Bridging the gaps: prevention, management, and future perspectives in hemolytic disease of the fetus and newborn

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III

HOW TO OPTIMIZE ANTENATAL TREATMENT

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Comparison of intrauterine transfusion techniques in hemolytic disease of the fetus and newborn

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Abstract

Objectives: Intrauterine transfusions (IUTs) are the cornerstone in treatment for hemolytic disease of the fetus and newborn (HDFN). It has been suggested that a non-vascular intraperitoneal blood transfusion used in conjunction with an intravascular IUT can slow the decrease in fetal hemoglobin (Hb) levels, potentially extending the interval between transfusions. Our aim was to evaluate the rate of decline in Hb levels and the interval between transfusions using different IUT techniques, including intrahepatic transfusions with and without intraperitoneal transfusion and transplacental transfusion at the site of the placental cord insertion.

Methods: We conducted a retrospective cohort study at the Leiden University Medical Center, the national referral center for HDFN, between January 2006 and December 2022. All cases that underwent intrahepatic (with and without intraperitoneal transfusion) and placental cord insertion IUTs during the study period were included. The primary outcome was the decline in Hb levels per week, measured by comparing the Hb level immediately after the IUT with the Hb level before the subsequent IUT or birth. The primary outcome was analyzed using generalized estimating equations with and without adjustment for confounders.

Results: We included 309 fetuses that received a total of 791 IUTs, of which 151 were intrahepatic-only transfusions, 273 were intrahepatic + intraperitoneal transfusions and 367 were placental cord insertion transfusions. We found an adjusted mean difference in the decline in Hb levels of 0.48 (95% CI, 0.29-0.66) g/dL/week between the group that underwent intrahepatic-only transfusion and the group that underwent intrahepatic + intraperitoneal transfusion ($P<0.001$). The adjusted mean difference between the intrahepatic-only IUT group and the placental cord insertion IUT group was 0.49 (95% CI, 0.05 – 0.94) g/dL/week ($P=0.030$). The median interval to the next IUT for the total cohort was 21 (interquartile range (IQR), 18-28) days. Similarly, in the intrahepatic-only and placental cord insertion IUT groups, the median interval to the next IUT was 21 (IQR, 19-28) and 21 (IQR, 15-26) days, respectively. In the intrahepatic + intraperitoneal transfusion group, the median interval was slightly higher (26 (IQR, 21-28) days).

Conclusion: Decline in Hb levels was slower when using intrahepatic + intraperitoneal transfusion compared with other IUT techniques and seemed to prolong the interval between IUT procedures. The potential clinical advantages of the intrahepatic + intraperitoneal transfusion technique need to be weighed against the increased complexity and extended duration of the procedure on an individual basis.

Introduction

Severe hemolytic disease of the fetus and newborn (HDFN) is a serious perinatal condition that can occur early in pregnancy. If left untreated, HDFN can result in fetal demise or long-term neurological sequelae[1]. Intrauterine transfusions (IUTs) are considered the cornerstone in treating fetal anemia and preventing severe morbidity and mortality[2,3]. Multiple IUTs may be needed if fetal anemia occurs early in pregnancy, yet there is a higher risk of complications when IUTs are performed at earlier gestation[4].

Various access sites can be used for IUT, including intravascular access into the umbilical cord at the site of placental cord insertion, transamniotic intravascular access via a free loop of the umbilical cord or access into the intrahepatic portion of the umbilical vein. Less commonly used sites are direct transfusion into the fetal heart or non-vascular intraperitoneal transfusion [2,3,5,6]. The decision to use a particular access site is based ultimately on the placental and fetal position, as well as the fetal surgeon's expertise, leading to significant variation between centers worldwide[3,7].

Smaller cohort studies have previously shown the potential benefits of combining intravascular and intraperitoneal transfusions, suggesting that this combination may extend the interval between IUTs[8,9]. Despite these promising findings, larger studies have not been conducted. In the DIONYSUS study, which is a retrospective review of practices among 31 participating centers, our center was, surprisingly, the only center to perform combined intrahepatic and intraperitoneal transfusions[7]. Therefore, we set out to evaluate the different IUT techniques used within our large cohort to determine the differences between them and the benefits of adding an intraperitoneal transfusion.

The aim of this study was to evaluate the rate of decline in hemoglobin (Hb) levels and the interval between transfusions using different IUT techniques, including intrahepatic transfusion with and without an intraperitoneal transfusion, and placental cord insertion transfusion.

Methods

Study design and population

We conducted a retrospective, observational cohort study, encompassing all IUTs performed for HDFN between January 2006 and December 2022 in the Dutch national referral center for HDFN, the Leiden University Medical Center (LUMC) (Leiden, The Netherlands). Data from the study period were obtained from LUMC patients included in the DIONYSUS database,[7] supplemented by data collected from patients treated at the

LUMC from June 2021 to December 2022. Given our focus on umbilical or intrahepatic transfusions, we excluded IUT procedures performed using techniques rarely used at our center, such as those involving accessing a free loop of the umbilical cord or the intracardiac approach. Additionally, IUTs deemed unsuccessful (i.e. where no blood transfusion was administered), were also excluded from analyses. The non-WMO (non-Medical Research Involving Human Subjects Act) medical review committee of the LUMC reviewed the study (G21.113) and waived the need for written informed consent.

Setting

A dedicated team at the LUMC performs IUTs, including a maternal–fetal medicine specialist – fetal surgeon, an ultrasound expert and a nurse practitioner. Patients are referred to the center if the serology results of antibody titers are above cut-off values, following the national guideline, and the fetus is positive for the implicated antigen. Since 2004, we have determined the fetal antigen status using cell-free fetal DNA. The decision to perform an IUT is based on weekly ultrasound examinations conducted by dedicated ultrasound experts who determine whether severe fetal anemia is suspected based on peak systolic velocity of the middle cerebral artery, using a cut-off of 1.55 multiples of the median (although higher cut-off values may be accepted in individual cases), and secondary signs of fetal anemia, such as (pre)hydrops fetalis, increased bowel opacity, decreased myocardial contractility and cardiomegaly. Ultrasound examinations at the LUMC are conducted on a Tuesday, and, if needed, an IUT is scheduled for a Thursday. At our center, it is customary to perform IUTs using the intrahepatic technique in cases with a posterior placenta. Conversely, the placental cord insertion technique is the primary choice in cases with an anterior placenta. A placental cord insertion transfusion is rarely combined with an intraperitoneal transfusion, as this requires repositioning the needle with an additional puncture in the fetus. The total intravascular volume transfused is calculated using the formula of Rodeck et al.[10], which considers the estimated fetoplacental volume (derived from estimated fetal weight), the pretransfusion hematocrit (Ht), the hematocrit of the donor blood and the desired end hematocrit value (which is also based on gestational age). There is no established protocol for deciding whether to combine an intrahepatic transfusion with an intraperitoneal transfusion, as this decision depends on several factors, including the gestational age, and the fetal condition and movements. For example, an IUT performed at an early gestational age (before 20 weeks' gestation) carries higher risks for the fetus. To navigate this high-risk period, adding an intraperitoneal transfusion can help to extend the time until the next transfusion when it may be safer for the fetus. Another example would be if an IUT is

planned to be administered at 33 weeks' gestation with the aim of bridging until 37-38 weeks' gestation without another transfusion, as we typically do not perform IUTs after 35 weeks' gestation. Extending the interval between IUTs can be beneficial, which is why adding an intraperitoneal transfusion may be advantageous. Conversely, excessive fetal movement during the procedure, despite administering atracurium beforehand, or needle dislocation could adversely affect the decision to proceed with an additional intraperitoneal transfusion.

At the start of the intravascular IUT procedure, a sample is taken for Hb measurement. This is repeated at the end of the procedure. As a general guideline, we administer 10-15 mL of intraperitoneal transfusion until 25 weeks' gestation, 20 mL of intraperitoneal transfusion between 25 and 30 weeks' gestation, and 30 mL of intraperitoneal transfusion between 30 and 35 weeks' gestation. The volume of transfusion further depends on the post-transfusion Hb value reached. The total intraperitoneal transfused volume does not exceed volumes defined previously that were set up to prevent excessive intra-abdominal pressure[11].

The decision to proceed with a subsequent IUT is influenced by several factors. Primarily, it depends on the presence and severity of fetal anemia, as described above, and how these features of fetal anemia compare with those observed during the last IUT. Other considerations include the end-Hb level, the volume transfused, the anticipated rate of erythrocyte breakdown and the total number of transfusions administered previously. Typically, the interval between IUT procedures increases with each additional transfusion as donor adult red blood cells accumulate in the fetal circulation. This accumulation suppresses fetal erythropoiesis, resulting in a minimal proportion of fetal red blood cells after two transfusions.[12]

Data collection

Both antenatal and postnatal data were collected from all included cases. This included, but was not limited to, laboratory results, Doppler measurements, gestational age at referral, IUT technique, mode of delivery, additional treatments and neonatal outcome.

Outcome measures

The main outcome for both research questions was the rate of Hb decline per week between subsequent IUT procedures, or the Hb decline from the last IUT to birth. For each interval between IUTs, the Hb decline was calculated by subtracting the pretransfusion Hb value at the next IUT or the Hb value at birth from the post-transfusion Hb value

from the preceding IUT. This difference was divided by the number of weeks between IUTs, or between the last IUT and birth. This creates the variable 'delta Hb' in g/dL/week, for which a higher value indicates a faster decline in Hb levels.

Exposure and potential confounders

Exposures considered were: the technique (intrahepatic without intraperitoneal transfusion vs intrahepatic + intraperitoneal transfusion) or access site used (intrahepatic vs transplacental placental at the site of the cord insertion) for IUT. The technique used in our center is determined mainly by the placental position. In rare instances, a different approach may be adopted during the procedure.

The following confounders were identified. (1) Placental location. This dictates whether the cord root or the fetal liver is used as the puncture site. In our center, in cases with an anterior placenta, the IUT is typically performed using the placental cord insertion technique, potentially boosting the production of alloantibodies because the needle is inserted through the placenta[3]. (2) Gestational age at IUT. A transfusion at an early gestational age (before 20 weeks' gestation) has significantly higher risks for the fetus, thus promoting the use of an intraperitoneal transfusion; although, in early gestation, an intraperitoneal transfusion is more difficult. (3) The order of IUT. If it is the first (if IUT is performed at an early gestational age) or last IUT, this may prompt the fetal surgeon to also administer an intraperitoneal transfusion. Additionally, the interval between IUTs increases the higher the order of IUT, as the Hb decline tends to slow down with each subsequent IUT.[13] (4) Pretransfusion Hb level. When Hb levels are lower, more donor erythrocytes that cannot be hemolyzed by alloantibodies are transfused, which may prolong the interval until the next IUT. (5) Type of alloantibody. K(ell) alloantibodies can cause a more severe and early onset of HDFN. Intraperitoneal transfusion may be considered in early IUTs to bridge the gap in time until a safer gestational age for a subsequent IUT.[14] (6) Presence of hydrops. In cases of hydrops, an intraperitoneal transfusion can serve as a potential rescue measure as it prevents the risk of intravascular overload. Additionally, intraperitoneal transfusion is relatively easy to achieve in the hydropic fetus. Conversely, some evidence suggests that hydropic fetuses may not absorb erythrocytes from the abdominal cavity effectively [8,15].

Hydrops was defined as mild when a small rim of fluid around the intestines was present with or without pericardial effusion, similar to our previous publication.[14] Excessive ascites with or without pericardial and pleural effusion and skin edema was defined as severe hydrops.

All included cases that underwent IUT were divided into three groups: intrahepatic transfusion, intrahepatic transfusion followed by an additional intraperitoneal transfusion and transplacental transfusion administered at the placental cord insertion site. Therefore, a single fetus that underwent multiple IUTs could be included in multiple groups if the IUT technique or access site differed between procedures. The outcome of each IUT procedure for the three different groups were then compared. To address the aims of the study, intrahepatic-only transfusions were compared with intrahepatic + intraperitoneal transfusion and intrahepatic-only transfusions were also compared with placental cord insertion transfusions.

For the secondary analysis, we re-evaluated group comparisons, categorizing the IUTs into first and second transfusions, with all subsequent IUTs grouped together. This method was used to identify any differences specific to each individual IUT procedure.

Statistical analysis

Linear regression was conducted using generalized estimating equations with an independent correlation structure to account for multiple IUTs within the same fetus. Analyses were performed with and without adjustment for the identified confounders. For the secondary analysis, a linear regression model was employed, adjusting for the same confounders as in the primary outcome.

Four sensitivity analyses were performed. In the main analysis, two extreme outliers were removed with a delta Hb >8.5 g/dL/week to prevent their influence on the analysis. To judge their influence, these outliers were included in the first sensitivity analysis. In the second sensitivity analysis, the data of the main analysis were taken and the variable 'transplacental approach' was included in the linear model to account for potential confounding due to this technique, in addition to confounding by placental location. In the third sensitivity analysis, fetuses with hydrops were excluded, as some evidence suggests that erythrocytes administered by intraperitoneal transfusion may not be well absorbed by the hydropic fetus[8]. In the fourth sensitivity analysis, multiple imputation methods were used to address missing values in the primary outcome.

Descriptive statistics were determined at both the fetus level and the transfusion level to provide a comprehensive overview of the data. Continuous variables are presented as median (interquartile range (IQR)) and categorical variables are presented as *n* (%). The main outcome of the generalized estimating equations is reported as mean ± standard deviation (SD). A P-value < 0.05 was considered to indicate statistical significance.

Results

Characteristics of fetuses and intrauterine transfusions

During the 17-year study period, a total of 820 IUTs were performed for HDFN in 311 individual fetuses. Twenty-nine IUT procedures were excluded due to unsuccessful procedure (n=4), intracardiac IUT (n=1), free-loop IUT (n=3), intraperitoneal transfusion only (n=11), placental cord insertion IUT combined with intraperitoneal transfusion (n=2) and IUT near the cord root (n=8) (Figure 1). Therefore, 309 fetuses, which underwent a total of 791 IUTs, were included in the study. The fetal and IUT characteristics are displayed in Tables 1 and 2, respectively.

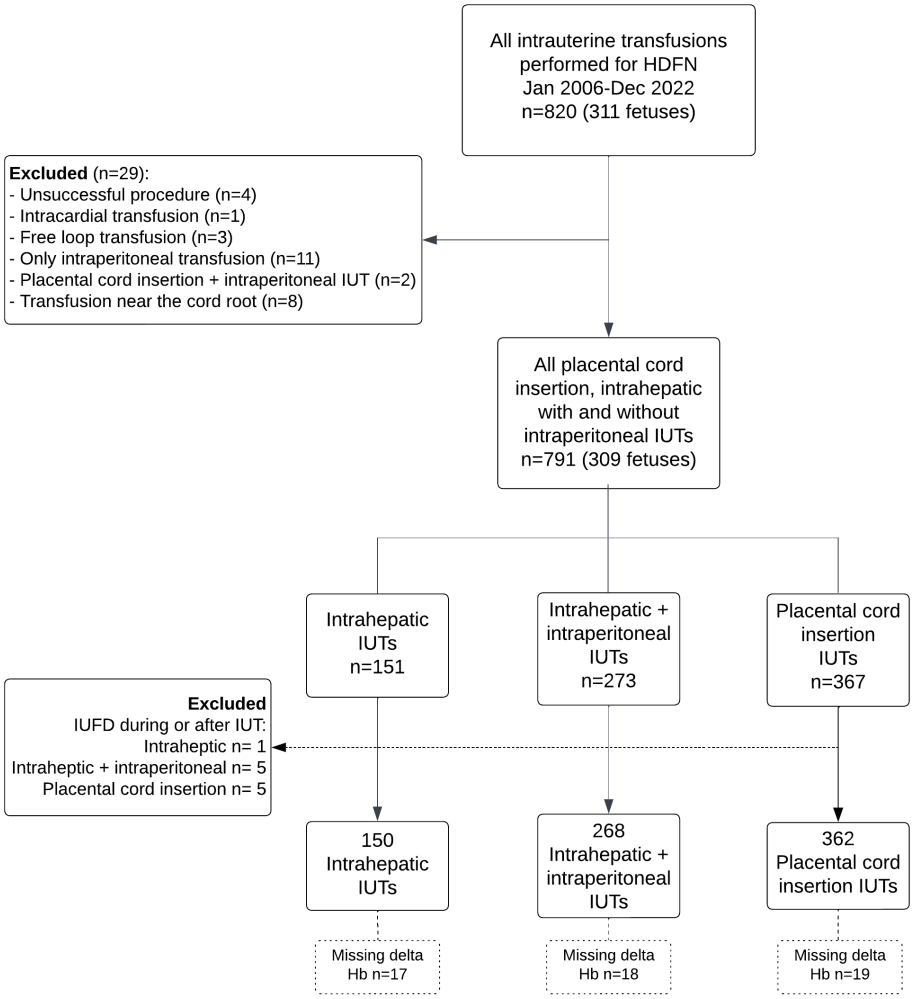

Figure 1. Flowchart summarizing inclusion in study of fetuses with hemolytic disease of the fetus and newborn (HDFN) that underwent intrahepatic intrauterine transfusion (IUT), intrahepatic and intraperitoneal IUT and/or placental cord insertion IUT, as well as numbers of cases missing delta hemoglobin (Hb) values.

The majority of transfusions were performed due to RhD-mediated HDFN. Furthermore, 42% of the total cohort of fetuses underwent IUT solely at the placental cord insertion. The rate of hydrops at first IUT was 13% (41/309), although hydrops was mild in most cases (90% (37/41)). The rate of fetal loss was 4% (11/309). There was one procedure-related fetal demise in each of the three IUT technique groups (n=3). The remaining eight cases of fetal loss were the result of: severe early-onset HDFN (before 20weeks' gestation), for which fetal demise followed shortly after a technically uncomplicated IUT procedure (n=4; three in the intrahepatic + intraperitoneal transfusion group and one in the placental cord insertion transfusion group); intrauterine infection (n=1); termination of pregnancy on maternal indication (n=1); unclear reason for demise which occurred 2.5 or 3weeks after IUT (n=2).

Table 1. Characteristics of 309 fetuses with hemolytic disease included in study, which were treated with at least one intrauterine transfusion (IUT).

Characteristic	Value
Primary alloimmunization	
RhD	233 (75)
K1 (Kell)	53 (17)
c	11 (4)
Other	12 (4)
Number of IUTs	2 (2-4)
IUT access site variation per fetus	
Only at placental cord insertion	130 (42)
Only intrahepatic	32 (10)
Only intrahepatic with intraperitoneal transfusion	53 (17)
Intrahepatic with or without intraperitoneal transfusion	58 (19)
Placental cord insertion/intrahepatic with intraperitoneal transfusion	10 (3)
Other	26 (8)
GA at first IUT (weeks)	28.3 (24.2-31.6)
Hydrops at first IUT	
No	268 (87)
Mild	37 (12)
Severe	4 (1)
IUFD during or after IUT	11 (4)
GA at birth* (weeks)	36.6 (35.9-37.1)
Hb at birth† (g/dL)	7.8 (6.9-8.9)
Phototherapy	294 (95)
Exchange transfusion	46 (15)

Data are given as n (%) or median (interquartile range). *11 values missing, mostly due to IUFD. †12 values missing, mostly due to IUFD. GA, gestational age; Hb, hemoglobin; IUFD, intrauterine fetal demise; IUT, intrauterine transfusion.

Table 2. Characteristics of intrauterine transfusions (IUTs), overall and according to transfusion technique

		Transfusion technique		
Characteristic	**All IUTs (n= 791)**	**Intrahepatic (n=151)**	**Intrahepatic + intraperitoneal (n=273)**	**Placental cord insertion (n=367)**
Primary alloimmunization				
RhD	580 (73)	107 (71)	192 (70)	282 (77)
K(ell)	165 (21)	32 (21)	62 (23)	70 (19)
c	22 (3)	4 (3)	13 (5)	5 (1)
Other	24 (3)	8 (5)	6 (2)	10 (3)
GA at IUT (weeks)	30.1 (26.4 – 33.0)	30.4 (27.0 – 33.3)	29.1 (25.2 – 32.4)	30.6 (27.3 – 33.3)
Hb before IUT (g/dL)*	7.4 (6.0 – 8.9)	7.8 (6.6 – 9.3)	7.4 (5.8 – 8.9)	7.3 (6.0 – 8.5)
Hb after IUT (g/dL)†	14.3 (13.5 – 15.0)	14.3 (13.5 – 15.1)	14.2 (13.5 – 15.0)	14.3 (13.5 – 15.0)
DHb change (g/dL/week)‡	1.61 (0.65 – 2.22)	1.49 (0.77 – 2.24)	1.43 (0.30-1.84)	1.88 (0.96 – 2.56)
Hydrops at IUT				
No	718 (91)	143 (95)	236 (86)	339 (92)
Mild	68 (9)	7 (5)	35 (13)	26 (7)
Severe	5 (1)	1 (1)	2 (1)	2 (1)
IUFD during or after IUT	11 (1.4)	1 (0.7)	5 (1.8)	5 (1.4)
Interval until next IUT (weeks)§	3.0 (2.6 – 4.0)	3.0 (2.7 – 4.0)	3.7 (3.0 – 4.0)	3.0 (2.1 – 3.7)

Data are given as n (%) or median (interquartile range). *Four values were missing: two in placental cord insertion (PCI) group, one in intrahepatic (IH) group and one in intrahepatic + intraperitoneal (IH+IP) group. †Forty-nine values were missing: 18 in PCI group, 15 in IH group and 16 in IH+IP group. ‡Sixty-five values were missing: 24 in PCI group, 18 in IH group and 23 in IH+IP group. §Thirteen values were missing: six in PCI group, one in IH group and six in IH+IP group. GA, gestational age; Hb, hemoglobin; IUFD, intrauterine fetal demise.

Most characteristics were comparable between the three groups, (Table 2). The most prominent difference found between the three groups was the rate of hydrops, which was 14% in the intrahepatic + intraperitoneal transfusion group, compared with 8% and 5%in the placental cord insertion and intrahepatic-only transfusion groups, respectively.

Delta Hb

A delta Hb was calculated for all IUTs, with the exception of 65 IUTs because of the inability to determine post-transfusion Hb values (n=46), intrauterine demise (n=11), no Hb value determined at the beginning of the next IUT (n=6), no Hb value determined directly postpartum (n=1) and delivery on the day of the IUT (n=1). There were two major outliers in delta Hb, which were excluded from all analyses except for one sensitivity analysis. Both major outliers were because of the short interval until birth (1 day) with a large difference between the post-transfusion Hb value and the Hb value from cord blood at birth. This discrepancy was considered as measurement error.

Difference in delta Hb between intrahepatic transfusions with vs without intraperitoneal transfusion

In 382 intrahepatic transfusions for which the delta Hb was known, the mean decline in Hb levels after an intrahepatic + intraperitoneal transfusion was 1.13 ± 1.07 g/dL/week, which was slower compared with that after intrahepatic transfusion alone (mean ± SD decline of 1.42±1.11 g/dL/week) (unadjusted difference in mean of 0.29 (95% CI, 0.07–0.51) g/dL/week; P=0.011) (Figure 2). The adjusted mean difference was 0.48 (95% CI, 0.29–0.66) g/dL/week (P<0.001) (Table S1). In the secondary analysis, an adjusted mean difference of 0.52 (95% CI, 0.17–0.86) g/dL/week (P=0.004) was found in the first IUT, 0.41 (95% CI, 0.13–0.70) g/dL/week (P=0.005) was found in the second IUT and 0.50 (95% CI, 0.21–0.79) g/dL/week (P<0.001) was found from the third IUT onwards.

Difference in delta Hb between placental cord insertion and intrahepatic transfusions

In the 474 IUTs for which delta Hb was known, the mean decline in Hb levels was greater for fetuses with placental cord insertion transfusion, with a mean ± SD of 1.83±1.41 g/dL/week compared to fetuses with intrahepatic transfusion alone (mean ± SD decline of 1.42±1.11 g/dL/week) (Figure 2). The unadjusted difference in mean between these two groups was 0.41 (95% CI, 0.18–0.64) g/dL/week (P<0.001). The adjusted mean difference was 0.49 (95% CI, 0.05–0.94) g/dL/week (P=0.030). In the secondary analysis, an adjusted mean difference of 0.99 (95% CI, 0.25–1.73) g/dL/week (P=0.009) was found in the first IUT, 0.001 (95% CI, −1.01 to 1.01) g/dL/week (P=0.99) in the second IUT and 0.35 (95%CI, −0.23 to 0.93) g/dL/week (P=0.24) from the third IUT onwards. Note that no significance was found in the last two analyses.

Interval until next transfusion

Overall, the median interval until the next IUT was 3.0 (IQR, 2.6–4.0) weeks. The median interval was similar for the intrahepatic-only (3.0 (IQR, 2.7–4.0) weeks) and placental cord insertion (3.0 (IQR, 2.1–3.7) weeks) transfusion groups, and was slightly higher in the intrahepatic + intraperitoneal transfusion group (3.7 (IQR, 3.0–4.0) weeks). This corresponds to an unadjusted 21-day interval for intrahepatic-only or placental cord insertion transfusions and a 26-day interval for the intrahepatic + intraperitoneal transfusion group.

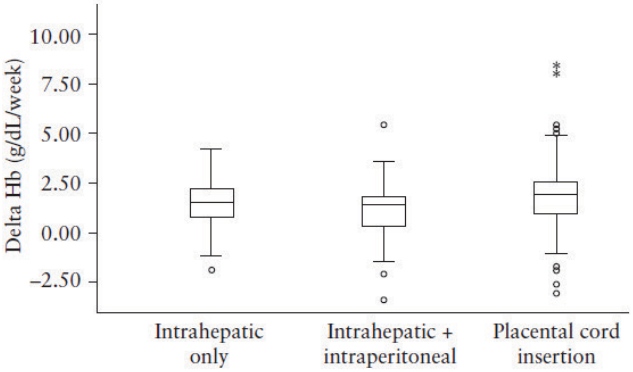

Figure 2. Box-and-whiskers plot showing unadjusted delta hemoglobin (Hb) according to whether intrauterine transfusion technique used was intrahepatic-only transfusion, intrahepatic + intraperitoneal transfusion or placental cord insertion transfusion. Boxes show median and interquartile range (IQR), and error bars depict 95%CI. Dots (°) represent outliers. Asterisks (*) represent extreme outliers, defined as 3× IQR. The two major outliers (one in the intrahepatic-only group and one in the placental cord insertion group) were not included in the main analysis and are therefore not displayed in this figure.

Sensitivity analyses

In Table S1, the outcomes of the four sensitivity analyses are displayed. As in the original analysis, intrahepatic transfusions were compared when administered with vs without intraperitoneal transfusion, and intrahepatic-only transfusions were compared with placental cord insertion transfusions. Estimated mean differences and adjusted mean differences are displayed in Table S1. The four sensitivity analyses showed similar results to the original analysis, except for the analyses that included the major outliers, especially in the analysis for the intrahepatic with vs without intraperitoneal transfusion, for which the estimated mean difference in delta Hb became 0.46 (95% CI, 0.05–0.88; P=0.028), with an adjusted mean difference of 0.66 (95% CI, 0.27–1.05; P<0.001), when the major outliers were included.

Discussion

Main outcome and clinical implications

Intrahepatic + intraperitoneal transfusion resulted in a slower Hb decline of almost 0.5 g/dL less per week compared with intrahepatic transfusion alone, which is consistent with previous findings.[8,9] This difference was consistent over all consecutive IUTs.

However, incorporating an intraperitoneal transfusion may prolong the procedure and potentially complicate it by causing an abdominal wall hematoma. Depending on how accessible the peritoneum is, the duration of transfusing the blood deposition may

be 10 min, or even longer with a more challenging case. As this was a retrospective study, without a set protocol for when to perform an intraperitoneal transfusion, it is impossible to determine whether any of the major complications were the result of the intraperitoneal transfusion itself or of an inherently higher risk of complications in the cases in which it was used. There were three procedure-related fetal demises, one in each of the three IUT technique groups. We have reported previously on these cases and other complications at our center.[4]

We observed a 5-day interval difference between intrahepatic with vs without intraperitoneal transfusion. Although this difference may appear minor, it could offer benefits in specific cases, such as managing early-onset severe HDFN or extending the interval until term gestation after the final IUT. In cases that need to start IUT at an early gestational age, this interval difference may even result in the reduction of the number of IUTs performed over the pregnancy. We recommend evaluating the necessity of adding an intraperitoneal transfusion on a case-by-case basis for each procedure, balancing the previously described risks and benefits.

Comparing placental cord insertion and intrahepatic-only IUTs showed a difference in delta Hb of nearly 0.5 g/dL/week, favoring the intrahepatic IUTs. However, this difference was observed primarily when comparing the first IUT procedure.

The interval between IUTs was 0.7 weeks longer for the intrahepatic + intraperitoneal transfusion group (3.7 weeks) compared with the intrahepatic-only and placental cord insertion transfusion groups (3.0 weeks), corresponding to a 26-day vs 21-day interval. This prolonged interval may be attributed partly to the fetal surgery team's decision to delay the subsequent IUT because of the addition of intraperitoneal transfusion. The reverse can be said about cases of placental cord insertion IUT needing the subsequent IUT sooner; however, this was not reflected in our data. Furthermore, as IUTs are administered on Thursdays in our center, this creates set intervals between transfusions of whole weeks, unless there is an acute reason for transfusion.

Pathophysiology of factors influencing Hb decline

By combining an intrahepatic transfusion with additional intraperitoneal transfusion, more volume of blood can be transfused. The peritoneal absorption into the fetal circulation occurs over 8–10 days[11,16], resulting in an overall slower decline in Hb.

Historically, survival rates for hydropic fetuses were low when only intraperitoneal transfusions were performed, suggesting that hydropic fetuses cannot effectively

absorb intraperitoneal red blood cells[8,16]. After reviewing the 37 intraperitoneal transfusions performed in fetuses with mild or severe hydrops in the present study, we found that the interval between procedures was at least 10 days, suggesting that the fetuses had a good ability to absorb the red blood cells. Notably, we only had two cases of severe hydrops (i.e. excessive ascites). In these cases, the ascites was not drained before transfusion. The volume of blood transfused in all cases of hydrops, both with and without excessive ascites, was consistent with the amount described in the Methods section. Our experience is that intraperitoneal transfusions are performed more commonly in hydropic fetuses, as the hemodynamic status of the fetus is overloaded earlier, resulting in less intravascular transfusion than desired. This can be partly compensated for by intraperitoneal transfusion. In addition, the intraperitoneal cavity is accessible more easily in a hydropic fetus. Severely ill fetuses may need another IUT sooner, potentially confounding results. In non-hydropic fetuses, Hb decline may be slower, as seen in the unadjusted sensitivity analysis.

The slower Hb decline in the intrahepatic-only transfusion group compared with the placental cord insertion transfusion group could theoretically be due to a higher breakdown of red blood cells in the placental cord insertion group. Placental cord insertion IUTs in our center are performed transplacentally. These procedures are associated with an increased risk of fetomaternal hemorrhage, which can cause an increase in the level of maternal alloantibodies and result in increased fetal hemolysis.[3,17,18] After the first transfusion, this effect was no longer observed, probably because of the accumulation of adult red blood cells in the fetal circulation, as mentioned previously.[12]

Strengths and limitations

The major strength of this study is the large dataset with limited missing values, allowing for correction of several confounders and suggesting limited residual confounding. However, it was not possible to determine a delta Hb for all IUTs, primarily because of an inability to obtain a fetal Hb sample after the procedure. Despite this, the number of missing delta Hb values was limited and results using multiple imputation methods were similar. However, the possibility of any residual confounding may still remain.

We learned from international collaboration that different techniques are used for similar cases.[7] The choice of technique depends largely on the training and experience at different centers. As this is a low-frequency procedure performed by a limited number of specialists, the training received plays a crucial role in determining the technique used. The technique with which practitioners are familiar is likely to result in

the fewest complications and is therefore employed. We can only speculate as to why the combined approach is not employed elsewhere after there has been evidence that it could be helpful to prolong the interval between IUT procedures.

Finally, one could hypothesize that if the same technique is applied at every IUT procedure, this may result in a cumulative effect. If each IUT results in a slower decline in Hb, extending the interval between transfusions by an additional 5 days could potentially reduce the number of IUTs by one. The number of fetuses treated uniformly in the three groups was unfortunately too small to perform robust analyses.

Conclusion

In this study, we found that the addition of an intraperitoneal transfusion during an intrahepatic intravascular IUT is associated with a slower decline of Hb levels and prolongs the interval between IUT procedures by 5 days. The slower decline (almost 0.5 g/dL/week slower) may be beneficial in individual cases, such as in early-onset severe HDFN, to bridge the gap in time to a more advanced gestational age (and possibly to prevent one IUT procedure) or when bridging the gap until term gestation after the final IUT. In the current study, we could not determine the effect of a possible cumulative effect of uniform treatment. International collaboration has shown that different techniques for similar cases depend on the training and experience of specialists, with familiar methods preferred to reduce complications. Sharing our experiences will enhance knowledge of the various techniques employed.

Acknowledgment

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Supplemental material

Table S1. Main outcome of the four sensitivity analyses performed.

Intrahepatic IUTs with vs without intraperitoneal deposition of donor blood		**EMD (95% CI)**	***P***	**Adjusted EMD (95% CI)**	***P***
Original analysis	N=382				
Delta Hb		0.289 (0.066-0.512)	.011	0.475 (0.292-0.657)	<.001
With major outliers	N=383				
Delta Hb		0.464 (0.051-0.878)	.028	0.660 (0.271-1.049)	<.001
Transplacental approach	N=382				
Delta Hb		0.289 (0.066-0.512)	.011	0.475 (0.293-0.657)	<.001
Without hydrops	N=341				
Delta Hb		0.355 (0.128 – 0.582)	.002	0.488 (0.300 – 0.675)	<.001
Multiple Imputation	N=423				
Delta Hb		0.273 (0.044-0.504)	.019	0.480 (0.277-0.683)	<.001
Placental cord insertion IUT vs intrahepatic IUT					
Original analysis	N=474				
Delta Hb		0.411 (0.181-0.640)	<.001	0.492 (0.046 – 0.937)	.030
With outliers	N=476				
Delta Hb		0.205 (-0.218-0.627)	.342	0.426 (-0.034-0.886)	.070
Transplacental approach	N=474				
Delta Hb		0.411 (0.181-0.640)	<.001	0.661 (-0.688 – 2.010)	.337
Without hydrops	N=441				
Delta Hb		0.405 (0.169 – 0.640)	<.001	0.444 (-0.022 – 0.911)	.062
Multiple Imputation	N=516				
Delta Hb		0.388 (0.144 – 0.632)	<.001	0.370 (-0.119 – 0.858)	.135

The main outcome is presented as estimated mean differences and adjusted estimated mean difference in delta Hb between the groups that are compared. CI: confidence interval; EMD: estimated mean difference; Hb: hemoglobin; IUT: intrauterine transfusion.