Chapter 3

Increased perinatal loss after intrauterine transfusion for alloimmune anaemia before 20 weeks of gestation

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Abstract

Objectives*:* To evaluate and compare perinatal outcome after intrauterine transfusions (IUT) performed before and after 20 weeks of gestation. To analyse contributing factors.

Methods: Fetuses were divided into two groups: fetuses requiring the first IUT before 20 weeks of gestation (Group 1) and those in which the IUTs started after 20 weeks (Group 2). The cause of perinatal loss was classified as procedure-related (PR) or not procedure-related (NPR). The cohort was divided into two periods to describe the change of perinatal loss over time. Primairy outcome was perinatal loss of fetuses requiring the first IUT before 20 weeks of gestation, compared with perinatal loss later in gestation.

Results: A total of 1422 IUTs were performed in 491 fetuses. Perinatal loss rate in Group 1 was higher (7/29 24% versus 35/462 8%, P = 0.002). Especially NPR was higher for IUTs performed before 20 weeks (4/37 11% versus 19/1385 1%, P < 0.001). Kell alloimmunisation was overrepresented in Group 1 (7/29 24% versus 52/462 11%, P = 0.04). In a multivariate regression analysis, only hydrops was independently associated with perinatal loss (P = 0.001). In recent years, a decline in total perinatal loss was found (36/224 16% versus 6/267 2%, P < 0.001), but perinatal loss in Group 1 did not decline (4/224 1.8% versus 3/267 1.1%, P = 0.5).

Conclusions: Perinatal loss after IUT performed before 20 weeks of gestation is increased compared with loss after IUT performed later in gestation. In addition, we confirmed earlier observations that hydrops is a major contributor to adverse outcome. Early and timely detection and treatment may prevent hydrops and improve outcome.

Introduction

Maternal red cell alloimmunisation is a potential cause of perinatal morbidity and mortality. Intrauterine intravascular transfusions (IUT) have been the mainstay of treatment of fetal alloimmune anaemia since the early 1980s.[1] Formerly, alloimmune haemolytic disease was the most important cause of perinatal mortality. Nowadays, perinatal survival rates exceed 90% in experienced centres.[2-4]

However, a few small studies suggest that IUT in the early second trimester is still associated with significant mortality.[2, 4-7] This is believed to be predominantly related to more difficult access to the fetal circulation because of the smaller size of the blood vessels. Fetal hydrops is a known poor prognostic factor for survival after IUT.[5, 8, 9] To what extent fetal compromise, i.e. presence of hydrops, contributes to higher perinatal loss rates related to IUT in early gestation has not been elucidated.

The aim of our study was to assess and compare the perinatal outcome after IUTs performed before and after 20 weeks of gestation in a large cohort and to analyse contributing factors. Such knowledge is essential for the development of innovative, safer approaches for the treatment of early, severe fetal anaemia.

Methods

Data were collected from all pregnancies treated with intrauterine intravascular transfusions for fetal alloimmune anaemia from March 1987 until August 2009 at the Fetal Medicine Unit of the Department of Obstetrics of the Leiden University Medical Centre, which has been the Dutch national referral centre for fetal therapy since 1965. Our methods for the management of alloimmunisation in pregnancy and technical aspects of intravascular fetal transfusion have been described previously.[10] In summary, after administration of local anaesthesia a 20- or 22-gauge spinal needle was inserted into the fetal circulation. Our first choice is to perform transfusion into the intrahepatic vein. At anterior placenta, we usually elected to use the cord insertion site. Exceptionally in the case of intrahepatic route, we additionally transfused some blood in the intraperitoneal space. Fetal paralysis was applied in the vast majority of procedures. The volume of blood to be transfused was calculated by using the formula by Rodeck et al.[11] Haemoglobin concentrations before and after the procedure were immediately assessed in the operating room. Fetal condition was monitored by ultrasound during IUT, and by fetal heart rate tracings before and after IUT. From 1987 onwards, data on all IUTs were prospectively collected in a database.

The treated fetuses were divided into two groups: a study group of fetuses requiring the first IUT before 20 weeks of gestation (Group 1), and a control group in which treatment with IUTs started at or after 20 weeks (Group 2). The limit of 20 weeks of gestation was based on our previous study on IUT-related complications, showing the highest risk of fetal demise before 20 weeks of gestation (5.6% per procedure).[2] Baseline characteristics studied included: type of antibody, gestational age at first IUT, number of IUTs per fetus, presence of hydrops and haemoglobin level at first IUT. Standardised *Z* scores of haemoglobin (*Z*-haemoglobin) were defined as the number of standard deviations (SDs) by which the actual value deviated from the normal mean for gestational age. Reference values for fetal haemoglobin were derived from Nicolaides et al.[12]

Primary outcome variable was perinatal loss in the group requiring the first IUT before 20 weeks of gestation, compared with perinatal loss in the control group. The main secondary outcome variable was the most likely cause of fetal demise: procedure-related (PR) or not procedure-related (NPR). Perinatal loss was classified by two independent obstetricians as PR when fetal condition before transfusion was satisfactory and fetal demise occurred during or following a complicated procedure and as NPR when fetal condition before an uncomplicated procedure was compromised.[2] The assessment of a compromised fetal condition was principally based on the presence of massive hydrops and on abnormal findings of the following examinations: fetal heart tracing, biophysical profile and initial fetal pH (fetal compromise if pH ≤7.25). Classification of complicated procedures was based on the number of needle insertions, occurrence and duration of bleeding from the puncture site and deterioration of fetal condition during transfusion being assessed by fetal heart rate and blood pH.[2] In addition, we assessed perinatal loss rates per procedure performed before or after 20 weeks of gestation.

To weigh the influence of additional potential risk factors on perinatal loss after IUT, a multivariate analysis was performed using variables that were significantly different between both groups. To quantify the effect of gestational age on perinatal loss after IUT, we used the natural logarithm (log odds). Apart from the total group, log odds were also calculated for fetuses with or without hydrops at the first IUT. To describe the change of perinatal loss and survival rates over time at our centre we divided our study group into two nearly equal periods.

Statistical analysis

Data were analysed in a database using SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). Chi-square test was used for comparing nominal data. We used Student's *t* test for the comparison of haemoglobin level between groups. Logistic regression analysis was used for comparing survival rates between the different categories of gestational age. We used univariate logistic regression analysis to test the association between perinatal loss after IUT and the potential risk factors. Multiple logistic regression analysis was used to measure the independent effect of potential risk factors for perinatal loss. Natural logarithm ('log odds') was used to assess the effect of gestational age on perinatal loss after IUT. A *P* value < 0.05 was considered to indicate statistical significance in all tests.

Results

In the study period, a total of 491 fetuses in 484 pregnancies of 400 mothers were treated with 1422 intravascular IUTs for fetal alloimmune anaemia (median 3 per fetus; range 1–8). Eight twins were included with IUT of 15 of the 16 fetuses, all starting IUT after 20 weeks of gestation. The overall survival rate was 91% (449/491). The overall incidence of hydrops was 29% (140/491). Our study group (Group 1) consisted of 29 singletons (of 491 fetuses, 6%), in which IUT started before 20 weeks of gestation. The control group of 462 fetuses (Group 2, 94%), received the first IUT at or after 20 weeks. Table 1 shows the baseline characteristics for both groups.

Table 1. *Characteristics of 491 fetuses requiring first intrauterine transfusions before (Group 1) and after 20 weeks' gestation (Group 2).*

	Group 1 <20 weeks n=29	**Group 2 >=20 weeks** n=462	**p-value**	**OR (95% CI)**
Type antibodies, *n (%)*				
- D	22 (76)	381 (82)	.4	1.5 (0.6-3.9)
- Kell	7 (24)	52 (11)	.04	2.5 (0.9-6.6)
- c		21 (5)	NA	NA
- other[A]		8 (2)	NA	NA
Gestational age at first IUT, *weeks*	18+1 (16-19+6)	27+5 (20-35+4)	-	-
Hydrops at first IUT, *n (%)*	13 (45)	127 (27)	.04	2.1 (0.9-4.9)
Haemoglobin at first IUT, *g/dl*	3.5 ± 2.6 (1.3-11.9)	5.7 ± 2.2 (1.1-13.2)	< .001	3.6 (1.3-2.2)
Z-haemoglobin at first IUT*, SDs*	-7.8	-7.2	0.2	1.9 per SD decrease (0.7-3.6)
Singleton and twin fetuses, *n*				
Singleton	29	461		-
Twins	0	15[#]		-

NA= Not Applicable. Gestational age and Hb in median (range), [A] Rh(E) (n=2), Rh (e), Duffy[a], Kpa, Kidd[a], Cw, Private antigen 'Verdegaal'

[#] Fifteen of the 16 twin fetuses were treated with IUT

In Group 1, seven of the 29 fetuses died (24%), all *in utero*, of which six died before 20 weeks of gestation (22%) and one at 37 weeks. In Group 2, the perinatal mortality was 8% (35/462, *P* = 0.002), including 24 (5%) intrauterine and 11 (3%) neonatal deaths. Primary outcome data including PR and NPR loss rates are given in Table 2. Loss rate per procedure in the total cohort was 2.9% (42/1422 procedures), 1.3% of which was classified as PR (19/1422) and 1.6% as NPR (23/1422).

Table 2. *Perinatal loss (per fetus and procedural loss) in relation to gestational age.*

	<20 weeks	**>=20 weeks**	**p-value**	**OR (95% CI)**
Per fetus	*(n = 29)*	*(n = 462)*		
Perinatal loss*, n (%)*	7 (24)	35 (8)	.002	3.9 (1.4-10.4)
Per procedure	*(n = 37)*	*(n = 1385)*		
PR, *n (%)*	2 (5)	17 (1)	.08	4.6 (0.0-21.9)
NPR, *n (%)*	4 (11)	19 (1)	< .001	8.7 (2.4-29.3)
Total perinatal loss, *n (%)*	6 (16)	36 (3)	< .001	7.3 (2.5-19.7)

PR = procedure-related

NPR = not procedure-related

Apart from gestational age, the following baseline characteristics that appeared significantly different between both groups were considered as additional potential risk factors for perinatal death: Kell immunisation, gestational age, fetal hydrops and haemoglobin level at first IUT. The association of all variables with perinatal death was first studied in a univariate analysis, the results of which are shown in Table 3. Subsequently, a multivariate logistic regression model was constructed to assess factors independently associated with perinatal loss after IUT. We excluded haemoglobin at first IUT from this multivariate model, as this variable is strongly associated with the presence of hydrops and including both could possibly bias our results. In the multivariate regression analysis, only fetal hydrops was independently associated with perinatal loss (P = 0.001).

Table 3. *Analysis of potential risk factors for perinatal loss after intrauterine treatment.*

	Perinatal loss (n = 42)	**No perinatal loss** (n = 449)	**p-value***	**OR (95% CI)***	**p-value****	**OR (95% CI)****
Kell immunisation, *n (%)*	9 (21)	50 (11)	.06	2.2 (1.0-4.8)	.35	1.5 (0.6-3.4)
Gestational age at first IUT***, *weeks*	25.3 ± 5.2	27.4 ± 4.7	.007	1.1 (1.0-1.2)	.73	1.1 (1.0-1.1)
Hydrops at first IUT, *n (%)*	24 (57)	116 (26)	< .001	3.8 (2.0-7.3)	.001	3.2 (1.7-6.3)
Haemoglobin at first IUT***, *g/dL*	4.6 ± 2.0	5.6 ± 2.3	.01	1.2 (1.1-1.4)	-	-

* Univariate analysis

** Multivariate analysis

*** Value given as mean ±SD

Figure 1 shows the effect of gestational age at first IUT on the log odds of perinatal loss. The linear black line shows the log odds. The grey area represents the 95% confidence interval (95% CI) which indicates the accuracy of the possible relation between gestational age and perinatal loss. A significantly declining perinatal loss with increasing gestational age was found for the total group (P = 0.008) and for fetuses without hydrops (P = 0.002). Both the log odds (black line) and the confidence interval (grey area) are declining. No significant effect was found in fetuses with hydrops (P = 0.54). Both a declining as well as an ascending line fits in the 95% confidence interval, indicating no significant relation between perinatal loss and gestational age at IUT in fetuses diagnosed with hydrops.

Figure 1. *The effect of gestational age at first IUT on the log odds (black line, confidence interval in grey) of perinatal loss showing a significant relation in the total group (middle: P = 0.008) and in fetuses without hydrops (left: P = 0.002). No effect was found in fetuses with the presence of hydrops (right: P = 0.54).*

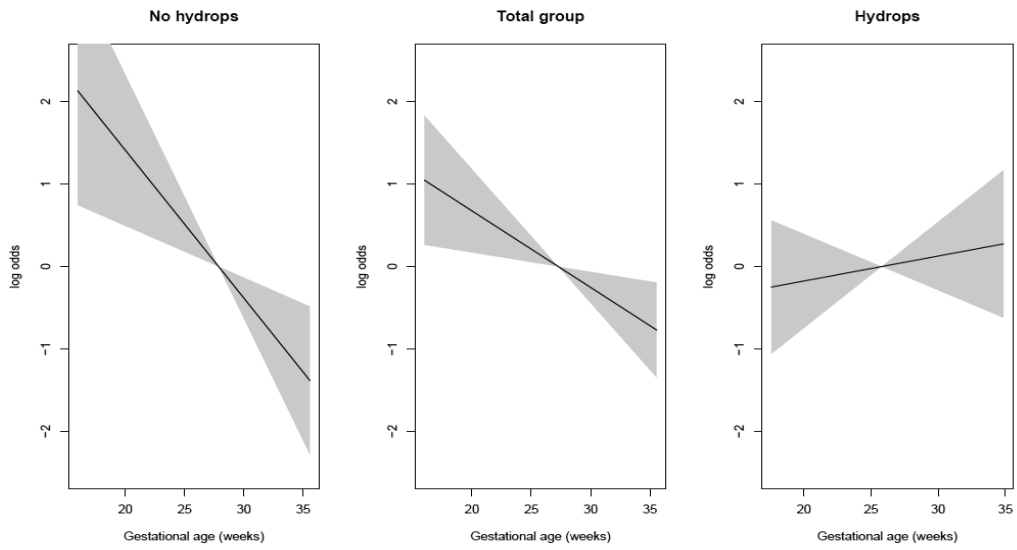
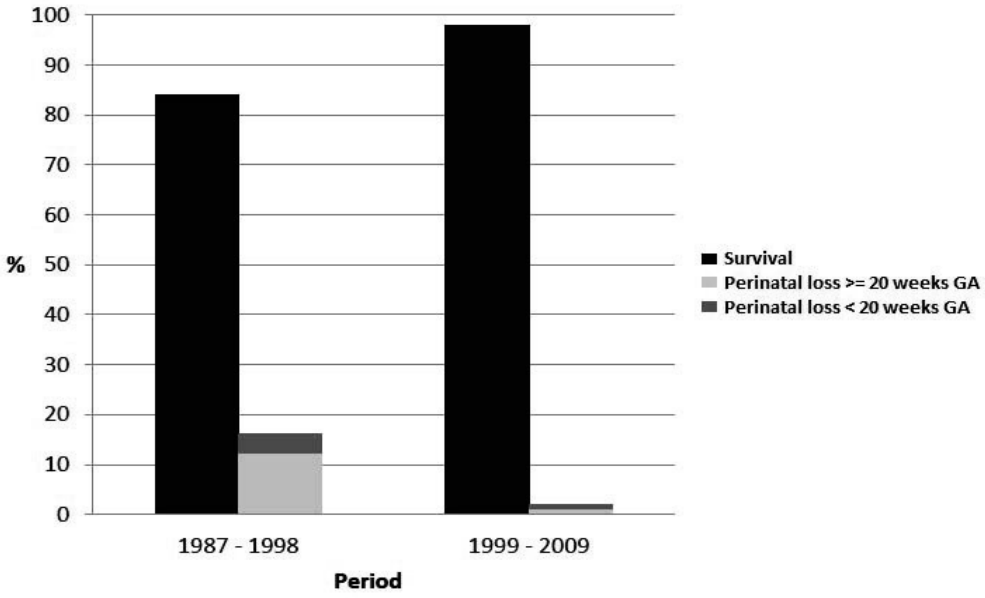


Figure 2 shows an increase of perinatal survival after IUTs in two consecutive periods (188/224 [84%] in 1987–98 versus 261/267 [98%] in 1999–2009, $P < 0.001$, odds ratio [odds ratio] 8.3, 95% CI 3.4–20.2). Although perinatal loss occurring in Group 2 declined significantly from 14% (32/224) to 1.1% (3/267) ($P < 0.001$, OR 14.8, 95% CI 4.5–49.1), no difference was found in perinatal loss in Group 1 between both periods (1.8% 4/224 versus 1.1% 3/267, $P = 0.5$, OR 1.8, 95% CI 0.4–8.4). The incidence of hydrops decreased from 86/224 (38%) in the first period to 54/267 (20%) in later years ($P < 0.001$, OR 2.6, 95% CI 1.6–3.7).

Figure 2. *Survival and perinatal loss rates after intrauterine transfusion in two consecutive periods at our centre. In Group 1 fetuses received the first IUT before 20 weeks of gestation, and in Group 2 treatment with IUTs started at or after 20 weeks of gestation.*



Discussion

In this study, we evaluated the outcome of early second-trimester IUTs for alloimmune fetal anaemia in a large cohort of more than 1400 procedures performed between 16 and 35+4 weeks of gestation. We found a nearly four-fold risk of perinatal death of fetuses requiring IUT for severe alloimmune anaemia before 20 weeks of gestation. These data confirm the findings of previous smaller studies.[2, 4-7] By comparing cohorts of fetuses treated before and after 1998, we showed a significant decline in perinatal loss. However, perinatal loss of fetuses treated before 20 weeks of gestation did not decline, indicating that early IUT therapy is still associated with a significant risk. Contrary to our expectations, the losses after early IUTs were more often not related to the procedure itself. This may be explained by a higher percentage of severely anaemic and hydropic fetuses in the early IUT group. This in turn might be related to an overrepresentation of Kell-immunised pregnancies, for which in the Netherlands routine screening in the first trimester was only introduced in 1998. In addition, fetal anaemia in Kell disease generally develops earlier in gestation, because of the presence of Kell antigens on erythroid precursor.[13, 14] This leads us to conclude that to increase the safety and improve outcome of IUTs before 20 weeks of gestation, our efforts should concentrate on the early identification of especially Kell immunisation in pregnancy.

To prevent early and severe fetal haemolytic disease and to overcome the additional technical difficulties related to early IUTs, treatment with intraperitoneal IUT, plasmapheresis, maternal intravascular immunoglobulin (IVIG) or combined treatment have been suggested and reported.[15-25]

Intraperitoneal transfusion was first reported by Liley in 1963.[15] Bennebroek Gravenhorst et al.[16] reported in 1989 a cohort of 154 fetuses treated with 270 intraperitoneal IUTs, which they found to be ineffective in young and hydropic fetuses. Fox et al.[17] in 2008 reported a case-study of six women who in previous pregnancies had evidence of severe anaemia before 20 weeks of gestation with mortality in four of the six previous pregnancies. All women received prophylactically serial IUTs between 16 and 21 weeks of gestation combined with adjuvant maternal IVIG in four of the six pregnancies. Six of the seven fetuses (one twin) survived, suggesting an advantage of this approach.

Maternal plasmapheresis to remove IgG anti-D was introduced in the early 1970s.[18] However, a rebound phenomenon to levels higher than before the pheresis has been seen.[19] The value of plasmapheresis in preventing severe alloimmune fetal anaemia has never been proven.

Since 1985, IVIG has been applied in severe red cell immunisation[20] and most studies reported a positive effect.[21, 22] Voto et al.[23] suggested that high-dose IVIG therapy followed by IUTs may improve fetal survival in severe cases. The population studied was assigned to one of the following two groups: (i) IVIG group: 30 women receiving IVIG before 21 weeks of gestation and IUTs after 20 weeks, (ii) IUT group: 39 women undergoing IUTs starting at 20–25 weeks of gestation. The incidence of hydrops at the first IUT and of fetal death was significantly higher in the IUT group compared with the IVIG group.

Combined therapy of plasmapheresis and IVIG for the treatment of alloimmunisation in pregnancy has been applied in three studies with a total of 16 women.[24, 25] In the most recent of these studies, Ruma et al.[25] presented a series of nine women with a history of previously affected pregnancies with fetal hydrops, anaemia or fetal demise before 24 weeks of gestation. Four of the nine pregnancies had Kell immunisation. Plasmapheresis was given every other day, starting after the 12th week of gestation. After the third plasmapheresis, IVIG was administered. All infants survived, maternal antibody titres were significantly reduced after plasmapheresis and remained decreased during IVIG therapy.

In addition to evaluation of the relation between gestational age and IUT outcome, we again found a strong association of the presence of hydrops and adverse outcome.[8] This predictor appeared to be of greatest influence on perinatal loss, relatively reducing the importance of gestational age.

As almost half of fetuses in the early IUT group suffered from hydrops, we expected and found a lower haemoglobin level in this group. Remarkably, when correcting haemoglobin for gestational age by calculating *Z* scores, differences between both groups were no longer significant. This substantiates the concept that tissue oxygenation depends more closely on the number of circulating red cells then on deviation of the haemoglobin level from the mean for gestational age. Since short-term and long-term outcome after IUTs appear to be better in nonhydropic fetuses, clinicians should try to prevent hydrops in fetuses at risk for fetal anaemia by frequent monitoring and starting treatment early.[8, 26]

Conclusions

Intrauterine transfusions indicated before 20 weeks of gestation still pose a great challenge to clinicians. Although technical aspects inherent to gaining intravascular access in a very small fetus may to some extent influence outcome, we found the strongest association of perinatal loss after IUT to be with the severity of haemolytic disease, i.e. presence of hydrops. We suggest that the emphasis for improving outcome in early severe red cell alloimmunisation should be on referral to specialised centres for the timely detection and treatment of fetal anaemia. Whether in addition alternative treatment options, such as IVIG to prevent or delay the development of early onset haemolytic disease, have additional value can only be assessed by well-designed, adequately powered international multicentre collaborative studies.

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