Intrauterine blood transfusion : indications, risks, quality control and long-term outcome

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Chapter 9

Summary, general discussion and future perspectives

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Fetal anemia is a serious complication in pregnancy, and is associated with perinatal mortality and morbidity. One of the major advances in the management of fetal anemia was the introduction of the intrauterine blood transfusion (IUT). This thesis presents several studies on IUT for fetal anemia including current indications and complications, analysis of the learning curve for IUT, and long-term outcome. In this chapter, all studies are summarized and then generally discussed according to different subjects, followed by future research perspectives.

Summary

In **Chapter 2,** current indications and complications of IUT are reviewed. In addition, we summarized known policies to improve outcome after IUT, such as the use of fetal paralysis, avoiding arterial puncture and preventing the development of fetal hydrops.

In **Chapter 3**, we analyzed perinatal loss after IUTs performed before and after 20 weeks of gestation and the contributing factors. 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). In this study, a total of 1422 IUTs were performed in 491 fetuses. Perinatal loss rate in Group 1 was higher than in Group 2 (24% versus 8%). Especially the not procedure-related loss rate was higher for IUTs performed before 20 weeks, compared to later IUTs (11% versus 1%). In a multivariate regression analysis only fetal hydrops appeared to be independently associated with perinatal loss. In more recent years, a significant overall decline in perinatal loss was found, however, in the group receiving IUTs before 20 weeks we found almost no reduction in perinatal loss over time. We conclude that IUTs 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 to be the presence of hydrops.

The aim of the study described in **Chapter 4** was to test the feasibility of CUSUM (Cumulative SUM) analysis for quality control in performing IUT. Formerly, CUSUM was designed to control the performance of industrial processes. Later on, the CUSUM method

has been increasingly used in medicine for quality control, and more recently for the assessment of learning curves. In this study, CUSUM was used to estimate learning curves for IUT and as a method to monitor individual performance after completing the learning process. All IUTs performed at our center from 1987-2009 were retrospectively classified as successful or failed. Individual CUSUM graphs were easily assessed and presented for 4 operators, who altogether performed nearly 1200 IUTs at our center. We showed that learning curves of an operator learning IUT in an experienced team is shorter than operators pioneering IUT, which urges the importance of centralization of knowledge and skills in order to create an optimal climate for trainees starting to learn this technique.

The full study protocol of the LOTUS study (LOng Term follow-up after intra Uterine transfusionS) is described in **Chapter 5**. All children treated with IUT for severe alloimmune anemia from 1988-2008 in the Leiden University Medical Center were invited to participate. All children were tested for their neurological, cognitive and psychosocial development, using standardized tests and questionnaires according to the children's age. The primary outcome was neurodevelopmental impairment (NDI), a composite outcome defined as any of the following: cerebral palsy, cognitive or psychomotor development < 2 standard deviation, bilateral blindness and/or bilateral deafness. Secondary, potential risk factors for NDI were investigated.

Chapter 6 reports the incidence of long-term NDI after IUT treatment for red cell immunization in the LOTUS study population. Secondary, potential risk factors for NDI were determined, including severity of fetal anemia, presence and severity of fetal hydrops at the start of intrauterine treatment, number of IUTs, gestational age at birth, severe neonatal morbidity and perinatal asphyxia. A total of 291 children were evaluated at a median age of 8.2 years (range, 2–17 years). Cerebral palsy was detected in 6 (2.1%) children, severe developmental delay in 9 (3.1%) children, and bilateral deafness in 3 (1.0%) children. The overall incidence of NDI was 4.8% (14/291). Several risk factors were found to be associated with NDI, including fetal hydrops and hemoglobin level at first IUT, number of IUTs, prematurity, and severe neonatal morbidity. Mean parental education was significantly lower in the NDI group compared with the no-NDI group. In a multivariate regression analysis, including prenatal and postnatal factors, the following risk factors were independently associated with NDI: number of IUTs performed, occurrence of severe neonatal morbidity and parental education. In a multivariate regression analysis including just preoperative risk factors, only severe hydrops was independently associated with NDI. Our findings suggest that the prevention of risk factors might improve long-term outcome.

Chapter 7 presents the long-term neurodevelopmental outcome in a relatively large cohort of children treated with IUT for fetal anemia due to parvovirus B19 (B19V) infection in pregnancy. The objective of this study was to evaluate the long-term neuromotor and cognitive development of children treated with IUT for severe anemia induced by intrauterine B19V infection. Primary outcome was a composite outcome termed neurodevelopmental impairment (NDI), defined and determined as described previously (Chapter 5). Secondary outcome was presence of minor neurological dysfunction. Presence of postnatal brain injury on neonatal cerebral imaging was recorded if available. NDI was present in 3 of 28 (11%) children. We conclude that NDI after B19V infection of the fetus treated with IUT is not an uncommon finding, and the incidence of severe developmental delay (11%) was higher compared with the Dutch normative population (2.3%). Similarly, the incidence of cerebral palsy (4%) was higher in this group than in the general population born at 32 to 36 weeks' gestation (0.7%) and at or after 37 weeks' gestation (0.2%).

In **Chapter 8** a review of the literature is presented that focuses on long-term neurodevelopmental and cardiovascular follow-up of survivors after IUT. The review is structured according to different but most prevalent IUT indications. Cohort studies from the literature evaluating the long-term outcome of fetal anemia treated with IUT were available for the indications maternal red cell alloimmunization and B19V infection. For other indications such as fetomateral hemorrhage (FMH) and twin-anemia-polycythemia-sequence (TAPS), data on long-term neurodevelopmental outcome are limited. The current knowledge on cardiovascular outcome after fetal anemia is also described in Chapter 8.

Discussion

Current indications and associated risks

The main indication for IUT is still fetal anemia due to red cell alloimmunization, with survival rates exceeding 90% in specialized centers worldwide [1-4]. IUT has also been reported to be successful in fetal anemia related to nonimmune causes, such as B19V infection, FMH, TAPS, twin-to-twin transfusion syndrome (TTTS), inherited red cell disorders and fetal/placental tumors.

IUT is a challenging and complex procedure, and can be associated with short-term and long-term complications. The most important short-term complication of IUT is perinatal death. Procedure-related fetal loss ranges from 0.9 to 4.9% per procedure [1-7]. Other short-term complications after IUT include preterm premature rupture of membranes and

chorioamnionitis. However, both complications appear to be extremely rare; 0 - 1.3% [1-3,5] and 0 - 1.0% respectively [1,3,6]. Long-term complications of IUT include formation of additional red-cell antibodies. These antibodies may cause problems in selecting compatible red blood cells for future fetal or maternal transfusions, and are capable of inducing delayed hemolytic transfusion reactions. Avoiding transplacental transfusion and carefully matching (single) IUT donors for immunogenic antigens, may prevent the induction of additional antibodies during IUT treatment for alloimmune anemia [8,9]. However, the mechanism of this immunization phenomenon in pregnant women is still not fully understood.

We found a nearly four-fold risk of perinatal death of fetuses requiring IUT for severe alloimmune anemia before 20 weeks of gestation. The losses after early IUTs were more often not related to the procedure itself, but to a compromised fetal condition prior to an otherwise uncomplicated transfusion. This may be explained by a higher percentage of severely anemic and hydropic fetuses in the early IUT group. This in turn might be related to an overrepresentation of Kell-immunized pregnancies, for which in the Netherlands routine screening in the first trimester was introduced in 1998. Fetal anemia in Kell disease generally develops earlier in gestation, due to the presence of Kell antigens on erythroid precursor cells [10,11]. In a subsequent evaluation of the relation between gestational age and IUT outcome, we again confirmed a strong association of the presence of hydrops and adverse perinatal outcome [12]. Due to the combination of routine first trimester screening for red cell antibodies (including Kell) in all pregnancies, and increased adherence to the advice to use only Kell and Rhesus c matched donor blood for transfusions in women up to the age of 45, late referral of an already hydropic fetus has nowadays almost disappeared. Nevertheless, we suggest that the emphasis for improving outcomes in early severe red cell alloimmunization (especially Kell) should be on immediate referral to specialized centers for the timely detection and treatment of fetal anemia, before hydrops develops.

Concerning alternative therapeutic options for intravascular IUT, such as single intraperitoneal or combined intraperitoneal and intravascular transfusions, maternal intravenous immunoglobulin (IVIG) administration and/or plasmapheresis, no conclusive proof of benefit has yet been demonstrated. Most studies suggest a positive effect of maternal IVIG treatment on preventing the early onset of fetal hemolytic disease, however, IVIG entails high costs per treatment. The possible additional value and cost-effectiveness can only be established by well-designed, appropriately powered international multicenter collaborative studies.

Given the potential short-term and long-term complications, careful individual risk-benefit analysis must be made at the start of IUT treatment. We suggest that an international web-based register to document management, complications and (long-term) outcome of intrauterine transfusions would be extremely helpful to centralize knowledge and skills and further improve the optimal management of all, but especially of rare cases of fetal anemia.

Quality control and Learning curve

In this thesis, we experienced that CUSUM may be easily accomplished and results in clarifying graphs as a visual guidance of individual performance. The operators pioneering IUT in the late 1980s had longer learning phases and this may be explained in the first place by the lack of any experience with this new technique. This accounts not only for those actually performing the procedure, but also for other team members being responsible for the ultrasound-guidance and the preparation of the blood product. In addition, ultrasound equipment and neonatal care facilities improved remarkably during the study period. The 2 operators learning IUT in later years in an experienced team performed acceptably from the start and reached a level of competence after 34 and 49 procedures respectively. They seem to have benefited from the earlier identification of pregnancies at risk and the concommitant reduction in cases of hydrops, resulting from the implementation in the Netherlands in 1998 of a routine red cell antibody screening of all women in the first trimester of pregnancy. In addition, the early diagnosis of fetal anemia was gradually facilitated by the use of middle cerebral artery (MCA) Doppler, allowing an accurate and timely diagnosis of fetal anemia and the start of intrauterine treatment before the development of fetal hydrops [13].

As an experienced operator contributes to a safe and successful procedure and thus to improved outcomes, methods to monitor quality control of performing IUT are essential. CUSUM is a feasible method for monitoring individual learning processes and long-term performance, not only of intravascular IUT but of all fetal therapy techniques, for which acceptable and up-to-date success and failure rates can be defined. A critical consideration of individual and team performance should be recommended for all procedures in fetal therapy.

Long-term outcome

Red cell alloimmunization

In the LOTUS study, we showed that the vast majority of children treated with IUT for alloimmune anemia had normal neurodevelopmental outcome. The incidence of severe

developmental delay (3.1%) was in line with the Dutch normative population (2.3%). In addition, the incidence of bilateral deafness in the general population was similar to what we found in our cohort. However, the rate of cerebral palsy (2.1%) was higher compared with the general population (0.7% at 32 to 36 weeks' gestation, and 0.2% at 37 weeks' gestation). Interestingly, the incidence of cerebral palsy decreased significantly over time, from 5.7% in 1987-1996 (6/106) to 1.1% in 1997-2007 (2/182, p=0.04, OR 2.3), the latter being comparable with the general population rate (*unpublished data*).

Our study shows a clear association between long-term impairment and the severity of the hemolytic disease, i.e. presence of fetal hydrops, fetal hemoglobin concentration, and number of IUTs. The underlying mechanism causing cerebral damage and long-term NDI in hydropic and severely anemic fetuses is not yet clearly established. Cerebral lesions may result from hypoxic injury related to severe (chronic) anemia. Because short- and long-term outcome appear to be better in nonhydropic fetuses, clinicians should try to prevent or reduce the development of hydrops in fetuses at risk for severe anemia. Another risk factor for NDI was severe neonatal morbidity. We found that the occurrence of severe neonatal morbidity and the incidence of NDI were both associated with prematurity. Severe prematurity is a well known risk factor for neonatal morbidity, cerebral injury, and long-term adverse outcome [14-17]. Finally, parental education was independently associated with NDI. Socioeconomic status and parental educational level are well known determinants of child cognitive development [18,19]. The importance of our analysis lies in the determination of potentially avoidable risk factors for long-term adverse outcome, such as prematurity and fetal hydrops.

Parvovirus B19 infection

Impaired neurodevelopmental outcome in children treated with IUT for B19V infection is increased compared to the general population. Our results suggest a possible association between intrauterine B19V infection, fetal cerebral injury and NDI. The causes for an increased rate of NDI after intrauterine B19V infection are still not fully understood and might be related to cerebral injury caused by the infection itself. Several brain imaging studies report a wide variety of cerebral lesions possibly related with B19V infection, including migratory abnormalities [20] and calcification in cerebral cortex and basal ganglia. [21,22]. However, the lack of neuroimaging in all prenatally B19V infected children during the perinatal period hampers our understanding of the possible mechanisms of the neurological injury. Cerebral injury caused by hypoxic-ischemic injury of severe fetal anemia and hydrops may provide an alternative explanation for the increased NDI-rate. Finally, fetal hydrops is a known risk factor for cerebral palsy and severe developmental delay in red cell

alloimmunization, as already described in Chapter 6. The association between neurological symptoms and the neuroimaging findings also remains to be further elucidated. The emphasis is on timely assessment of pregnant women exposed to B19V for the presence of the infection. If the woman has been infected, the fetus should be monitored by ultrasound examination for MCA Doppler measurements and the possible development of hydrops.

Other indications

FMH is defined as the passage of fetal blood into the maternal circulation, and may lead to severe anemia. Other fetal problems include distress, hydrops, hypovolemic shock and death. These pregnancies often present clinically with decreased or absent fetal movements. The fetal heart rate may be sinusoidal, and ultrasound examination often reveals hydrops and increased middle cerebral artery peak systolic velocities (MCA-PSV). A Kleihauer–Betke test for detection of fetal blood in the maternal circulation may aid in the diagnosis of FMH. Perinatal death rate is high and varies between 31 and 50% [23-25]. The outcome of long-term survivors after severe anemia due to FMH is not well established, but includes neurological sequelae [24,25]. Whether outcome could be improved by IUT treatment is not known because of the rarity of this condition and the lack of follow-up studies.

TAPS is a newly described disease in monochorionic twin pregnancies that is characterized by large intertwin hemoglobin differences, without signs of oligo-polyhydramnios sequence [26]. TAPS occurs spontaneously, but also in TTTS cases after laser surgery ('post-laser TAPS'). There are several treatment options in TAPS, including expectant management, induction of labour, selective feticide, (repeated) fetoscopic laser surgery or IUT. IUT treatment is considered as a temporary symptomatic treatment (for the donor) and not as a causal treatment. Transfusing the donor may potentially result in worsening the polycythemia in the other twin, which may also lead to serious complications such as cerebral injury and limb necrosis [27]. Therefore, a combination of IUT to the donor with a partial exchange transfusion (PET) in the recipient twin may be considered [28,29]. Follow-up data on the long-term neurodevelopmental outcome in TAPS are limited. Recently, the first study evaluating long-term neurodevelopmental outcome in children after post-laser TAPS was reported by Slaghekke and collaegues [30]. The incidence of NDI was 9%, without differences between donors and recipients. Larger studies are needed to evaluate all risk factors for adverse outcome and to determine whether IUT is a successful option in the management of TAPS.

Cardiovascular follow-up

Chronic, progressive fetal anemia caused by alloimmunization in pregnancy results in an increase of cardiac output. Chronic cardiac compromise may lead to myocardial hypertrophy. Fetal myocardial hypertrophy in anemia may thus be the result of an increased cardiac workload. Only a few small studies have evaluated the cardiovascular impact of severe fetal anemia after birth. Myocardial hypertrophy in neonates with hemolytic disease was first described in 1990 [31]. Echocardiograms were performed in the first 48 hours of life in ten newborns. In five of these, disproportionate septal hypertrophy was demonstrated. Neonates who had not received IUTs had a higher mean septal to left ventricular freewall ratio than neonates after IUT treatment, which may indicate a protective effect of IUTs, leading to less myocardial mass in childhood.

Parvovirus B19-induced fetal anemia may also lead to cardiovascular sequelae on the long-term, which may result from either severe anemia or viral-induced myocarditis, or a combination of both. However, this is currently speculative and limited by small sample sizes, requiring validation from further studies.

Although there are some data to assess the cardiovascular impact of fetal anemia, no studies on long-term cardiovascular effects of fetal anemia into adulthood have been reported to date. The first survivors after IUT treatment are now older than 30 years. According to the developmental origins of adult disease (Barker) hypothesis, accurate monitoring of the cardiovascular impact and general health in these adults would be of great interest. This is currently being investigated in the ANAEMIA study at the University of Auckland in New Zealand, the same place where Sir William Liley actually performed the first in utero transfusion in 1963 (http:// www.liggins.auckland.ac.nz/uoa/anaemia-study).

Conclusions

From the studies described in this thesis, we conclude that after more than 20 years of improved knowledge and skills, IUT is to be considered a safe and successful method to treat severe fetal anemia for different causes. The vast majority (over 95%) of children treated with IUT for severe alloimmune anemia have a normal neurodevelopmental outcome, confirming the success of this antenatal treatment.

The strongest association of adverse perinatal and long-term outcome appears to be with fetal hydrops. Hence, for improving outcome, clinicians should try to prevent hydrops in fetuses at risk for fetal anemia by early identifying pregnancies at risk, timely referral to specialized centers, frequent monitoring and starting treatment in early stages of the disease.

Centralization of knowledge and skills is of great importance in order to maintain successful outcomes and to create the optimal management of fetal anemia detected in pregnancy for any indication. This indicates the need for an international register to document management, complications and (long-term) outcome of cases. Outcome data of neonatal and long-term outcome are extremely important in counselling parents considering IUT. Learning IUT for new operators in an experienced team leads to shorter learning phases, which confirms the importance of centralized care of fetal anemia.

Future research perspectives

Despite significant improvement during the last decades, the optimal management of fetal anemia remains challenging. Moreover, several research questions are still unanswered. This paragraph focuses on future research perspectives.

Prenatal management

Alloimmunization

In July 2011, an addition to the national screening program has been implemented in the Netherlands: a second antibody screening for Rhesus c at 27 weeks of gestation. The clinical impact and evidence of the introduction of extra screening for Rhesus c antibodies on perinatal survival was recently evaluated by Y Slootweg et al., and appears beneficial and cost-effective (*personal communication*).

As IUT in very early gestation is generally still associated with increased perinatal loss, prospective, ideally double blind, randomized controlled trails are necessary to evaluate the benefits and adverse effects of immunoglobulins (IVIG) for the (early) treatment and prevention of fetal alloimmune anemia.

Timing of subsequent IUT

Without additional risks to the fetus or mother, serial MCA-PSV measurements are most predictive for fetal anemia. After the first IUT, fetal erythropoiesis is suppressed and the majority of circulating red cells are adult donor cells. This alteration may affect the correlation between the MCA-PSV and the fetal hematocrit. The reliability to apply MCA-PSV for determining the need for subsequent IUTs will likely be clarified by the still ongoing international multicenter 'MCA Doppler Study' (http://www.adelaide.edu.au/arch/research/clinical_trials/MCADoppler/). A few (older) studies suggest advantages of a combined intravascular and intraperitoneal transfusion: mainly longer transfusion intervals [32,33]. More evidence is needed to elucidate the optimal timing of IUT and to determine possibilities to decrease the number of IUTs and associated risks per pregnancy.

Parvo B19V

In parvovirus B19 (B19V) infection, cost-effectiveness studies on universal screening and/or a possible vaccination strategy to prevent perinatal morbidity and mortality of intrauterine infection would be of great value. In case of infected pregnancies, future studies should focus on a reduction of the high rate of fetal hydrops due to B19V infection. A reduction of fetal hydrops by early recognition may lead to an improvement of both short-term as well as long-term outcome. Adverse long-term outcome after B19V infection of the fetus treated with IUT is not an uncommon finding. To increase our knowledge and understanding of the possible deleterious effects of B19V infection in fetuses, international collaboration between study groups involved in the care of these children, is of great importance. In this respect, studies on neuroimaging in fetuses or neonates with anemia due to B19V are required to gain further insights in central nervous system damage.

Quality control of performing IUT

The basic technique for performing IUT is to insert a thin needle safely and accurately into the fetal circulation, and the operator preferably must have both ultrasonographic experience and excellent hand-eye coordination. More (CUSUM) studies are needed to estimate individual learning curves for IUT with up-to-date predefined limits for acceptable/unacceptable performance. CUSUM should ideally be used in future prospective studies, both as a tool for assessing learning curves and for continuous monitoring of individual performance. CUSUM is eligible for all invasive procedures in pregnancy, for which success and failure rates can be clearly defined.

Neonatal management

The mainstays in postnatal treatment for hemolytic disase of the newborn are intensive phototherapy, exchange transfusion (ET) and top-up transfusion. The introduction of the national restrictive ET criteria in 2005 at our center, led to a significant decrease in the rate of ET from 70% to less than 20% [34]. Although the mortality rate associated with ET is nowadays lower than 0.5%, the morbidity rates remain high [35]. Potential risks associates with ET include neonatal infection, thrombotic events and electrolyte imbalance. Further research is needed to reduce the risks of ET. Several pharmacological antenatal management options such as corticosteroids, phenobarbital and the earlier mentioned IVIG might play an important tole in the reduction of ET and warrant further research. This reduction of ET has led to an increase of top-up transfusions during the first 3 months of life,

especially in neonates treated with IUTs [36]. More studies are needed on the use of erythropoietin to prevent late anemia and to reduce the need for top-up transfusion [37].

Long-term outcome

The risk of neurodevelopmental impairment after IUT appears to be low. However, in specific risk groups including those with fetal hydrops and parvo B19 infection, the incidence of adverse neurodevelopmental outcome is still higher compared to the general population. Further research to determine the optimal management in these high-risk groups is still warranted and should always include long-term follow-up. As neurological sequelae in children after IUT treatment for anemia is not the only clinical meaningful outcome, more studies are needed to determine school performance and quality of life of these children. More research is also required to investigate the cardiovascular impact of fetal anemia into adulthood en general health.

High red cell antibody titers during pregnancy together with new antibody formation after IUT are likely associated with a higher human leukocyte antigen immunization rate or so-called ‘high respondership’ [38]. The clinical relevance and consequences of leukocyte antibodies are not very clear as both harmful and beneficial effects have been described. Prospective studies are required on the long-term immunohematologial sequelae after IUT treatment.

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