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

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SUMMARY AND GENERAL DISCUSSION

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Since the naming of the disease in 1941, numerous developments have shaped the current management of HDFN. What was once a common condition associated with high mortality and morbidity has now become rare in the Netherlands.[1] This progress is the result of many important prevention measures and innovation in treatment protocols. In this thesis, we aimed to recalibrate and evaluate multiple aspects of management of D-immunization. We revised our current D-immunization prevention program to find possibilities to optimize it further in light of current challenges. We evaluated the nowadays used antenatal clinical intrauterine transfusion techniques and studied next pregnancies after a pregnancy with IUTs to improve counselling for subsequent high-risk pregnancies. We also studied the effect of IUTs on the placenta. Finally, we conclude with the translation of all the evidence on prevention and management of HDFN collected over the last few decades and the insights presented in this thesis to update and extend the guideline "Erythrocyte immunization in pregnancy". Together with a multidisciplinary group of experts the new Quality Standard on screening, monitoring and treatment of HDFN in pregnancy and postpartum was developed to be used by all professionals involved in the chain of care of this rarely occurring disease in pregnancy.

In this this thesis four different parts are studied. Here, I summarize and discuss the individual chapters and provide a reflection. Innovation in prevention and management of HDFN is an ongoing process, in which we can learn from the various settings worldwide and in which we face challenges how to use new developments. Although the research presented in this thesis advances our understanding and closes important gaps in knowledge, some questions remain. Therefore, this chapter concludes with a section on future perspectives.

Part 1: Overview

In **Chapter 1**, we provide an overview of the current laboratory and clinical management strategies used to detect HDFN and to assess the severity of fetal anemia.[2] We conclude that with adequate early identification of at-risk pregnancies, effective prevention programs, timely referral to a maternal-fetal medicine center, and the use of non-invasive diagnostic tools, the occurrence of fetal hydrops, intrauterine demise, and long-term morbidity can be significantly reduced or even prevented. We emphasized the importance of optimal prevention of D immunization through the administration of RhIg immunoglobulin to those who require it.

Reflection

Effective prevention and management require continuous adaptation to emerging developments, including novel discoveries, novel insights on the effectiveness of

interventions, regulatory aspects related to diagnostic tests and changes in perspective on benefits of interventions. The focus is gradually shifting: from simply keeping the fetus alive and ensuring it is born relatively safe by any means necessary, e.g. timely delivery (but avoiding preterm delivery), toward considering the optimal outcome for the newborn and its quality of life in terms of long-term outcome.

Another focus that is shifting is on the requirements of determination of fetal blood groups by means of laboratory testing, both for D-immunization prevention and for pregnancies at risk of HDFN because RBC antibodies are found. Since 1997, these laboratory tests have continued to evolve and remain subject to ongoing refinement. Current developments focus on expert insights into qualitative aspects, regulatory considerations, and the interpretation of false-negative and false-positive results, particularly when these are influenced by the ethnicity of the mother and/or fetus. In **part 2** of this thesis, we recalibrate the since 2011 existing Dutch fetal *RHD* genotyping screening platform to accommodate for these changes.

Part 2: How to improve a screening platform

An effective RhIg prophylaxis program requires a systematic, evidence-based approach incorporating both fetal and maternal *RHD* testing. By addressing some of the existing gaps we work towards safer, more efficient, and more resource-conscious prevention of D alloimmunization.

Fetal *RHD* genotyping

Many countries have adopted and implemented programs to prevent D immunization, though the level of detail and implementation varies widely. Some countries only administer RhIg postpartum to RhD-negative postpartum women after determining the neonatal blood group, and only administer RhIg to those with an RhD-positive neonate. A better protection is achieved when all RhD-negative pregnant women receive RhIg during pregnancy, often around week 30 of pregnancy, to protect against small amounts of fetal-maternal transfusion during the last trimester of pregnancy. Though small, these amounts are already capable of immunization. However, RhIg is an immune product derived from plasma from intentionally hyperimmunized donors who are undergoing repeated plasmapheresis and therefore both a precious and a limited resource.

A more resource conscious option is to target and protect only the pregnant women carrying an RhD-positive fetus, as they are the only ones at risk and in need of RhIg. This can be done by determining the fetal blood group using cell-free fetal DNA (cff-DNA), present in maternal plasma, before administering RhIg.[3,4] In the Netherlands, 38% of RhD-negative women carry a RhD-positive fetus.

In the Netherlands, fetal *RHD* genotyping for RhIg administration has been introduced in 2011 and subsequently, after extensive verification of its performance in 2013, routine cord blood RhD typing was stopped, making fetal *RHD* genotyping the only test to target both antenatal and postpartum RhIg.[5] This implies that robust genotyping quality controls are of critically importance. In the initial Dutch set up we found a high sensitivity and specificity with only 0.03% false negatives before discontinuing cord blood typing. Therefore, we concluded that a control to confirm for the presence of sufficient fetal DNA in every test, as is used in clinical testing for pregnancies at risk for HDFN, was not necessary in a screening setting and would increase the costs. However, since three of the false negatives were found to be related to errors in the process of DNA extraction and PCR amplification, Sanquin started to investigate how to include a so called in-process control by addition of an artificial PCR template to an individual maternal plasma sample. In 2022, the cfDNA subgroup of the International Society of Blood Transfusion (ISBT) Working Party on Red Cell Immunogenetics and Blood Group Terminology (RCIBGT) released expert guidance on validating and ensuring quality in non-invasive prenatal testing for fetal blood groups.[6] Their recommendations included incorporating a control for the extraction and/or amplification process. Fortunately, the Dutch platform now is in line with this recommendation. **Chapter 2** reports on the performance of several aspects of the Dutch national fetal *RHD* genotyping platform during the first two years after implementation of the in-process control. To comply to regulatory advice, we designed a multicenter study involving 19 midwifery practices across the Netherlands and to collect a sufficient number of cord blood samples for assay verification, as RhD typing of cord blood remains the reference ("gold standard"). Our in-process control consists of an exogenous DNA sequence that is spiked into the plasma sample (spike-in control). We found that implementing this spike-in is feasible and adds value: next to complying to its purpose to further reduce the risk of issuing false-negative results, it also provided benefit in indicating perturbations in PCR assay conditions. In total, we prevented eight false-negative results during the two-year study period through use of the spike-in control. Prevention of about three to four false-negative results per year is in concordance with the expectations derived from the first validation study performed in 2011-2012. Furthermore, apparently, addition of the spike-in control also improved the protocol for test result issuing: test repeat rates reduced from 0.46% to 0.35%, which further adds quality to the process.

RhD variant alleles

While the concept of administering RhIg to RhD-negative mothers based on fetal *RHD* typing is in general straightforward, certain cases are more complex. Some mothers carry genetic variation of the *RHD* allele, leading to loss of D antigen epitopes. Consequently, they

can form alloantibodies against the missing epitopes and require RhIg administration for protection. Genetic variation in either the maternal or fetal *RHD* gene occurs at different frequencies in different ethnicities and can lead to serological positive, negative or weak results. The African Black population shows particularly high variability in the *RHD* gene. Unlike most individuals of European ancestry, where RhD negativity typically results from complete absence of the *RHD* gene, approximately 66% of RhD-negative individuals of African descent carry non-expressing *RHD* alleles (pseudogenes).[7] Because women with a partial D variant allele or *RHD* null allele possess large part of *RHD* gene sequences, fetal *RHD* genotyping becomes more challenging, as the maternal DNA can obscure fetal results. This may lead to inconclusive or even false positive results. This challenge in fetal *RHD* genotyping, which makes that fetal *RHD* genotyping may not be equally reliable across all ethnicities, has been previously recognized.[3,8] Thus far, in the Dutch situation, as in other designs, such as used in the UK[9] and France[10], this resulted in an assay in which also in mothers carrying the *RHD* pseudogene an RhD-positive fetus can be recognized.

We have previously investigated the *RHD* variant alleles present in the Dutch third-trimester screening population (i.e., serologically RhD-negative women).[11] In **Chapter 3**, we evaluated the screening process from the start of RhD typing of the pregnant women in the first trimester of pregnancy to the different fetal *RHD* test outcomes in week 27 of pregnancy. Our earlier research identified the most common *RHD* variants among serological RhD-negative pregnant women in the Netherlands as the *RHD* pseudogene (highly prevalent in African Black population) and DVI variants (mostly prevalent in the White European population), with D_{el} variants (prevalent in the Asian population) also playing an important role.[11] In **Chapter 3**, we show that almost all Weak D types 1, 2, and 3 are already correctly identified in the first trimester, reducing unnecessary fetal *RHD* typing in this group of women, who can be regarded as RhD positive. Furthermore, we demonstrate that the current algorithm provides conclusive and, for the most prevalent variants, correct results in 99.34% of cases. We emphasized the need for additional testing in specific result categories, such as a maternal or fetal signal in only one of the two exons employed as is with the DVI or pseudogene. By determining these variants, more conclusive results can be provided for all type of fetus independent on their ethnicity and this will ensure appropriate RhIg administration; both preventing unnecessary use and promoting correct application of RhIg.

Fetal genotyping in presence of antibodies

When RhD-negative women do become immunized, confirmatory fetal *RHD* typing is held to stricter standards than screening tests. A false-positive result in a non-immunized woman may lead to unnecessary RhIg use; an unfortunate and wasteful but currently

harmless outcome. However, a false positive in an immunized pregnancy triggers intensive weekly ultrasound monitoring and, due to the relatively high false-positive rate of ultrasound monitoring, increases the risk of invasive procedures such as cordocentesis. In future, it could also mean unnecessary start of intensive immunotherapy such as the currently investigated FcRn blockers. Therefore, diagnostic tests must be highly sensitive (to avoid missing RhD-positive fetuses) and highly specific (to minimize false positives). In **Chapter 4**, we describe key considerations for using non-invasive prenatal testing (NIPT) in alloimmunized pregnancies, including those with RBC antibodies and fetal/neonatal alloimmune thrombocytopenia (FNAIT).[12] These recommendations are intended not only for laboratory- and transfusion specialists but also for clinicians, outlining the reliability of various methods and guiding decision-making when commercial tests are required. While real-time quantitative PCR (RQ-PCR) has long been the standard, measuring the specific DNA sequences during amplification and thereby providing relative quantification, droplet digital PCR (ddPCR) and next-generation sequencing (NGS)-based typing are quickly replacing this. The advantage of ddPCR is that there is less background amplification from maternal DNA sequences. It was also used to introduce a universal fetal DNA marker as control for sufficient fetal DNA in the assay, making the assay in principle suitable for all pregnancies. The more recent development of assays with next generation sequencing (NGS) has the advantage that more DNA sequences can be read in parallel, offering a broad view of both genetic variation and on sufficient amplification of fetal DNA sequences, making genotyping for fetal *RHD* highly accurate. Clinicians should be aware of the limitations of commercial tests offered in their setting, understand how to manage *RHD* variants, and recognize how these variations influence testing strategies.[12]

Reflection

In **Part 2**, we evaluated our current fetal *RHD* genotyping program to ensure it accommodates evolving techniques, regulations, and population dynamics. Even in highly efficient processes, there comes a time to review if an assay is still complying to the state of the art. While some changes are driven by shifting regulations, such as those in **Chapter 2** influenced by the EU In Vitro Diagnostic Regulation (IVDR), others are shaped by the populations they serve.[13] With current migration patterns, our pregnant population no longer resembles that of twenty years ago. At this moment, our evaluation showed that on a population scale only minimal improvements can be achieved, however, for individual cases these small improvements could have significant impact. Accounting for genetic *RHD* variants that may become more frequent in this light, will provide equitable accessibility to the screening program and correct administration of RhIg. Accounting

for these changes and combining laboratory data with clinical data from immunized pregnancies provides rare and highly valuable insight into whether we are still on the right track or in need of improvement.

However, accounting for ever-changing regulations and processes comes at a cost. The aforementioned IVDR requires for clinical assays for which the intended scope is changed, a dataset of approximately 1,000 samples to be collected to demonstrate performance. Even if the platform used is already widely used and extensively validated, as is the case in our setting.[5] These regulations could raise the overall standard within fetal *RHD* testing in Europe, but also leads to costly validation studies and form a barrier to continue with in-house developing and innovating laboratory tests. The personnel effort, documentation burden, and logistical demands on investigators, midwives, pregnant women, and the broader healthcare system risk exceeding what is reasonable for incremental validation. It is worth asking whether regulatory expectations in this context may have become disproportionate; a concern colleagues have previously raised in an open letter.[14]

Part 3: How to optimize antenatal treatment

Antenatal course of disease

When maternal RBC antibodies are detected, incompatibility between mother and fetus have been shown and ultrasound findings suggest severe fetal anemia, IUTs remain the gold standard treatment.[15] The choice of puncture site varies between centers, as demonstrated in the Dionysus study comparing HDFN management across 32 centers internationally.[16] In that study, our center was the only one performing an additional IP transfusion following a transfusion into the intrahepatic portion of the umbilical vein. Because evidence on the added benefit of this combined approach was limited,[17,18] we analyzed our own data in **Chapter 5** comparing Hemoglobin (Hb) levels after IUT with Hb levels before the subsequent IUT. We found that intrahepatic IUTs supplemented with IP transfusion were associated with a slower Hb decline (approximately 0.5 g/dL less per week) compared to intrahepatic transfusion alone, consistent with earlier reports.[19] This effect persisted across all consecutive IUTs. Furthermore, the interval between transfusions was longer in the combined-treatment group: 3.7 weeks (26 days) compared to 3.0 weeks (21 days) in the intrahepatic-only or placental cord insertion groups. Although these results are promising, they may be influenced by selection bias, such as the surgeon's choice to delay the next procedure. Therefore, prospective studies are needed to confirm this effect and to determine whether repeated IP transfusions provide a cumulative benefit. A slower Hb decline after IP may be particularly advantageous in specific scenarios, such as very early-onset severe HDFN allowing pregnancy to advance to a safer gestational age, or when bridging to term after the final IUT, potentially avoiding an additional procedure.

After a pregnancy requiring IUTs, both parents and clinicians are faced with burning questions: *What is the likelihood that this will happen again?* And *if so, when will it occur?* Because antibody titers and ultrasound measurements are poor specific predictors, these were important questions to address. The prevailing consensus has long been that hemolytic disease worsens in each subsequent pregnancy, but an incidental finding in previously published data from our group pointed out that around a third of patients with a history of very severe cases of HDFN had a later onset of fetal anemia in the subsequent pregnancy.[20] In **Chapter 6**, we examined in a larger cohort what happens in a subsequent pregnancy with an antigen-positive fetus after a pregnancy treated with IUTs. We found that IUTs were required again in the vast majority of subsequent pregnancies (86%). Moreover, in 60% of these cases, IUTs were initiated significantly earlier than in the previous pregnancy. Even in subsequent pregnancies that did not require IUTs, delivery was often induced preterm, and neonates frequently presented with low hemoglobin levels. This suggests that fetal anemia severe enough to warrant an IUT was likely present, but delivery was chosen over performing the procedure. When IUTs were required in a subsequent pregnancy, the first procedure typically occurred a median of 3 weeks earlier than in the previous pregnancy, though the timing varied substantially. Interestingly, in 25% of women, the first IUT was performed later than in the preceding pregnancy, and in a small number of cases, IUTs were not required at all.

These findings have important implications for preconception counseling. Women who have experienced a pregnancy with severe HDFN requiring IUTs are often told that a subsequent pregnancy with another antigen-positive fetus carries extreme risk and a high likelihood of adverse outcomes. Many interpret this as advice to avoid future pregnancies. However, our study provides more accurate information, and prior research has shown that outcomes in these pregnancies can be very good.[21]

In **Chapter 7**, we zoom in on the placenta, aiming to examine its characteristics in case with mild disease and assessing the impact of donor RBCs (containing mainly HbA) on placental histology. Recently, efforts started to transfuse cord blood containing high levels of HbF to severely premature babies to provide them with blood cells with similar characteristics and oxygen affinity as they were used to before birth. A recent RCT study (BORN trial), found that cord blood had indeed a favorable clinical impact, reducing the risk and severity of retinopathy of prematurity (ROP) and bronchopulmonary dysplasia (BPD).[22] We therefore wondered if adult donor RBCs might also have impact on the placenta tissue. Historically, placental descriptions have primarily come from the era before intravascular transfusions were possible, focusing largely on cases involving severely hydropic fetuses or those that succumbed to the disease. In this study, we compared placentas from HDFN cases with those from a healthy cohort and found that HDFN placentas exhibited significantly higher rates of cord hypocoiling ($p<0.001$), maternal vascular malperfusion ($p=0.005$), fetal hypoxia

($p<0.001$), and placental hydrops ($p<0.001$) relative to controls. No histological differences were detected between IUT-treated and untreated cases, indicating that donor RBCs do not affect placental structure or function.

Reflection

In **Part 3** we evaluated antenatal care. Reflecting on common practices for us, such as transfusion techniques, prompts us to question whether these approaches are still truly best practice. In rare diseases such as HDFN, gathering evidence on best practices and identifying opportunities to improve care can be challenging. Therefore, in addition to maintaining a healthy degree of self-reflection, it is advisable to look beyond national borders and foster international collaboration to find the answers. In the Dionysus study, Derek de Winter of our group did just that, identifying variations in practice that warrant further investigation for their effectiveness.[16] Not only did we find that we were the only center adding IP to intrahepatic transfusions, but we also observed substantial variation in almost all aspects of antenatal and postnatal management. These differences ranged from referral structures (early vs. late) to the frequency of repeat titers (weekly vs. bi-weekly) and the use and timing of IVIg, to name a few. Such variations raise new questions and drive further studies, but it also initiates reflection on whether one's own unique practice still represent best practice, or if they should be updated in light of new evidence. In this thesis, we evaluated our series of intra uterine transfusions for transfusion techniques and we found evidence that, in some cases, adding IP remains the best option, therefore we continue our management accordingly. In the end, there is value in remaining open to how others address similar issues, as this may broaden understanding and insight even further.

Prediction model

Collaborations also helps to gather larger datasets, necessary for instance, to inform prediction models. In **Chapter 6**, we showed that we can reasonably predict outcomes in subsequent pregnancies following a pregnancy with IUTs. However, our understanding is less clear regarding the counseling of women and their partners who have had a pregnancy with RBC antibodies but without the necessity to treat with IUTs, or who experience a first immunized pregnancy. In a previous study on D-immunized pregnancies, we found that certain factors, such as the timing of antibody detection and the severity of disease in the first affected pregnancy, were most strongly correlated with outcomes in subsequent pregnancies.[23] However there is a clear need for better prediction and prediction models as only part of the puzzle is known and involves mostly D-immunized pregnancies. Our ultimate goal is to predict which women will develop severe disease and which will have a milder course. Currently, we rely on titers and the ADCC (predicting biological antibody

activity), but a large group of patients is still monitored intensively via weekly ultrasound, many of them never requiring IUTs. In **Chapter 6**, we observed a wide range of outcomes in subsequent pregnancies, which underscores the necessity to identify more factors that could be beneficial for a prediction model, such as the IgG subclass of antibodies and IgG-Fc glycosylation patterns.[24] Such a prediction model would help triage pregnancies to ensure that the right patient receives the right care at the right center. If the currently investigated FcRn blockers prove effective for HDFN, a prediction model will also help identify which patients should receive this treatment. This is also necessary to prevent overtreatment. Risk profiling will likely differ across populations, so international collaboration will be crucial for establishing a model that is broadly applicable or can be adapted across borders.

Part 4: How to move from evidence to guideline

In **Chapter 8**, we culminate all findings, together with prior research, into the new Dutch guideline* for RBC alloimmunization in pregnancy. Key recommendations emphasize early identification and risk-tailored management to reduce fetal and neonatal complications. Regular ultrasound surveillance guides care in moderate- to high-risk pregnancies and helps identify those requiring intrauterine intervention. Postnatal management includes close bilirubin monitoring to prevent kernicterus and hemoglobin surveillance to detect and treat anemia. With timely detection and appropriate treatment, HDFN has become a highly manageable condition with generally favorable outcomes.

Reflection

Writing this multidisciplinary guideline* was a challenge, but very valuable, since it was the first time a guideline for RBC alloimmunization in pregnancy was developed by a multidisciplinary working party in the Netherlands. Stakeholders from all specialties involved in HDFN care and the patients themselves have very different viewpoints on what is important in antenatal and postnatal care and during this process it was important to find consensus. As this is a rare disease, we had to balance evidence-based care, seeking supporting data wherever possible, and, where evidence was lacking, engage all stakeholders to agree on expert opinion. A clear example of this was the discussion on timing of delivery. A few additional weeks in utero can bring benefits such as a more mature neonatal liver to process bilirubin and stronger lungs to reduce respiratory complications. However, the predictive value of ultrasound for detecting fetal anemia decreases with gestational age, and the risk of fetomaternal transfusion (highest in the last trimester) increases the chance of severe anemia. In the Dionysus study, gestational age at birth was also an outcome measure, and the results showed a broad variation with medians of international centers from 33+2 weeks to 37+3 weeks

gestation. We finally concluded that based on RBC alloantibody specificity a tailor-made advice was formulated in the guideline*. Furthermore, within the working party, we examined different antibodies and the scarce evidence available for some of the RBC alloantibody specificities to induce severe HDFN, ultimately reaching consensus on monitoring and diagnostic policies. This was incorporated in a risk-based flowchart.

Personally, this was one of the most challenging projects of my PhD trajectory. Not necessarily because of differences of opinion, that part was motivating and taught me a great deal about how perspectives can shift when you see an issue from another angle. What I found more challenging was the time required to establish common ground with the diverse professional organizations involved even though some are stakeholders in only a small part of the RBC alloimmunization prevention program. In the end, that might have taught me even more, and it reflects the central theme of this thesis: there is a gap in knowledge that can be bridged with evidence, but there is also a gap to bridge where it concerns the various perspectives that different professionals can have given their expertise and daily practice. Advancing any professional field therefore demands constant recalibration, evaluation, and lots of time to push the change to come to improvement, only for the cycle to start all over again. I am deeply proud of the delivery of the guideline, as so many research findings never make their way into daily practice. With this guideline*, I had the privilege of honoring both my predecessors and scientists around the world who are inching toward better care, and of translating my own and their findings into a practical compass for clinicians. I can only hope that all studies in this thesis bridge part of the gap in HDFN management.

*Officially this document is a quality standard and not a guideline according to the guideline development rules in the Netherlands. This is because the format in which guidelines have to be made, was not thought to be suitable for a rare disease such as HDFN where background information is warranted.

Future perspectives

First trimester *fRHD*

In the current Dutch screening program, fetal *RHD* genotyping is performed at 27 weeks' gestation. We are now conducting a large multicenter study to obtain maternal blood samples from RhD-negative women already at 11–13 weeks' gestation and test them on the same platform. Earlier determination of the fetal RhD status would allow us to withhold RhIg prophylaxis at potential sensitizing events (e.g., abdominal trauma, invasive diagnostic procedures, late miscarriage) when the fetus is D negative. In the Netherlands, this applies to 38% of RhD-negative pregnant women. These women would also no longer require repeat

alloantibody screening at 27 weeks. This together with earlier reassurance represents a clear benefit for pregnant patients and for obstetric care providers. Many countries have already implemented early testing algorithms.[4] The design of our early-gestation study is building further on the clinical study described in **Chapter 2** (i.e. collecting for a small cohort of women cord blood), but instead of the addition of an in-process control, gestational age is the key difference. As mentioned in the reflection of **Part 2**, for this study there is an equal high burden in personnel, logistical challenges, a huge demand of midwifery practices and pregnant women making the efforts needed for this relatively simple study quite disproportionate. However, the added value of first trimester (week 11) fetal RhD testing continues to be recognized. We hope that, in the near future, fetal RhD typing can be incorporated into routine first-trimester blood sampling in the Netherlands. In Figure 1 we describe a possible future PSIE program according to suggestions from this thesis and discussion.

RhIg production - polyclonal versus monoclonal

In this thesis, we have taken steps toward optimizing RhIg prophylaxis and improving equity in care for women from diverse genetic backgrounds. A key motivation is the need to use RhIg judiciously. Because RhIg is a human plasma-derived product, supply can be limited, as was evident in some countries during the COVID-19 pandemic. Moreover, a global shortfall has been recognized: hundreds of thousands of women (but maybe more) remain at risk of D alloimmunization due to inadequate access to RhIg. It is estimated that only half of the necessary need of postnatal RhIg is fulfilled.[25] This shortage demands coordinated international attention, as was also addressed by the European Medicines Agency (EMA).[26] Currently, most RhIg is produced from a relatively small pool of healthy, male, hyperimmunized plasma donors, most based in the United States. Until recently, the Netherlands maintained a plasma collection program that included immunized men and women immunized during pregnancy: a small but highly committed donor group that reliably met national needs. The program was discontinued because fractionation of Dutch RhIg plasma was stopped by the Dutch manufacturer. Re-establishing such donor initiatives, potentially supported through EU-level collaboration, could form part of the solution, both domestically and internationally, if similar programs were reinstated elsewhere.

Another long-term strategy is the development of a reliable laboratory-produced monoclonal RhIg. Although substantial research has been devoted to monoclonal approaches, none has yet yielded an agent proven to provide effective prophylaxis.[27]

In Figure 2 possible future developments mentioned in this thesis and discussion are placed on a timeline. The gaps in HDFN management may never be fully closed, but these future advancements will play a vital role in bridging the most significant gaps we face today.

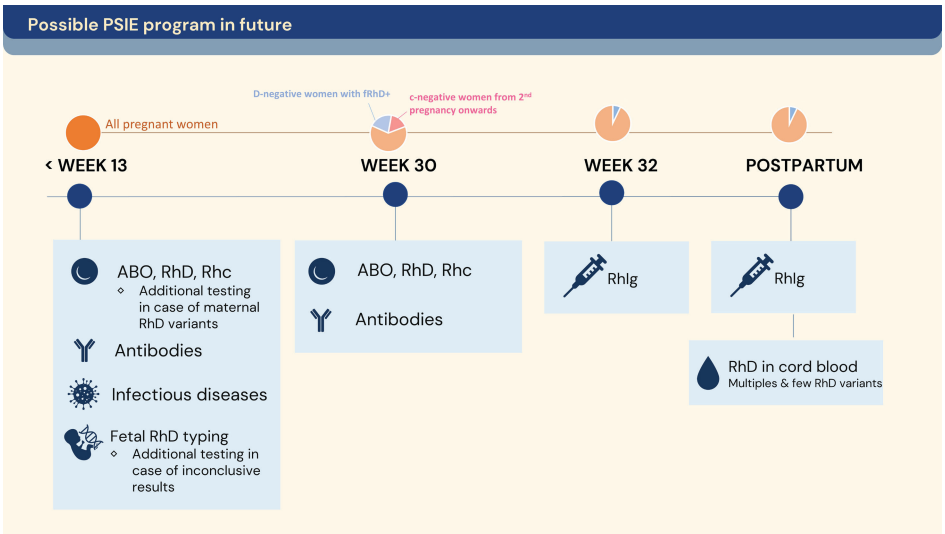

Figure 1. How the Dutch screening program could possibly be if all studies from this thesis, and future perspectives, were incorporated. RhIg: Rhesus D immunoglobulin.

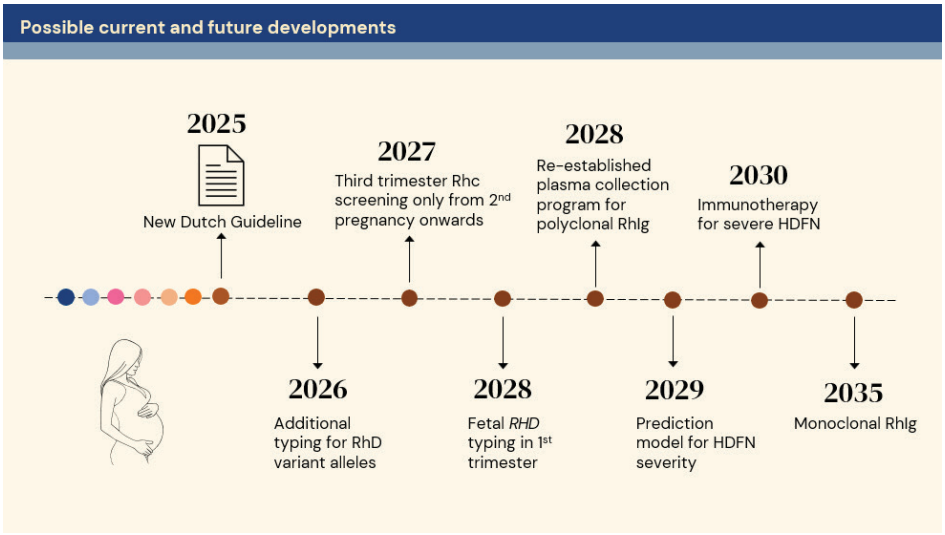

Figure 2. timeline of developments found in this thesis and possible future perspectives. Immunotherapy such as FcRn-blockers. RhIg: Rhesus D immunoglobulin.

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