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

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I

OVERVIEW

General Introduction

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Hemolytic disease of the fetus and newborn (HDFN) is a condition in which a mismatch between paternally inherited red blood cell (RBC) antigens on fetal RBCs and maternal RBC antigens have led the maternal immune system to produce alloantibodies which bind to the fetus' RBCs in the current pregnancy. These antibodies can cross the placenta and may mediate hemolysis of fetal RBCs, and in some cases, suppress fetal erythropoiesis. This can result in severe fetal anemia, which may manifest as cardiomegaly, hepatosplenomegaly, hydrops fetalis and, if left untreated, can lead to fetal demise. In the neonate, severe hyperbilirubinemia resulting from hemolysis (even with adequate treatment) can cause kernicterus, a form of lifelong neurological disability. Finally, (delayed) neonatal anemia can occur up to three months of age.

While many blood group antigens are known to trigger HDFN, the most clinically significant cause for severe disease are those in the Rh blood group system, particularly RhD and to a lesser extent Rhc, and the Kell (K) blood group system.[1,2]

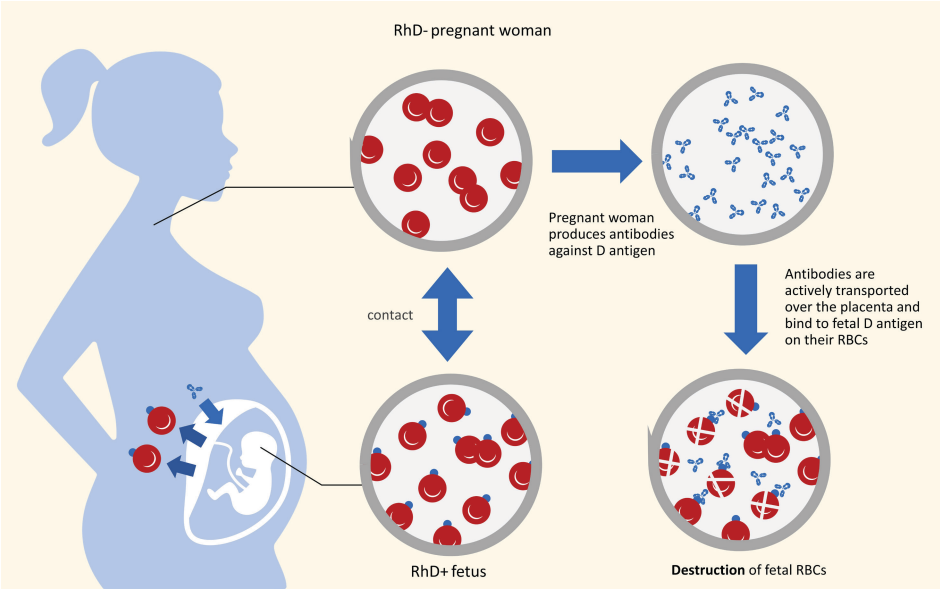

Figure 1. process of hemolytic disease of fetus and newborn (HDFN). Red blood cells (RBCs) from fetus cross the placenta and come in contact with the maternal immune system. As the pregnant women does not have the D antigen on her RBCs, alloantibodies can be created which cross the placenta by active transport, bind to the fetal D antigen on fetal RBCs and cause destruction. Adapted from L. Tollenaar.

Historic Overview

Before effective treatment became available, HDFN was one of the leading causes of perinatal mortality. With the discovery of blood group systems in the 1930s and 1940s, previously unexplained conditions were finally unraveled. Hydropic, stillborn, and jaundiced neonates were all found to be suffering from the same underlying cause: HDFN.[3] Significant advances in treatment soon followed. In the 1940s, exchange transfusions for neonates became feasible, and by the early 1960s, the first successful intrauterine transfusions (IUTs) were performed.[4] The greatest improvement on perinatal health however came with the arrival of Rhesus D Immunoglobulin (RhIg) prophylaxis. Developed, tested, and widely implemented in the late 1960s, RhIg dramatically reduced the incidence of maternal sensitization.[5,6] Prior to its use, approximately 15% of RhD-negative women became alloimmunized following a pregnancy with an RhD-positive fetus.[3] After the introduction of postpartum RhIg prophylaxis, this rate dropped significantly, and with the addition of antenatal prophylaxis, it declined even further to 0.3%-1.3%.[7]

To identify individuals who should receive RhIg prophylaxis or who are already at risk for HDFN, screening programs were implemented at national or regional levels. Initially, these programs focused solely on blood group determination and antibody testing, with RhIg added in 1969 in the Netherlands. Today, however, more comprehensive and sophisticated screening protocols are in place. An overview of the current possibilities and treatment options is given in **Chapter 1.**

Dutch history of HDFN

In the Netherlands, we followed the global trend by initiating a national screening program for blood groups and antibodies in the 1950s.[8] From its beginning, this program was centralized at the Central Laboratory for Blood transfusion services (CLB), which later became part of the national blood supply organization Sanquin and at the Institute for specialized blood investigations (in Dutch BIBO), currently part of the University of Groningen. Following the groundbreaking publication by New Zealand physician William Liley on the first successful IUT,[9] one of our colleagues from Leiden (prof. Jack Bennebroek Gravenhorst) traveled there to learn this innovative technique and to introduce it in the Netherlands. This led to the establishment of a national center for treatment of HDFN at the Leiden University Medical Center (LUMC).

Collaboration between laboratory testing in Amsterdam and the clinical treatment center at Leiden University was integral from the outset, making the evaluation and continuous

improvement of clinical processes more the rule than the exception. This reciprocal relationship not only enhanced clinical care but also contributed to the evolution of the screening program itself. While the program in the 1950s focused solely on determining blood groups and detecting antibodies, the protocols for RhIg administration, timing, and follow-up have undergone significant refinement over the past 70 years. Today, the national screening program is executed by the RIVM (National Institute for Public Health and the Environment) that coordinates the so called PSIE (Prenatal Screening for Infectious Diseases and Erythrocyte Immunization) program, which is offered free of charge.[10]

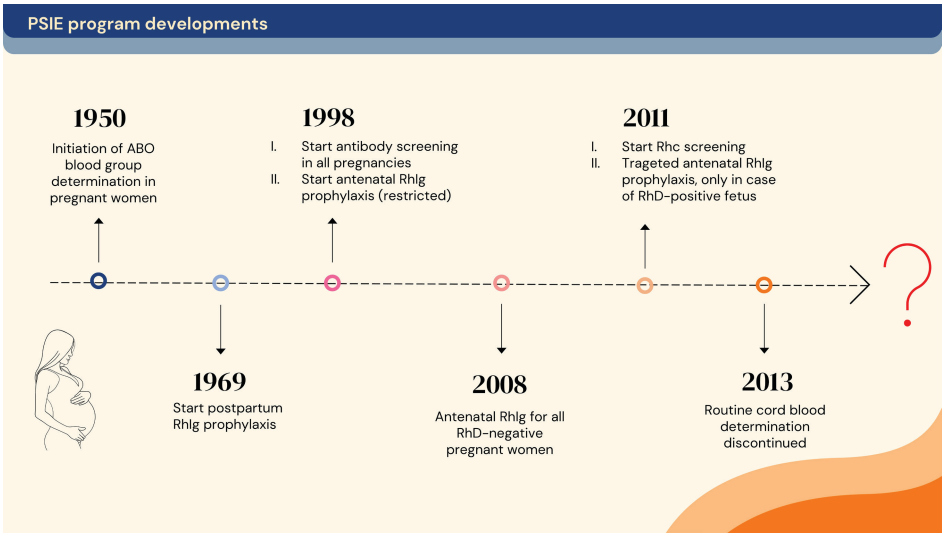

Figure 2. Highlights of developments of the Prenatal Screening for Infectious Diseases and Erythrocyte Immunization (PSIE) program throughout the years in the Netherlands. RhIg: Rhesus D immunoglobulin.[8]

Laboratory screening and RhIg prophylaxis program

Screening program

In the current screening program, maternal ABO, RhD and Rhc blood groups and the presence of RBC alloantibodies are determined in the first trimester of pregnancy. RhD- and Rhc-negative women are rescreened at week 27 of gestation. At this time point, the fetal RhD blood group is also determined in pregnancies of RhD-negative women. If the fetus is RhD-positive, RhIg immunoglobulin is administered in week 30 of gestation. This approach avoids unnecessary RhIg administration in approximately 38% of cases (Dutch numbers), hence where an RhD-negative mother is carrying an RhD-negative fetus.[11]

Postpartum, RhIg administration is repeated based on the fetal RhD typing result; cord blood testing is only performed in case fetal RhD typing was not possible because of genetic *RHD* variation or in case of a multiple pregnancy since one needs to determine the individual RhD typing results of all newborns to adjust RhIg dosage if more than one newborn is RhD positive.[10] Genetic variation occurs in people from all ethnicities, but some *RHD* variants occur more often in RhD-negative people with African or Asian ancestry.[12] **Chapter 3** of this thesis discusses whether *RHD* variation should be given greater consideration.

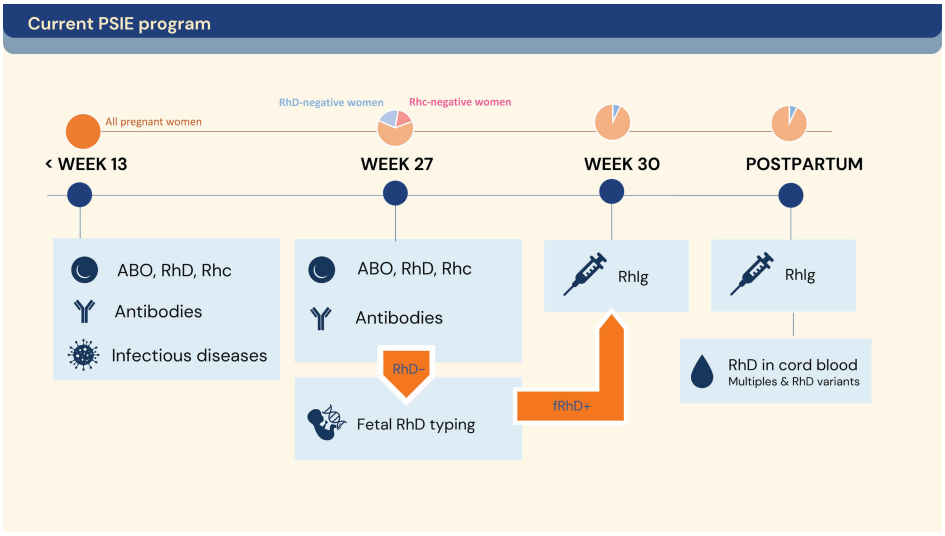

Figure 3. Current Prenatal Screening for Infectious Diseases and Erythrocyte Immunization (PSIE) program in the Netherlands. RhIg: Rhesus D immunoglobulin, fRhD: fetal RhD. Adapted from L. Tollenaar.

cell-free fetal DNA

The fetal RhD blood group is determined using cell-free fetal DNA (cff-DNA). The presence of cff-DNA in plasma of pregnant women and its suitability for non-invasive prenatal diagnosis was first demonstrated by Professor Lo and his colleagues in 1997.[13,14] Since then, the range of clinical applications for cff-DNA has expanded rapidly. Beyond RBC or platelet blood group determination, it is now also used to detect various gene-based conditions.[15] In the Netherlands, fetal RhD typing with cff-DNA is centralized at Sanquin Diagnostic Services and performed with a blood sample taken in week 27 of pregnancy by using real time quantitative PCR (RQ-PCR). The fetal *RHD* typing platform has been in use since July of 2011. After 1,5 years of testing and comparing fetal *RHD*

test results with cord blood results, the platform was found to perform robustly with a false negative rate of only 0.03% and therefore routine cord blood testing has been discontinued since 2013.[11] In this thesis we discuss the routine laboratory performance of the recently modified fetal *RHD* typing platform, which, after an extensive in house development and validation trajectory by Sanquin Research now includes an additional DNA extraction control. In this thesis we report on both the small clinical verification study, as regulatory demanded, and a large two-year cohort study showing the benefits of this modification (**Chapter 2**).

After Denmark, the Netherlands was among the first countries to adopt the approach of administering RhIg based on the fetal RhD blood group.[11,16] While some countries have since followed, the majority still administer RhIg prophylaxis to all RhD-negative pregnant women and determine the neonates' blood group only after birth to target postpartum RhIg. In light of RhIg shortages a fully targeted system is the more favorable option administering only to those who need protection.

In contrast, when maternal RBC alloantibodies are found and fetal blood group determination is essential for HDFN risk stratification, a different PCR assay design is employed to mitigate the risk of false negative results further. The application of this method in daily practice, along with the key considerations and potential pitfalls in designing the testing algorithm, is discussed in **Chapter 4**.

Antenatal Management of HDFN

When RBC alloimmunization does occur, during pregnancy the severity of fetal anemia has to be assessed. Not all type of RBC alloantibodies have the same risk to induce fetal hemolysis so the alloantibody specificity has to be taken into account; antibodies against RhD, Rhc or K (Kell) can lead to severe HDFN. To further assist the clinicians in risk stratification, RBC alloantibody levels can be semi-quantified by determining the alloantibody titer (i.e., a measure for the concentration of the alloantibodies in the maternal plasma), and in the Netherlands, the antibody-dependent cell-mediated cytotoxicity (ADCC) test is also routinely performed to predict the biological activity of the alloantibodies. Clinical care can be provided by midwives if risks are acceptable, but most often alloimmunized women are referred to a care giver in a hospital setting. Once a critical threshold or cut-off value is reached, further evaluation via specialized ultrasound is necessary in a secondary or tertiary center. Above the high-risk cut-off where the risk of severe fetal anemia is suspected, women are referred to the LUMC for this specialized care. Using Doppler ultrasound, the peak systolic velocity in the

middle cerebral artery (PSV-MCA) is measured, which correlates well with the degree of fetal anemia.[17,18] If severe fetal anemia is suspected, an IUT can be performed to deliver donor blood to the fetus in utero. When Dr. Liley first introduced IUT, it was performed intraperitoneally (IP), guided by X-ray imaging to position the needle.[9] Advances in technology, particularly the introduction of ultrasound, enabled safer and more precise intravascular transfusions. At the LUMC, the current standard approach is to administer the IUT in the hepatic portion of the umbilical vein when the placenta is posterior, and at the umbilical cord root at the placental site when the placenta is anterior. In some cases, an additional intraperitoneal deposit of donor blood is given alongside a hepatic IUT. Through the recently completed DIONYSUS study, which examined international IUT practices, we found significant variation in the choice of puncture sites.[19] Notably, IP transfusion is not commonly used in the centers contributing to DIONYSUS. In **Chapter 5** we evaluate the effect of this practice.

Something still rarely described is the effect of IUTs on the placenta. Literature on placenta's of pregnancies complicated by HDFN is mostly from the 1970s and 1980s, mostly concerning description of placenta pathology from pregnancies with severely hydropic or stillborn fetuses.[20-28] In **Chapter 7** we provide an overview on findings in placentas from not only severe, but also mild cases of HDFN (i.e. without IUTs) and compare them to both a healthy control group and a group of severe HDFN cases treated with IUTs to evaluate the placental changes after multiple IUTs with donor RBCs.

When a pregnancy has been complicated by IUTs, it is common practice to counsel parents that the condition is likely to be more severe in a subsequent pregnancy, with IUTs expected to be required at an earlier gestational age. However, this assumption had not previously been confirmed with data. In **Chapter 6** we report on our study to determine if and when in a subsequent pregnancy IUTs are needed.

Given the numerous studies conducted over the past two decades, an update to our national guideline on RBC immunization in pregnancy was warranted. Furthermore, since HDFN is rarely occurring and concerns both care to the pregnant couple and to the newborn, there was a wish to extend the former guideline, which made a multidisciplinary approach including experts from different fields, e.g. midwives, obstetricians, perinatologists, neonatologist, laboratory professionals and patient representatives a prerequisite. These multidisciplinary efforts are presented in **Chapter 8.** As demonstrated by the international variations observed in the DIONYSUS study, we think that sharing our updated guideline will foster global collaboration and improve clinical management by enabling mutual learning.

Aim and outline of this thesis

This thesis evaluates a broad range of topics related to the prevention and management of HDFN, culminating in updated Dutch clinical guidelines.

Part 1 provides an overview of both laboratory and clinical management strategies when maternal alloantibodies are detected during pregnancy.

In **Part 2**, we examine the screening platform used in the Dutch RhIg prophylaxis program. We assess additional quality control measures necessary to ensure the platform is in line with new regulatory quality demands to maintain its accuracy. This evaluation is of importance for others that consider implementing high-throughput fetal RhD typing platforms and omitting postpartum RhD typing with cord blood. Furthermore, we explore strategies to improve equity in access to screening. This section concludes with an evaluation of non-invasive fetal RBC typing when alloantibodies *are* present, highlighting important pitfalls that must be considered when implementing this approach.

Part 3 focuses on antenatal HDFN treatment aspects. We compare different transfusion techniques to evaluate the potential added benefit of IP blood deposition IUTs. We also investigate outcomes in pregnancies following a previous IUT-treated pregnancy. Notably, there was previously no empirical data to support the commonly held belief that subsequent pregnancies are invariably more severe. This part concludes with an analysis of the impact of donor blood on the placenta and whether the transfusion process itself should be evaluated.

Finally, **Part 4** presents an updated version of the Dutch guideline on HDFN, which was accomplished by a multidisciplinary expert group and is based on new evidence from recent studies including those presented in this thesis.

Part 1: Overview

General introduction

Chapter 1 - "Identification and management of fetal anemia due to hemolytic disease"

Part 2: How to improve a screening platform

Chapter 2 – " Routine use of a spike-in DNA control as process control for foetal *RHD* typing: real life performance of this canary"

Chapter 3 – " Creating an inclusive platform in a multi-ethnic population for fetal *RHD* genotyping to target RhIg immunoprophylaxis"

Chapter 4 - "How I use noninvasive prenatal testing for red blood cell and platelet antigens"

Part 3: How to optimize antenatal treatment

Chapter 5 – "Comparison of intrauterine transfusion techniques in hemolytic disease of the fetus and newborn"

Chapter 6 – "Severity of haemolytic disease of the fetus and newborn in patients with a history of intrauterine transfusions in a previous pregnancy: A nationwide retrospective cohort study"

Chapter 7 – "Histopathology of the placenta in mild to severe cases of HDFN with and without IUTs – the influence of donor blood"

Part 4: How to move from evidence to guideline

Chapter 8 - "Management of alloimmunization in pregnancy: screening, monitoring, and treatment; the Dutch Quality Standard"

Summary and General Discussion

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