	IUT	21 WG + 2	No	20 WG + 0	5.6
3	Kell	IUT and TOP	21 WG + 0	No	17 WG + 5	9.6
4	D+C	IUT	21 WG + 3	No	25 WG + 0	6.4
5	D	IUFD	NA	No	17 WG + 0	IPT
6	D	Neonatal death	24 WG + 0	No	22 WG + 3	4.3
7	Kell	Neonatal death	NA	No	18 WG + 5	1.3 (IPT)
8	Kell	IUT †	18 WG + 5	No	15 WG + 5	IPT
9	D	IUT	22 WG + 6	No	18 WG + 0	4.8
10	D+E	IUT	18 WG + 0	No	19 WG + 3	2.3
11	D	IUT	24 WG + 0	No	30 WG + 0	7.0
12	D	IUT	22 WG + 0	No	22 WG + 6	4.8
13	D	Neonatal death	NA	No	28 WG + 0	6.4
14	Kell	IUFD	NA	No	24 WG + 0	7.5
15	c	Neonatal death	NA	No	24 WG + 5	2.2
16	D	IUFD	NA	No	27 WG + 2	3.5
17	D	IUFD	NA	No	28 WG + 3	3.4
18	D+C+S	IUT †	20 WG + 0	No	21 WG + 5	3.4
19	D	IUT †	23 WG + 2	No	25 WG + 1	2.6
20	D+C	Neonatal death	C/S at 34 WG	No	21 WG + 5	3.8
21	Kell	IUT Neonatal death	25 WG + 4 C/S at 26 WG	No	23 WG + 2	5.0
22	Kell	IUFD	17 WG	8	24 WG + 4	3.9
23	D+C+G	IUT	18 WG + 0	5	17 WG + 0	IPT
24	D	Neonatal death	C/S at 34 WG	8	24 WG + 0	5.0
25	D	IUT †	24 WG + 0	9	26 WG + 0	9.0
26	D+C	IUT	21 WG + 5	9	20 WG + 0	3.5
27	D+G	IUT †	19 WG + 6	10	21 WG + 4	3.3
28	D+C	IUT	21 WG + 0	11	23 WG + 6	6.0
29	Kell	IUFD	27 WG + 0	18	29 WG + 5	9.9
30	c	IUT †	21 WG + 1	25	<i>No IUT / transfusion at DOL 14</i>	
31	D+C+S	IUT	21 WG + 5	16	29 WG + 3	9.1
32	D+C+S	IUT	21 WG + 5	6	18 WG + 2	2.6 (IPT)
33	D	IUT	20 WG + 0	11	21 WG + 4	3.8 (IPT)
34	D	IUT	20 WG + 0	13	24 WG + 4	3.8
35	D	IUT	18 WG + 1	8	20 WG + 1	IPT
36	D+s	IUT †	18 WG + 0	8	21 WG + 3	3.2
37	D	IUT	23 WG + 0	24	<i>No IUT / transfusion at DOL 24</i>	
38	D+C+E	Neonatal death	C/S at 32 WG	8	20 WG + 4	7.3
39	Kell	IUT †	17 WG + 0	9	20 WG + 3	2.6
40	D+C	IUT	24 WG	15	30 WG + 5	9.0
41	D+C	IUT	24 WG	21	32 WG + 0	9.0

Table 3: Description of first intrauterine transfusions at previous and index pregnancies according to IVIG treatment

Fetuses were tested by non-invasive fetal *RHD*, *KEL1* or *RHc* genotyping in case of anti-D (RH1), anti-Kell (KEL1) or anti-c immunization respectively. At first intravascular IUT, they were also tested for ABO, Rh and Kell blood groups in order to confirm fetomaternal incompatibility. In addition, a direct antiglobulin test was performed on fetal blood.

In cases 18, 31 and 32, the fetuses were incompatible concerning D, C and S. In case 36, incompatibility could be confirmed only for RHD.

Legend:

C/S: cesarean section; DOL: days of life; IPT: intraperitoneal fetal transfusion; IUFD: intrauterine fetal death; IUT: intrauterine transfusion; IUT †: intrauterine transfusion followed by intrauterine fetal death; IVIG: Intravenous immunoglobulins; NA: non-available; TOP: termination of pregnancy; WG: weeks of gestation