Hemolytic disease of the fetus and newborn

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CHAPTER 1

INTRAUTERINE TRANSFUSION AND NON-INVASIVE TREATMENT OPTIONS FOR HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN – REVIEW ON CURRENT MANAGEMENT AND OUTCOME

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ABSTRACT

Introduction

Hemolytic disease of the fetus and newborn (HDFN) remains a serious pregnancy complication, which can lead to severe fetal anemia, hydrops and perinatal death.

Areas covered

This review focusses on the current prenatal management, treatment with intrauterine transfusion (IUT) and promising non-invasive treatment options for HDFN.

Expert commentary

IUTs are the cornerstone in prenatal management of HDFN and have significantly improved perinatal outcome in the past decades. IUT is now a relatively safe procedure, however the risk of complications is still high when performed early in the second trimester. Non-invasive management using intravenous immunoglobulin may be a safe alternative and requires further investigation.

INTRODUCTION

Hemolytic disease of the fetus and newborn (HDFN) is still a serious complication in pregnancy. The condition is caused by maternal alloimmunization to fetal red cell antigens, inherited from the father, leading to fetal hemolysis and anemia. Untreated, progressing fetal anemia may result in hepatosplenomegaly, cardiomegaly, cardiac decompensation and eventually in fetal hydrops and perinatal death. If the fetus survives, persistent hemolysis may lead to severe neonatal hyperbilirubinemia and brain injury, an irreversible condition known as 'kernicterus'. [36] Antibodies associated with severe HDFN are mostly of the anti-Rh(D) type, and to a lesser extent of the anti-Kell (anti-K1) or anti-Rh(c) type. Severe HDFN is occasionally caused by other Rh-antibodies, and only very rarely by non-Rh antibodies (Duffy, Kidd, or S).[37]

Prenatal screening for red cell antibodies and several preventive measures, such as matched blood transfusions for Rh- and K antigens and the antenatal and postnatal administration of anti-D immunoprophylaxis, have significantly reduced the incidence and severity of HDFN.[5,38] Nowadays, approximately 1/300-1/600 pregnancies ending in live births are complicated by red cell immunization.[39]

If antibodies are detected in pregnancy, the risk of HDFN is estimated using maternal serum testing for antibody levels (quantification or titers) and, mainly in the Netherlands, antibody-dependent cell-mediated cytotoxicity (ADCC) assays for RhD immunizations.[24,40-42] In most countries, a critical titer around 16, varying from 8 to 32, is used as a cut off for fetal monitoring,[23,41] although this value has a false-positive rate of 77% for predicting fetal anemia.[24] Recent studies on antibody characteristics showed that lower core fucosylation of RhD-antibodies significantly correlated with increased disease severity[28] and in anti-c immunizations, antibody galactosylation and sialylation best predicted fetal/neonatal disease.[27] Furthermore, IgG1 anti-D subtypes are associated with increased severity, in contrast to IgG3.[26] To our best knowledge, these interesting novel insights are not yet implemented in general practice. If either of these serum tests suggest an increased risk on fetal hemolysis, the patient will be monitored by serial Doppler measurements, as the peak systolic flow velocity (PSV) of the middle cerebral artery (MCA) is considered the most accurate noninvasive predictor of fetal anemia.[30,43-48]

Until the 1960s, no prenatal treatment options for severe HDFN were available. The only possible intervention in case of suspected fetal anemia was to deliver the baby

prematurely, to enable neonatal treatment. HDFN was until then a major cause of perinatal mortality. In 1963, Liley described the intrauterine intraperitoneal blood transfusion (IPT), which considerably reduced mortality rates.[34] However, the outcome of fetuses with alloimmune anemia <26 weeks' gestation and of those with hydrops remained poor (32 and 42%, respectively).[49] In 1981, direct intravascular intrauterine transfusions by fetoscopy (IUT) were first described,[50] with initial survival rates around 85%.[51] In the years that followed, ultrasound guidance gradually replaced fetoscopy[52] and since then, intravascular IUT has been the cornerstone of treatment for fetal anemia due to red-cell alloimmunization.[50,51,53] This review focusses on the current transfusion techniques, complications and promising non-invasive treatment options for HDFN.

PRENATAL TREATMENT OF HDFN

Intravascular intrauterine blood transfusion (IUT)

Transfusion preparation and details

Indication

IUT should be urgently performed if MCA-PSV Doppler exceeds 1.5 multiples of the median (MoM) and/or if signs of hydrops are present, as both correlate strongly with moderate to severe fetal anemia.[30,39,46] Timing of subsequent IUTs can be done by calculating the expected decline in hematocrit and by MCA-PSV Doppler measurements.[54] Nowadays, since the prediction using the MCA Doppler is highly reliable, fetal blood sampling is preferably directly followed by IUT and not performed as a diagnostic tool without blood available for immediate transfusion.[55,56] However, the degree of fetal anemia, assessed by the hemoglobin concentration in the pre-transfusion fetal blood sample, finally sets the conclusive IUT indication. The cut offs used for this decision differ amongst the various fetal therapy centers. However, authors agree that IUT should only be performed in case of moderate to severe anemia, usually defined as hemoglobin concentrations of four to five standard deviations below mean/median for gestational age[23,57-59] or a hemoglobin deficit of 5 g/dL or more.[60,61]

Setting

In the Leiden University Medical Center (LUMC), the Dutch national referral center for fetal therapy, the operating team performing IUTs is composed of a staff-perinatologist, an experienced ultrasonographer and one or two operating nurses.[55] This corresponds

to the approach of other centers, although in some centers an additional perinatologist or pediatrician is present.[62-64]

Authors agree that IUTs need to be performed under aseptic conditions, guided by continuous ultrasound/Doppler, using a 20-22 gauge needle [55,62,63,65,66]. No data are available on the influence of needle size on procedure complications.

Premedication

Maternal premedication varies from local anesthetics only to routine indomethacin and/or pethidine/promethazine to combined spinal epidural analgesia,[55,58,63] the latter being used to facilitate an emergency caesarian section if needed. There is no expert uniformity or scientific evidence supporting routine use of prophylactic antibiotics or corticosteroids[60,63,67,68] at IUT and these prophylactic measures are not routinely used at our center.[55]

Fetal premedication consists of an intramuscularly (or intravenously) administered paralytic agent and/or fetal pain medication. Because of the reported lower risk of complications following IUT when applying fetal paralysis in all cases, routine use is advocated.[55,57] For fetal paralysis, atracurium (0.4 mg/kg), vecuronium (0.1 mg/kg) or pancuronium (0.1 mg/kg) are the most commonly used products.[69-72] Atracurium or vecuronium are often used as first-line premedication option due to the fact that these short-lasting agents give sufficient paralysis for IUT completion. Furthermore, pancuronium is associated with several cardiovascular side-effects.[73]

As the neurologic basis for nociception is present from 24-28 weeks' gestation and hormonal and circulatory stress responses have been reported from as early as 18-20 weeks', fetal analgesia should be considered when performing invasive fetal procedures.[74] Authors advocate 10 µg/kg fentanyl to reduce the fetal stress response and possible fetal pain sensation.[74] However, other authors have found that these fetal hemodynamic and stress hormone changes are more likely to be caused by volume expansion than by fetal stress, as the response was independent of insertion site.[75,76]

Transfusion volume

The transfusion volume is calculated by the method described by Rodeck in 1984,[51] making use of estimated fetoplacental volume (V), fetal hematocrit in pre-transfusion

sample (Ht_1), donor blood hematocrit (Ht_2) and the aimed fetal hematocrit post-transfusion (Ht_3):

$$Transfusion\ volume = V(Ht_3 - Ht_1)/Ht_2$$

Examples of used calculations for computing the fetoplacental volume (V) are:

- *0.1 mL volume/g of estimated fetal weight,*[77] or
- *0.15 mL volume/g of estimated fetal weight,*[78] or
- *1.046 + (fetal weight in grams) x 0.14.*[79]

In order to simplify these formulas, Giannina et al.[77] introduced a simplified equation and compared this to previously described methods:[79,80]

Transfusion volume = 0.02 x target increase in fetal Ht per 10% x g of estimated fetal weight,

assuming that donor blood hematocrit is approximately 75%. This equation was shown to be equally accurate as the formula introduced by Rodeck and is therefore very useful as a simplified calculation method for transfusion volume.[51,77] Target hematocrit should be around 45%.[55,68,72,78,80]. Furthermore, fetal hemoglobin (Hb) testing prior to IUT is nowadays often used to precisely calculate the volume to transfuse.

Blood source

Intrauterine transfusions are usually carried out with O-negative, washed, irradiated, leukocyte depleted blood, negative for the antigens against which the mother is immunized.[55,81] In the Netherlands, donor blood for IUTs is additionally matched with the maternal Duffy, Kidd and S blood group, to reduce the high risk on the formation of new antibodies.[82] Donations are usually from an allogenic donor, as multiple maternal blood donations have been associated with adverse pregnancy outcome,[83,84] although a direct cause–effect relation seems unlikely.[85] Altogether, proposed advantages[69,81,85,86] usually do not outweigh these possible adverse effects of autologous donations.

Simple vs. exchange

Intrauterine exchange transfusion (IUET) has been proposed as an alternative to simple IUT as exchange transfusions may result in a more stable hematocrit[87], potentially decrease the risks of (temporary) volume overload and increase the interval between

procedures. However, the risk of procedure-related complications associated with IUETs may be higher, due to longer duration and needle movements.[88] Furthermore, the excess volume after simple IUT is thought to exit the intravascular compartment, decreasing the risk of volume overload and fetuses seem to tolerate single IUT quite well.[89,90] In a recent (relatively small) cohort study in which IUT and IUET were compared,[62] no differences in benefits or complications were found. However, data on the duration of the procedures were not available. Nowadays, most fetal therapy centers opt for simple transfusions rather than exchange transfusions.

Puncture site

Possible puncture sites or procedure access sites are: intrahepatic, placental cord insertion, transamniotic 'free loop' needling, intraperitoneal and (exceptionally) sites as the fetal heart or chorionic plate vein.[57] All transfusions should be aimed intravenously, as arterial punctures are associated with high complication rates.[57,91] The fetal liver and placental cord insertion are shown to be the safest puncture sites, whereas free loop needling is a higher risk procedure and should in our view best be avoided.[57,58,66-68]

Authors have postulated a beneficial effect of combined intravascular and intraperitoneal transfusion on the inter-procedure interval.[89] In our center, the liver gained more and more popularity as a puncture site in the last decades and currently 57.1% of transfusions are performed intrahepatic, frequently combined with intraperitoneal transfusion. Analysis of the effect of this combined technique on transfusion interval is planned. Our recent cohort study showed that transplacental cord punctures were nowadays performed in 41.3% of procedures and a free loop of cord was the chosen puncture site in only 1.1% of IUTs. In 0.5% of procedures, blood was transfused intraperitoneally only.[57]

Outcome

Many fetal therapy centers have reported on their IUT results in recent years. Table 1 contains a summary of survival after intravascular IUT from a selection of studies published in the last 10 years. Reported live birth rates after IUT vary from 81.9-100%.[57,58,64-68,92-94]

(Procedure-related) complications

Possible complications during or following IUT are: bleeding from the puncture site, cord occlusion, brady- or tachycardia and PPROM or preterm (emergency) delivery.[91,92] Furthermore, an intrauterine infection might be diagnosed following any invasive

procedure. These IUT complications may lead to maternal morbidity, an emergency cesarean section (CS) or even fetal death.

Table 1. Overall survival after intrauterine transfusion

Author, year	N	Hydrops (%)	GA at first IUT[a]	Technique	Preferred puncture site	Overall survival (%)
Somserset, 2006	221	26.9	25 (16-32)	IUST	Liver	91
Weisz, 2009	154	11.1	*26 (-)*	IUET	-	88,9
Tiblad, 2011	284	11.8	-	IUST	Liver	91.8
Johnstone-Ayliffe, 2012	114	13	26 (17-35)	IUST	Liver	93.5
Birchenall, 2013	256	-	30 (16-35.4)	-	Liver or PCI	95.3
Walsh, 2013	242	16	29.1 (19.2-34.4)	-	PCI	95.1
Pasman, 2015	135	14	-	IUST	PCI	100
Sainio, 2015	339	11.5	29 (18-36)	-	Free loop	96.2
Deka, 2016	303	21.6	*26.9 (19.7-33.8)*	-	PCI	96.1
Zwiers, 2016[b]	937	12.9	27 (16-36)	IUST	Liver	97
Overall						***95.2***

N: number of transfusions; GA: gestational age; IUT: intrauterine transfusion; Overall survival: live birth rate; IUST: intrauterine single transfusion; IUET: intrauterine exchange transfusion; PCI: placental cord insertion.
[a]weeks, median (range) or *mean (range).*
[b]result of cohort since 2001 shown.

We recently reported the largest cohort study on procedure-related complications after 1678 IUTs (741 unto 2000 and 937 from 2001 onwards).[57] We found a 1.2% procedure-related complication rate per procedure in the new cohort (3.3% per fetus), compared to 3.4% per procedure (9.8% per fetus) before 2001 (P=0.003 and 0.001, respectively). In experienced hands, 1.8% of fetuses died as a direct result of the procedure, indicating a 0.6% procedure-related fetal demise rate per procedure. Refraining from fetal paralysis, arterial and free loop needling were found to be important risk factors for adverse outcome.[57] Furthermore, operators should perform at least 10 IUTs per year to retain their competence.[95]

A summary of (procedure-related) complications in recent studies is shown in Table 2.[57,58,65-68,92,93]

IUTs performed early in the second trimester, before 20-22 weeks of gestation, carry substantially higher risks on (procedure-related) complications and fetal loss compared to later IUTs, due to technical challenges of early procedures and severity of disease

[57,66,91,96]. Reported survival rates for IUT series started before 20-22 weeks lie around 76-88%.[96-99] Lindenburg et al. found a fourfold risk of perinatal death after IUTs <20 weeks' gestation, compared to IUTs later in gestation.[98]

Table 2. Procedure-related complications and fetal loss

Author, year	N	PR complications (%)	Fetal loss (%)	PR fetal loss[a] (%)
Somserset, 2006	67/221	-	2.1	-
Weisz, 2009	54/154	-	11.1	-
Tiblad, 2011	85/284	16.5/4.9	5.9	4.7/1.4
Johnstone-Ayliff, 2012	46/114	13/5.2	6.5	2.1/0.9
Pasman, 2015	56/135	3.6/1.5	0	0
Sainio, 2015	104/339	23.1/7.1	3.8	3.8/1.2
Deka, 2016	102/303	8.8/3	3.9	4.9/1.65
Zwiers, 2016[b]	334/937	3.3/1.2	3	1.8/0.6
Overall	***848/2487***	***7.8/2.7***	***3.9***	***1.9/0.8***

N: number of fetuses/transfusions; PR: procedure related.
PR complications: infection, PPROM or preterm delivery within 7 days, emergency cesarean section, fetal loss. Numbers shown per fetus/per procedure.
[a]per fetus/per procedure.
[b]result of cohort since 2001 shown.

To improve the perinatal outcome in this specific group with fetal anemia early in the second trimester, several alternative strategies have been proposed. The intrahepatic transfusion route is probably the preferred route, as the surrounding tissue makes it easier to keep the needle in place, despite the small vessel size in early pregnancy (<3-5 mm before 20 weeks' gestation).[66,78] Some authors promote intraperitoneal transfusions instead[100] or noninvasive treatment options to postpone early intravascular transfusions.[98]

Non-invasive options

Several non-invasive treatment options have been proposed to postpone IUT in early severe HDFN, not as a sole treatment if fetal anemia is already present.[78]

Therapeutic plasma exchange (TPE)

In TPE, the patient's plasma is removed and replaced with albumin-rich fluid by passing the patient's blood through a cell separator.[101] The maternal antibodies directed against fetal red cell antigens are then removed. TPE may cause a decrease in antibody titers of as much as 75%, resulting in a reduction of the risk of fetal hemolysis.[101,102] However,

the beneficial value of TPE alone to postpone IUT in early severe HDFN was found to be disappointing.[78,98,101-103] The deficiency of therapeutic plasma exchange as a single treatment is possibly due to a rebound effect, causing a rapid rise in antibody levels to amounts equal as or higher than before TPE was performed, even if the pheresis is continued.[98,102]

Although the use of TPE is considered safe in pregnancy,[101] side and adverse effects do occur. For example, an increase in antibody-dependent cell-mediated cytotoxicity after TPE has been described, apart from the above-mentioned rebound phenomenon of antibody concentrations.[104] Second, placental blood flow might be altered during TPE, as fluctuations in pressure or electrolyte levels may cause variations in maternal blood pressure. Furthermore, with maternal serum extraction, coagulation factors and immunoglobulin levels in maternal blood fall, causing increased risks on postpartum hemorrhage and maternal and neonatal infections.[103,105]

As all available knowledge regarding TPE for severe HDFN is derived from observational case series, only category III recommendations can be made and decision-making should be individualized.[101] The decision to apply this technique for postponing an early IUT could be made in cases with a history of severe HDFN and should be individualized. Authors agree that, if at all, it is best used combined with intravenous immunoglobulins (IVIG), as described below.[105,106]

Intravenous immunoglobulins (IVIG)

The effect of IVIG in HDFN may result from various mechanisms including (1) inhibition of Fc-mediated antibody passage across the placenta, (2) negative feedback on maternal antibody production and/or (3) reticuloendothelial Fc-receptor saturation/blockage, amongst others resulting in decreased uptake of opsonized fetal cells by macrophages.[102,107-111] Although IVIG may prevent or reduce fetal hemolysis, it does not treat fetal anemia.[112]

In most fetal therapy centers applying IVIG, it is started at 400 mg/kg maternal weight/day for 5 consecutive days, repeated every 2-3 weeks.[108,110,112,113] An alternative regime could be 500 mg-1 g/kg maternal body weight weekly.[106,114] At our center, the first administration of IVIG is preferably planned in the 12th week of pregnancy in an outpatient setting usually at a dose of 500 mg/kg, followed by weekly infusions of the same dose. These follow-up infusions are offered as a home service by Sanquin Diagnostics, the national

blood and plasma product supply organization in the Netherlands, reducing the burden of frequent hospital visits.

Few fetal therapy centers administrate IVIG directly to the fetus after gaining intraperitoneal or intravascular access.[115-117] The increased volume given to the fetus could lead to cardiac compromise.

IVIG as a sole treatment to postpone early IUTs has shown promising results in several case series.[111,113] The only prospective study on maternal IVIG administration was performed by Margulies et al. and reported on 24 severely Rh-sensitized patients. In total, three fetal demises occurred (12.5%), in hydropic fetuses. IVIG treatment caused a significant decline in anti-D titers and hemolysis rate and even averted invasive IUT in this severely affected group if started before 28 weeks' gestation. Nevertheless, they concluded that for hydropic fetuses and for fetuses with advanced fetal anemia, IUT is inevitable.[118] In a retrospective study of the same group, patients receiving IVIG before 20 weeks had significantly less hydropic fetuses and a lower fetal mortality rate compared to patients treated with IUT alone.[112]

However, in another small series of four cases of severe RhD immunization, IVIG did not seem to have any effect on transfusion frequency, maternal antibody titers or hydrops.[119] Recently, a prospective case-control study in 34 women compared IUT with fetal IVIG infusion to IUT alone and described a slower hematocrit decline after IUT in the IVIG group.[117] Similar results were found before, but no impact on perinatal outcome was reported.[115,116]

An occasionally used strategy is the combination of IVIG and TPE. This treatment strategy is thought to oppose the previously mentioned rebound effect of TPE alone and might intensify the effect of IVIG and TPE on perinatal outcome. IVIG transfer to the fetus in HDFN is thought to start from 10–12 weeks' gestation, supporting treatment schedules starting from this gestational age.[102] A possible schedule could be: 3 serial TPE treatments in the 12th week of pregnancy, followed by weekly IVIG administration,[106] although evidence for this schedule is scarce.[120] Authors agree that this combined technique should be reserved for the most severe cases. The largest series reports on 9 severe HDFN cases, in which a combined regimen of three TPE procedures and weekly IVIG was used. Maternal antibody titers were reduced, IUT seemed to be postponed and all babies

were alive and well at birth.[106] Several case reports have been published with favorable outcome following this combined approach.[114,121-125]

Reported side effects of IVIG are rare but may include: headache, fever, myalgia and low back pain, rush or chills, urticaria, nausea and vomiting, tachycardia, chest tightness, hypotension and shortness of breath.[72,78,126,127] These events usually occur 30-60 min after admission and especially the headache could be prevented by 1000 mg acetaminophen before infusion.[72] Although very rare, renal failure, aseptic meningitis, anaphylaxis (mainly in case of IgA deficiency), hemolytic anemia, thromboembolism and pulmonary edema are described.[78,126,127]

Last, IVIG is an expensive treatment, with reported prices around $6000/week.[128] The costs and benefits of IVIG should be weighed against those of early IUT. Importantly, early IUT may result in more complications, which also carries along additional costs.[98] Furthermore, IVIG treatment might even prevent perinatal deaths to occur, a situation associated with a high psychological burden and of which the costs are difficult to quantify.

Recommendations are based on observational studies only and are therefore weak. However, there seems to be a beneficial effect of IVIG (with or without TPE) to postpone early transfusions and therefore it should be considered in patients with a history of severe HDFN.

EXPERT COMMENTARY

In the past decades, improved prevention and screening strategies have greatly reduced the incidence of severe HDFN and it is nowadays considered a rare disease. Consequently, yearly IUT numbers per fetal therapy center decrease and centralization of this highly specialized care becomes more and more important. Fetal therapy centers from all over the world collaborate and invest in high-quality research to deliver the best possible patient care in this field. Not only did the incidence of HDFN decline, a dramatic improvement in prognosis of HDFN was achieved. With the introduction of intravascular IUTs and the availability of valuable noninvasive diagnostics for fetal anemia, survival increased remarkably. Several adjustments in transfusion technique, such as transfusing through the intrahepatic vein or placental cord insertion instead of using a free loop of cord and applying routine fetal paralysis, have further improved outcome. Procedure-

related complication rates are currently as low as 3.3% per fetus and 1.2% per procedure in experienced hands.[57] Nonetheless, preventable fetal losses do occur, especially in fetuses in need for IUT before 20–22 weeks.[98] A truly evidence -based noninvasive approach to further reduce fetal loss rates is however not yet available. Nevertheless, the use of intravenous immunoglobulins to postpone these hazardous early IUTs, potentially combined with TPE, shows promising results in case series and single center case-control studies.[112,118] Therapeutic plasma exchange alone seems unable to create similar results.

FIVE-YEAR VIEW

Ideally a randomized controlled trial should be performed to assess the efficacy and benefits of IVIG in early severe HDFN. However, the auspicious results of IVIG published so far have possibly made it unethical to randomly assign patients with a history of severe HDFN to a 'non-IVIG' study group. Therefore, an international multicenter cohort study on the effect of IVIG is currently performed, in which IVIG cases are compared to a reference group with similar disease severity but without IVIG treatment. Depending on the results of this study, a randomized trial could still be considered, taking above-mentioned ethical dilemmas into account. If IVIG treatment proves to be disappointing, other options such as stem cell treatment and gene therapy will be investigated.

KEY ISSUES

- Although prophylaxis has strongly reduced the incidence of maternal immunization against fetal red cell antigens, HDFN is still a serious pregnancy complication which may lead to severe fetal anemia, hydrops and perinatal death.
- Intravascular intrauterine transfusions are the cornerstone in prenatal management and have significantly improved perinatal outcome in the past decades.
- Several risk factors for adverse outcome after IUT have been defined, such as refraining from fetal paralysis, arterial puncture and free loop needling. Avoiding these hazardous techniques causes continuously rising survival rates worldwide.
- Performing IUTs before 20-22 weeks' gestation greatly increases the risk on complications.
- The use of IVIG is an emerging non-invasive strategy to postpone these early IUTs. More research is needed to support its assets.