Hemolytic disease of the fetus and newborn

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GENERAL INTRODUCTION

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Hemolytic disease of the fetus and newborn (HDFN), caused by maternal red cell alloimmunization, has long been a major cause of perinatal morbidity and mortality. No antenatal treatment was available up to the 1960s. The only option was thus (preterm) induction, to enable neonatal treatment. This changed with the introduction of intrauterine intraperitoneal transfusion in 1963 by professor William Liley. However, in the early years, the complication risk of this X-ray guided intraperitoneal procedure was substantial. Outcomes gradually improved with more experience, the introduction of ultrasound-guided intravascular transfusions in the late 1980s, and advances in neonatal care.

Nowadays, both the incidence and risks of (antenatal treatment of) HDFN seem to have reached an 'as good as it gets' state in developed countries. In this thesis we aimed to summarize and evaluate current best practice, but often expert-based, management of HDFN. Furthermore, we assessed factors influencing the immunization risk and the severity of disease and evaluated standard and alternative treatment options.

In this chapter, an introduction to red cell alloimmunization and hemolytic disease of the fetus and newborn is provided.

RED CELL ALLOIMMUNIZATION

The origin of red cell alloantibodies

Red cell alloimmunization results from fetal-maternal blood group incompatibility: the fetus carries a red cell antigen, inherited from the father, that is unknown to the maternal immune system. Over 300 blood groups have been discovered so far, contributable to over 30 blood group systems[1] and incompatibilities between mother and child can exist in every single one of these blood groups. Apart from the ABO blood group system, the Rh(esus) system is the most well-known. It was discovered in the Rhesus macaque in the 1940s by Landsteiner and Wiener. This scientific milestone marks a major breakthrough in HDFN research and is therefore memorialized by the schematic illustration of a Rhesus macaque on the cover of this thesis.[2]

Fetal-maternal blood group incompatibility may lead to alloimmunization when fetomaternal hemorrhage (FMH) occurs during pregnancy or delivery (Figure 1). It is

known that FMH (often microtransfusions) can be detected in 45% of uncomplicated pregnancies during the third trimester and after 60% of deliveries.[3]

ABO incompatibility between mother and child is known to have a protective effect, reducing the chance of the alloimmunization. In the 1940s, Levine et al. already postulated this preventive effect when they noted that ABO incompatibility was less common among couples with D (formerly known as RhD) immunization in pregnancy, when compared to uncomplicated pregnancies.[4] In this thesis, we aimed to evaluate whether ABO incompatibility is also preventive for non-D immunizations (other Rh such as C, c, E or e, non-Rh such as K (Kell), Fy (Duffy), Jk (Kidd), etc.).

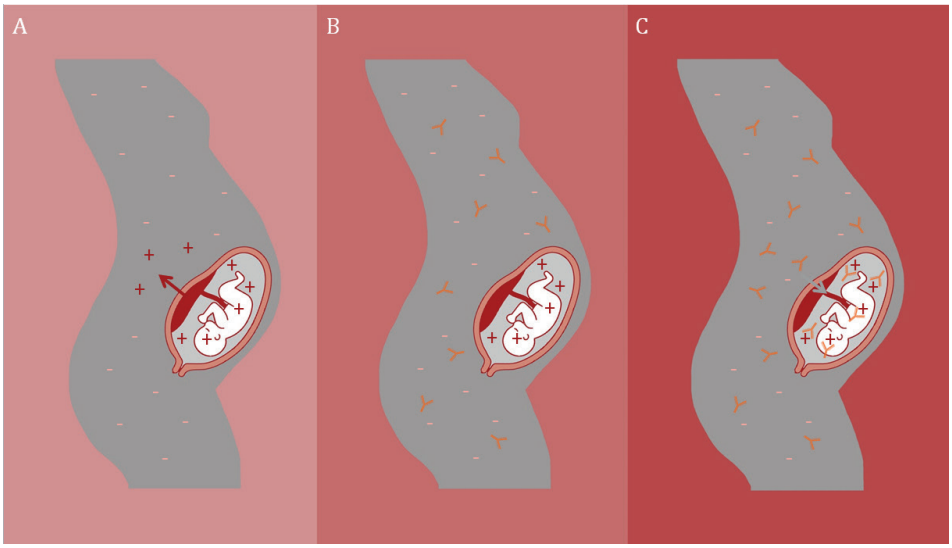

Figure 1. The process of red cell alloimmunization. A: the mother is negative for a blood group antigen, the fetus is antigen-positive. During pregnancy or after delivery, fetal red cells enter the maternal circulation. B: maternal antibodies are formed against the fetal antigen. C: in the same or a next pregnancy, the antibodies are transported across the placenta and bind fetal red cells.

Prevention, screening and incidence

In order to prevent D immunization, D-negative women carrying a D-positive child receive both antenatal (at 28-30 weeks) and postnatal anti-D prophylaxis (RhIg) in most developed countries.[5-7] Targeting the antenatal administration only to women with D-positive fetuses became possible with the introduction of cell-free fetal genotyping in maternal plasma.[8] This test can prevent that 40% of D-negative women unnecessarily receive antenatal RhIg, and is therefore progressively implemented throughout the developed world.[9,10]

Another common measure to prevent alloimmunization is matching blood transfusions for women of reproductive age for the D antigen, and, in the Netherlands, additionally for Kell, c and E.[11,12]

To date, the working mechanisms of RhIg remain partially unclear. To evaluate whether RhIg might function in a non-epitope specific manner, and because no prophylactic agent is available to prevent non-D immunization, the effect of RhIg on the occurrence of non-D antibodies was assessed in this thesis (Chapter 2).

As a result of the abovementioned prophylactic measures, the D immunization rate after delivering one D-positive child has decreased dramatically over the past decades, from 5.0% in the 1960s to 0.3% in 2008.[5,13] The prevalence of D immunization amongst all pregnant women was 0.09% in 2016, based on our nationwide prospective study (this thesis, unpublished data) and the total number of screened pregnant women.[14] To timely identify these pregnancies in which alloantibodies associated with HDFN (mainly Rh and K antibodies) do occur, all pregnant women are screened at the time of the first antenatal visit (preferably before 13 weeks' gestation)[15] as part of our free national population screening programme carried out by the National Institute for Public Health and the Environment (RIVM). Within this programme, D-negative women are again screened at 27 weeks, before the prophylactic administration of RhIg. In addition, in the Netherlands, c-negative pregnant women are also screened for relevant antibodies at 27 weeks.

RISK OF HEMOLYSIS

Clinically relevant antibodies

The clinical relevance of alloantibodies in pregnancy depends on whether the fetus carries the antigen against which the antibodies are directed, whether it concerns IgG or IgM antibodies (IgM is not transported across the placenta) and on the ability of the antibody to induce hemolysis.[16] In general, severe antenatal HDFN is most frequently caused by anti-D, anti-K or anti-c and only exceptionally by other Rh (E, C) and non-Rh antibodies such as anti-Fy or anti-Jk.[17,18]

Fetal phenotype

The first step to predict whether a pregnancy with alloantibodies is at risk for fetal hemolytic disease, is to determine whether the fetus carries the antigen against which the

antibodies are directed. If the father is found or known to be antigen-negative, no further fetal assessment is advised.[19,20] Otherwise, non-invasive fetal genotyping by polymerase chain reaction (PCR) on cell-free fetal DNA in maternal plasma is nowadays usually the next step. This test is available with a high sensitivity (>95%) for D, C, c, E, (e) and K[21] and can reliably be performed from the first trimester onwards(slightly later in gestation for K-typing). Therefore, the Royal College of Obstetricians and Gynaecologist (RCOG, United Kingdom) nowadays proposes that paternal testing might even be omitted.[22]

Serological testing

Assessment of the risk of fetal anemia in pregnancies with a positive antibody screening and an antigen-positive fetus is usually based on obstetric history and antibody titers, which is the highest dilution with positive agglutination test.[17,23] In the Netherlands, an antibody-dependent cell-mediated cytotoxity assay (ADCC) is additionally performed for risk assessment. This bioassay measures the percentage of hemolysis that the maternal antibodies induce in vitro and shows a higher specificity (and equal sensitivity) compared to antibody titer for predicting fetal anemia in D immunization.[24]

The 'critical titer' to identify pregnancies at risk for hemolysis is set on 1:16 for most (clinically relevant) antibodies in the Netherlands, the ADCC on 10% for D and 30% for non-D antibodies.[20] The ideal titer cut-off for K antibodies was long unclear and therefore set at 1:2. This was evaluated in a recent large study on K immunization in pregnancy, resulting in the recommendation to clinically monitor Kell-positive fetuses if antibody titer rises to or above 1:4.[25]

Recent studies have indicated that antibodies differ in their effector functions, as a result of differences in subclasses and glycovariants.[26-28] Furthermore, IgG-Fc receptor polymorphisms, influencing the clearance of anti-D sensitized fetal red cells, are found to be associated with HDFN severity.[29] These differences might be applicable in the future to predict which pregnancies are at risk for severe HDFN and which will only be subject to mild hemolysis.

Although most authors agree that HDFN severity increases in every subsequent pregnancy at risk, it is not well studied if and how obstetric history predicts outcome. We aimed to evaluate this by performing a nationwide cohort study amongst pregnant women with D immunization (Chapter 3).

MONITORING OF PREGNANCIES AT RISK FOR FETAL ANEMIA

If the results of serological risk assessment tests rise above a set cut-off value, weekly fetal monitoring is indicated. From the late 1990s onwards, peak systolic velocity measurement of the middle cerebral artery (MCA-PSV) blood flow by Doppler is the golden standard for predicting fetal anemia.[30] If MCA-PSV values exceed 1.5 multiples of the median (MoM) for gestational age, intrauterine transfusion (IUT) should urgently be performed, as measurements in this range strongly correlate with moderate to severe fetal anemia (sensitivity 100%, 12% false positive rate).

In the past, hydrops was often the first sign of fetal anemia due to red cell alloimmunization. This was alarming, as hydrops was associated with poor outcome on both the short and the long term.[31,32] The current well-organized health care system of routine early alloantibody screening, national guidelines for management and referral of cases and pooling of expertise in national reference laboratories and a referral center for fetal therapy aims to enable intervention before the development of hydrops. In this thesis, we evaluated how this system influenced hydrops rates and outcome of hydropic fetuses (Chapter 4).

INTRAUTERINE TRANSFUSIONS

The cornerstone in antenatal therapy for fetal anemia is the ultrasound guided intravascular intrauterine transfusion (IUT), replacing intraperitoneal transfusion in the 1980s.[33] In Chapter 1 of this thesis, we summarize the literature on the preparations, setting and (long-term) outcome of this treatment.

Whereas outcome after treatment with IUT(s) was fairly poor in the early years after implementation in 1980s, it is now considered relatively safe in experienced hands. Transfusion techniques and complication rates however vary between fetal therapy centers, although it is know that transfusion into the intrahepatic part of the umbilical vein or the placental cord insertion are safest (Figure 2 and Chapter 5). The current transfusion techniques and risk factors for complications are assessed in this thesis (Chapter 5). Overall, the survival after IUT in experienced centers has now reached approximately 96% (range 89-100 %, see Chapter 1).

We hypothesize that this increase could grossly be the result of three important changes: a reduction in complication rates, 2) more timely referral and therefore prevention of hydrops and 3) improvements in neonatal care. Both the complication rates and the prevention of hydrops were evaluated in this thesis (Chapters 4 and 5).

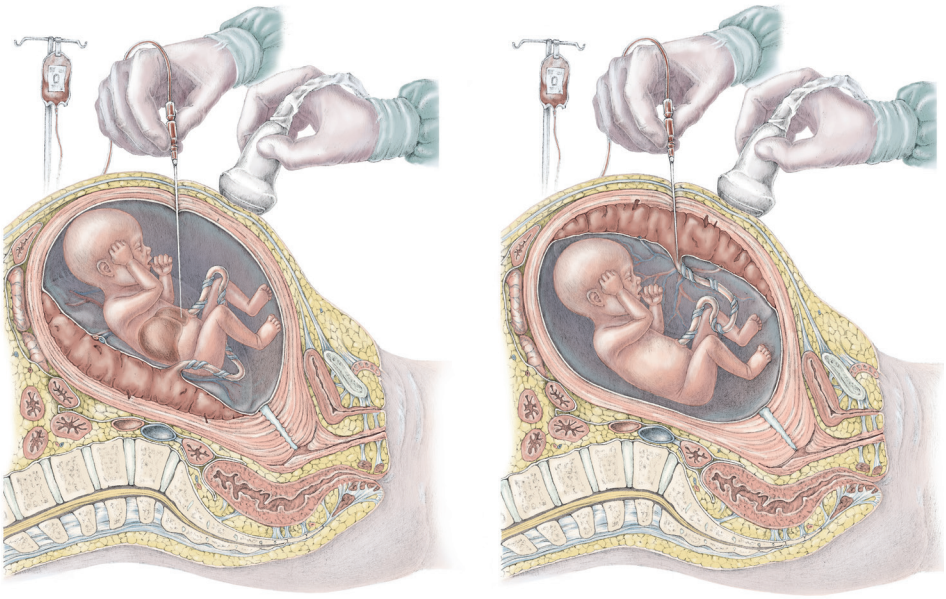

Figure 2. Intrauterine transfusion. Left: intrahepatic transfusion. Right: transfusion into the placental cord insertion (anterior placenta).

ALTERNATIVE TREATMENT

Several attempts have been made to assess the efficacy of non-invasive maternal treatment in pregnancies with severe (and early) HDFN. Most is known about therapeutic plasma exchange and/or intravenous immunoglobulins (IVIg). The literature on both of these alternative treatments is summarized in this thesis. Furthermore, we assessed the efficacy of IVIg in the multinational PETIT study. In this study, a cohort of patients with a history of early severe HDFN that was treated with IVIg, was compared to an equally severe non-IVIg group. The results are presented in this thesis (Chapter 6).

AIM AND OUTLINE OF THIS THESIS

The first attempts for intrauterine treatment by Liley et al. arose from a back-against-the-wall position: fetuses often died in-utero or suffered from extreme prematurity.[34] The founder of intrauterine transfusion in the Netherlands, professor Jack Bennebroek Gravenhorst, empirically introduced this treatment and stated:

'Hoofdzaak is het bestrijden van de anemie. Bij het voortschrijden van de technische mogelijkheden en door uitgebreidere toepassing van de laatstgenoemde methode zal ongetwijfeld een elegantere methode gevonden worden voor de toediening van het bloed, waardoor bezwaren, die thans ongetwijfeld bestaan, uit de weg geruimd zullen worden.'

In English, this quote would be: *'Main issue is to counter the anemia. The broadening of technical possibilities and more extensive application of the abovementioned method will undoubtedly lead to a more distinguished method for the administration of blood. Hereby the objections, that at present surely exist, will be eliminated.'*[35]

This thesis aims to describe the gradual disappearance of these objections by addressing our unique care system for alloimmunized mothers, assessing transfusion techniques and complications and evaluating alternative treatment. Thereby, we wish to contribute to converting the management of and intrauterine transfusion for hemolytic diseae of the fetus from expert-based to evidence based.

PART 1: OVERVIEW

General introduction

Chapter 1 – Review of the available literature on antenatal management and outcome of HDFN. This includes a detailed description of important aspects of intrauterine transfusion, the golden standard treatment for fetal anemia, and evaluates the evidence on alternative therapeutic options.

PART 2: PATHOGENESIS AND SEVERITY OF HDFN

Chapter 2 – Case-control study including pregnant women with newly detected non-D antibodies, assessing whether ABO incompatibility has a preventive effect on formation of non-D alloantibodies. Subsequently, we evaluated a possible protective effect of RhIg in a subgroup with non-Rh antibodies only.

Chapter 3 – National cohort study amongst all pregnant women with D immunization, in which we assessed if, and how disease severity increases in subsequent pregnancies complicated by HDFN. Furthermore, we aimed to identify factors from the first pregnancy with D antibodies that predict severe disease in the subsequent affected pregnancy.

Chapter 4 - 30-year cohort study evaluating trends in the condition of fetuses treated with intrauterine transfusion for red-cell alloimmunization, at the time of first transfusion and at birth. This, in relation to our well-organized health care system for alloimmunized mothers.

PART 3: INTRAUTERINE TRANSFUSION AND OTHER TREATMENT OPTIONS

Chapter 5 – 27-year cohort study, assessing trends in complication and fetal death rates after intrauterine transfusion. We evaluated how IUT is most safely performed and aimed to identify factors leading to improved outcome.

Chapter 6 – International cohort study comparing pregnancies of women with a history of severe HDFN that are treated with or without intravenous immunoglobulins (IVIg) in a subsequent pregnancy. We aimed to assess whether IVIg could be a non-invasive alternative for (hazardous) early intrauterine transfusions.

Chapter 7 – Cochrane systematic review evaluating neonatal treatment with IVIg to reduce the need for neonatal exchange transfusions.

PART 4: SUMMARY AND DISCUSSION

This section summarizes the findings of the abovementioned studies, discusses their implications and addresses future perspectives in the field.