Improving care for red blood cell alloimmunized pregnant women

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Chapter 9

General discussion

The primary aim of this thesis was to evaluate if the high level of care to pregnant women with red blood cell (RBC) alloantibodies could be improved, starting with the perspective of the obstetric care provider, and by collecting input from pregnant women on their experiences. We designed studies to evaluate the performance of new components of the current policies to prevent RBC immunization and to early identify the risk of severe HDFN during pregnancy. Based on our studies, we strive to make recommendations to further tighten preventive measures, and to gain insight into how the patient and the obstetric care provider can be optimally supported in this process.

Pathogenesis

HDFN is caused by maternal RBC antibodies being transferred to the fetus and is usually provoked by fetomaternal hemorrhage during pregnancy or delivery. HDFN is most frequently caused by RhD alloantibodies, although alloantibodies with other Rh specificities (c, C, E, e) or non-Rh alloantibodies (especially K) may also induce fetal hemolysis. Other type of RBC alloantibodies (Fy, Jk, M, S and s) rarely induce severe disease in the Netherlands.(10) Untreated HDFN may result in progressive fetal anemia, hydrops, neonatal icterus and even perinatal death. Preventive measures have substantially reduced the risk on maternal alloimmunization and improved the outcome of HDFN over the past decades.(14, 182) Both Rh and non-Rh alloimmunization in pregnancies is thus becoming a rare condition. The last report on the performance of the national prevention program (2020), showed that among 172,000 pregnant women there were 480-522 (0,28-0,30%) pregnancies in which RBC alloantibodies were identified, including 235-372 (0,14-0,22%) with clinically relevant RBC antibodies.(62)

Prevalence and prevention of RhD immunization

After the introduction of the antenatal RhIg prophylaxis in 1998, at that time only to pregnant women without a living child, the risk of a new RhD-immunization in the next pregnancy in RhD-negative women who gave birth to an RhD-positive (first) child decreased from 0.67% to 0.31%.(44) This rate is comparable to the observed prevalence in three meta-analyses conducted in the UK by the NICE (National Institute for Clinical Excellence).(183)

The extension of the antenatal RhIg prophylaxis to all RhD-negative pregnant women in 2008 (44) and the targeted RhIg prophylaxis exclusively to women with an RhD positive fetus (2011)(24), did not result in a further reduction of the risk for RhD immunization. The prevalence of newly detected RhD immunizations in 2016 was

0.31% (79/25,170) of all RhD-negative pregnant women in the Netherlands. This can be explained by the average rate of 1.7 children per woman (CBS 2008-2016), implicating that only 21% of women experience more than two pregnancies.(184) With a predicted rate of false-negative fetal *RHD* typing of 0.03%, the occurrence of unforeseen and unexpected severe HDFN was estimated as 1 case every three years. (24) Targeted administration of RhIg based on fetal RHD typing simplifies the process, because RhIg can be administered immediately after childbirth, without additional neonatal typing. However, the effect of the latest adjustments on the prevention of RhD immunization seem of minor importance.

Evaluation of repeated RBC antibody screening in Rhc-negative women

In our nationwide cohort of Rhc-negative women (2011-2013), we found 99 (0.16%) Rhc-negative women with newly detected RBC antibodies at the third trimester screening (at 27 weeks) **(Chapter 3)**. This is in line with reported incidences of late alloimmunization, varying between 0.06 and 0.43%. Remarkably, the incidence of severe HDFN in cases with late alloimmunization appeared to be considerably lower than expected, resulting in a NNS (number need to be screened) to detect one case of severe HDFN of 31,048. From earlier research an NNS of about 9000 was expected. This may be explained by the fact that timely detection of alloimmunized cases at risk for fetal hemolysis, followed by induction of labor at week 37, as advised in the Dutch Guideline on maternal alloimmunization, may have prevented the development of severe HDFN. The downside of this, being a potential negative feature of screening, might be several relatively early and unnecessary inductions of labor, performed purely because of the maternal alloimmunization, despite laboratory test results being below the cut-offs. We observed that a foregoing delivery was a risk factor for Rhc alloimmunization detected late in pregnancy. Furthermore, only three nullipara had late RBC alloimmunization and no HDFN due to RBC alloimmunization occurred. Therefore, it could be evaluated if the RBC alloantibody screening in week 27 could be restricted to para 1 and higher.

During pregnancy there are now valid cut-off values available for laboratory management to predict HDFN prenatally **(Chapter 5 and 6)**. However, these are not valid to predict neonatal disease and the need for neonatal phototherapy and/or exchange transfusions. Whether it is necessary to clinically observe a neonate, in order to monitor bilirubin and hemoglobin levels, if maternal titers were low early in pregnancy, requires further studies.

Future adjustments to the screening and prevention program

Most western countries have maternal RBC alloimmunization screening programs. A wide variation in design of these programs exists between and within countries, ranging from several screenings in all pregnant women to a single screening of RhD-negative women only.(21, 25, 26, 183) In the Netherlands, there is a high uptake of both the screening and the prevention program for RBC alloimmunization.(62) As a result, the current numbers of pregnant women with RBC alloimmunization, followed by HDFN with long-term sequelae, are low (described in **chapters 2, 3 and 4**). Since the disease can be serious in antigen-positive fetuses, it is of great value to further reduce the number of red cell immunizations as much as possible. Based on our findings, the options to prevent RhD immunization mainly lie around childbirth and miscarriage. In pregnancies with complicated deliveries, including cases of major bleeding and surgical interventions, such as cesarean section and surgical (manual) removal of the placenta, determination of FMH volume and adjustment of RhIg dosing is necessary to further reduce the RhD alloimmunization rate.

The mechanism of risk factors that are associated with RhD alloimmunization assumes that a complicated delivery gives an additional risk of a larger FMH. On the other hand, one third of the women who had previously given birth to an RhD positive baby, had none of the risk factors that we reported. Possibly, a larger but subclinical FMH than could be covered by the RhIg prophylaxis occurred, as has been reported earlier.(94) Alternatively, some women would respond more strongly to a relatively low volume of fetal blood entering their circulation. (185) The finding that 27% of the women included in our risk factor study was either nulliparous or had an RhD-negative child in history, supports this hypothesis.

The Dutch Association of Obstetrics and Gynecology (NVOG) advice to administer RhIg to all women with a (missed) abortion past 10 weeks, or when invasive treatment is used after 7 weeks of gestation. We found a higher miscarriage rate in RhD-negative women with anti-D detected early or late in their first ongoing pregnancy with an RhD-positive child, as compared with the general population (35% vs 12.5%). We also found that not in all cases RhIg was administrated, according to current protocol. These findings seem to support the policy to administer RhIg in all cases of miscarriage or abortion, irrespective of gestational age or instrumentation. Observational studies on the effect of RhIg after miscarriage/abortion were mostly performed in the early days after the start of RhIg prophylaxis, and randomized controlled trials are unfortunately lacking.(21, 183, 186) As our study, those early studies showed that anti-D is found late in the first ongoing pregnancy with an RhD-positive child, most likely because immunization already occurred around the miscarriage/abortion, but anti-D is only produced at detectable levels during this first ongoing pregnancy.(69, 95, 96)

In RhD-pregnant women with a previous pregnancy with an RhD-positive child, the significance of potential risk factors for a FMH in that previous pregnancy, such as: external cephalic version, abdominal trauma and antenatal bleeding, but also invasive diagnostics in the current pregnancy, is still controversial in the literature. (72-74) Absence of an association of current pregnancy-related risk factors with D-immunization, suggests that the adherence to current indications for RhIg administration is sufficient in the Netherlands.

Availability of RhIg

For prevention of RhD immunization, we are dependent of plasma from RhD-immunized donors. Until 2020 in the Netherlands, RhIg was part of the product portfolio of the plasma fractionation, by collecting plasma from RhD-immunized donors. Nowadays, all RhIg products used in the Netherlands originates from international operating pharmaceutical companies. Since 'natural RhD-immunized' donors (e.g., women immunized by pregnancy) are becoming more and more rare, mainly donations from actively RhD-immunized donors are used. Although RhD immunization may not implicate a donor's health, the presence of RhD antibodies can delay the process of preparing suitable donor blood, especially if RhD-negative blood is not sufficiently available, such as in Asia.(187) If, 'naturally immunized' women can be motivated to become plasma donor, this reduces such undesirable risks for other volunteers. In addition, voluntary unpaid blood donation is recommended by all international authorities (World Health Organization/Council of Europe/ International Society of Blood Transfusion/European Blood Alliance) (122), because it is the best way to strive for self-sufficiency of all blood products, while maintaining an optimal level of quality and safety for both recipients and donors. (121) Since the process of immunization, repeated boosting and frequent donations ask a lot of the donor, alternatively women already being immunized during pregnancy and being aware of the importance of donorship may serve as highly motivated donors. Our work (**Chapter 4**) showed that a way to tackle this challenge is to intensify the collaboration between obstetric care providers and blood banks. Tailored recruitment strategies could be designed for this group of potential donors, with the obstetric care provider having a major role in creating awareness of potential plasma donorship in women with RhD antibodies. This fits well with one of the CanMed roles of the caregiver, for example health advocate and collaborator. Ideally, an international donor program would be designed to always have sufficient plasma available.

Laboratory management

This thesis shows that although K-immunized pregnancies with a K-positive fetus nowadays occur seldomly (6 per year in the Netherlands), the screening and subsequent management of these high-risk cases are of value, as 50% of affected children need intrauterine (IUT) or postnatal transfusion therapy.

We showed that in K-immunized pregnancies with a K-positive fetus, an anti-K titer of 4 identifies all cases with a high risk for severe HDFN defined as the need for IUT or postnatal transfusion therapy **(chapter 5).** Remarkably, the test results of the titer and ADCC did not change significantly during pregnancy. The first titer appeared therefore to have the highest power to predict the necessity of transfusion therapy in K-alloimmunized pregnancies. Our proposed cut-off value of 4 for the titer is on the safe side and in contrast with those proposed by other authors.(5, 147) These studies included cases of severe HDFN and retrospectively described the titers in those pregnancies. In our study we had the opportunity to describe all pregnancies in the Netherlands between 1999 and 2015 with a K-positive fetus and collect all available titer and ADCC results. Therefore, we can accurately conclude that K-mediated HDFN with need for transfusion therapy in cases with titers <4 is very rare. The ADCC test was not suitable to select high risk K-alloimmunized pregnancies. This could be explained by the pathogenesis of anti-K mediated HDFN, in which both the suppression of erythropoiesis and hemolysis of fetal RBC occur. The ADCC test may generally be more correlated with the level of hemolysis. Cost-effectively, the ADCC will not contribute as the specificity of detecting HDFN is not increasing if ADCC test results are added (**Chapter 5**). Moreover, every pregnancy with a titer above the cut-off value will have to be clinically followed with Doppler ultrasound examination to timely detect fetal anemia.(26) Based on our results, we propose not to continue testing with the ADCC assay in K-alloimmunized women.

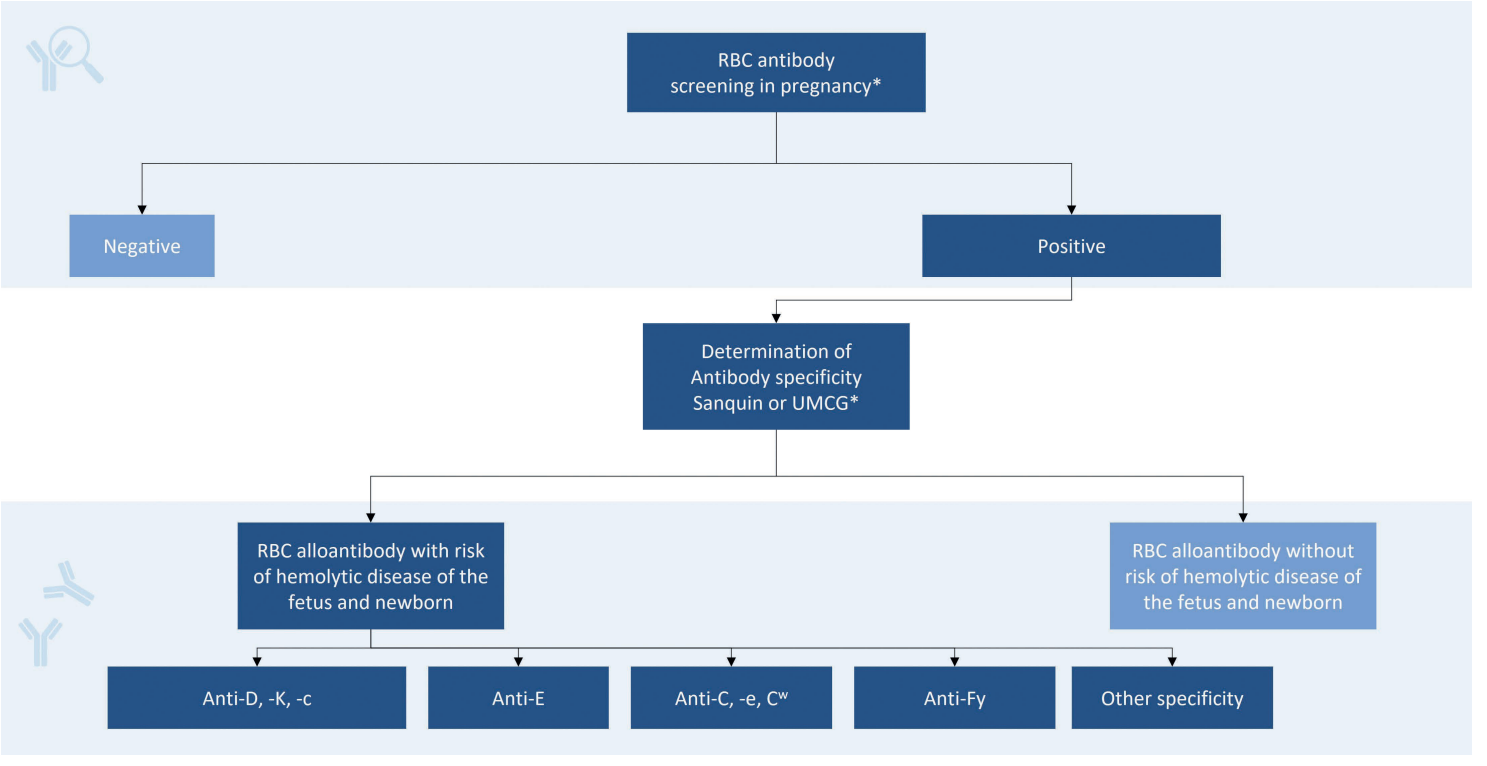
Most cases of severe HDFN are caused by anti-D, less frequently by anti-c and anti-K, and in a rare case by other Rh antibodies.(10) Anti-Fy type of antibodies increase the risk for neonatal icterus, needing phototherapy treatment.(140) For almost all other RBC alloantibody specificities there is casuistic evidence that they may cause severe HDFN disease, underscoring the fact of the very low frequency of those events. In our nationwide prospective cohort study, including pregnant women with RBC alloantibodies with a specificity other than anti-D or anti-K and with an antigen-positive fetus, we found that a maximum titer of ≥16 was the best cut-off to differentiate between pregnancies at low and high risk for severe HDFN. The cut-off of ≥16 obtained in our study is close to the cut-off of 32, derived from other studies. In the follow-up of pregnancies with RBC antibodies with other specificities than Rh, the ADCC test appeared to have no additional value (chapter 6).

Up until now, there is evidence that the glycoprofile of RBC alloantibodies may influence antibody pathogenicity and therefore may be considered a putative diagnostic marker.(153) The OPZI 2.0 cohort, which is described in **chapter 2**, has also been designed to collect a cohort of samples and clinical data to investigate the value of RBC alloantibody glycoprofiles in the prediction of HDFN. Study results are expected in the coming years.

Proposed laboratory management

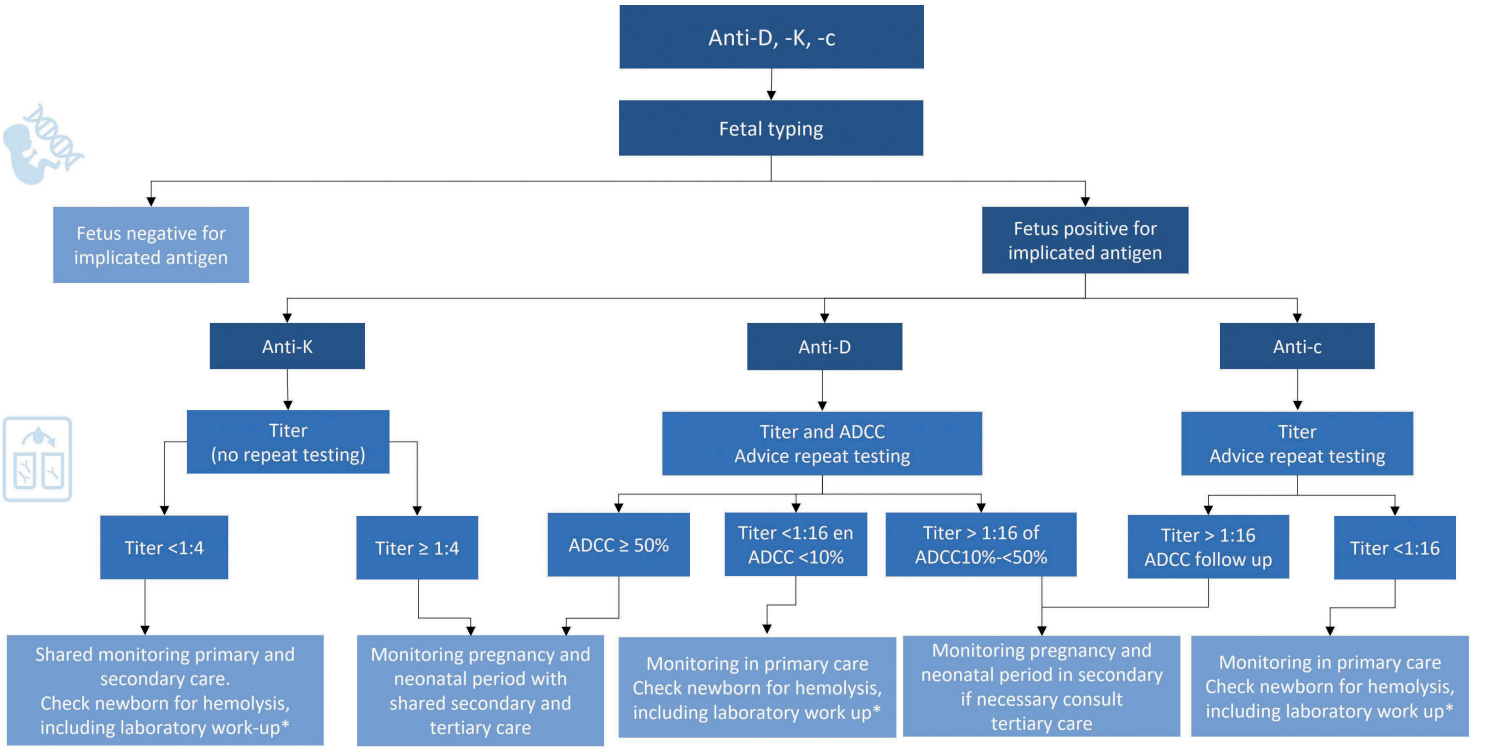
Evaluating the current laboratory management of alloimmunization in pregnancy in the Netherlands, in the context of the results of our studies, we were able to make useful suggestions for adjustments and finetuning of the protocol. In figure 1A,B,C we show how current laboratory management should look like, based on the findings in **chapters 3, 5, and 6**. Our research outcome can be used to update the Guideline Erythrocyte Immunisation and Pregnancy from the Dutch Society of Obstetrics and Gynecology (in Dutch: NVOG), as published in 2009.(26) To further improve the laboratory and clinical management, the care to RBC alloimmunized women would benefit from implementation of a process of continuous monitoring the predictive value of laboratory testing and clinical management in relation to HDFN disease outcome in the newborns. Due to the rarity of the disease, prospective studies to validate traditional and new laboratory tests or to judge necessity of clinical monitoring will be very difficult to perform and take a long time period. Ideally, a process would be developed enabling continuous centralized data collection on RBC alloimmunized pregnancy, making it possible to review laboratory test results and clinical data obtained during pregnancy and after birth.

Figure 1A



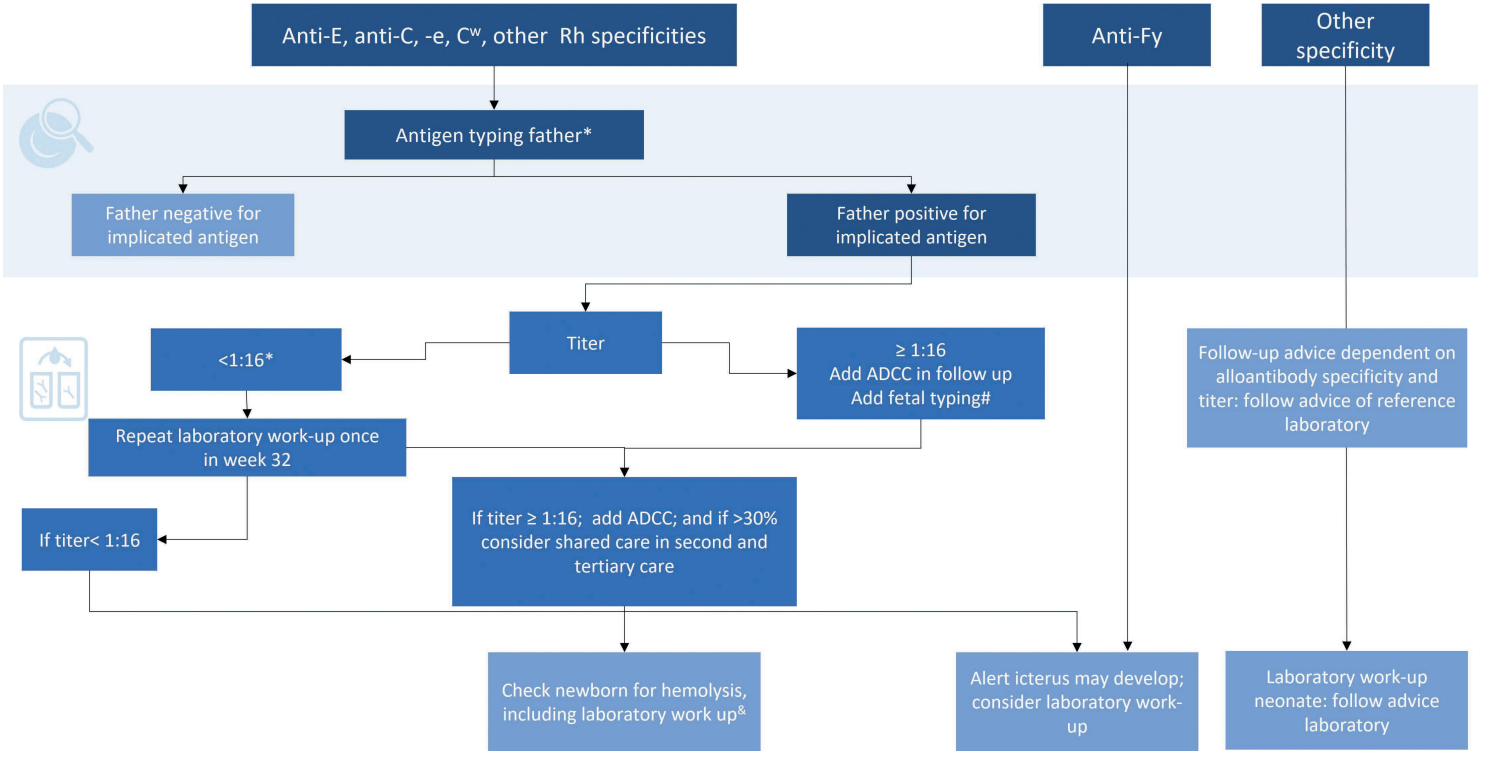
**According to national program: prevention and screening infectious diseases and erythrocyte antibodies in pregnancy (75); #no risk, if of IgM class; or low/absent expression of the antigen on fetal RBCs and no association with HDFN.*

Figure 1B



**For anti-K: if transfusion before 2004 or abroad: consider first K typing of partner: #Laboratory work up: Typing of implicated RBC antigen; Direct Antiglobulin Test, Hb, bilirubin*

Figure 1C



**Fetal typing is possible, but –especially if low are absent titers- antigen typing of the father can be used to reduce costs; #Consider ADCC or fetal antigen typing in follow-up, based on antibody specificity.*

&Laboratory work up: typing of implicated RBC antigen; Direct Antiglobulin Test, Hb, bilirubin

Evaluation prevention program in the framework of the Can MEDS model

Nowadays, due to the success of all preventive measures, obstetric care providers see only few pregnant women with a pregnancy complicated by RBC alloimmunizations This may result in insufficient knowledge, inadequate information transfer and substandard care for women, who are diagnosed with RBC antibodies. In the Netherlands, annually 170-180 000 pregnant women are entering the screening program; the uptake of the RBC alloantibody screening program is very high. Thanks to a well-organized network that has evolved over the years with central coordination of the prevention program by the RIVM, the laboratories and obstetric care providers, the RhD immunization rates are low ((53) and chapter 2) and the timely identification of high-risk pregnancies is successful.(14)

The prevention program contains multiple safety nets during the process of case identification. For example, the obstetric care provider receives all necessary information on RBC alloantibody risks from the reference laboratory, including advice (often also by phone) if a pregnant woman must be referred to a regional hospital or to the fetal therapy center, the LUMC. Presumably, this adds to the prevention of delay or misjudgments of test results, but this also means that the care provider is comforted in such a way, that there is no need to take the effort to be up to date and well-informed concerning current knowledge.

Viewed from the perspective of the Can MEDS framework, several caregiver roles are well represented in the prevention program, such as global leadership in preventing alloimmunization and a high standard of treatment and care. There is a national collaboration of the reference laboratories, the fetal expert center and the National Institute of Public Health and Environment. Centralizing care thus enables a high standard of prevention and treatment. Knowledge obtained from the Dutch program and experience may be shared with international colleagues, to further improve all programs in terms of prevention, diagnostics and clinical monitoring.

Nevertheless, there is also room for improvement regarding the competencies needed to run the screening and prevention program properly. The obstetric care provider can think of education and keeping him- or herself up to date. Since the last adjustments in the screening program in 2011, there was an e-learning accessible for all health care providers, involved in the care of alloimmunization in pregnancy. In 2017, several medical experts did a tour through the Netherlands, to provide healthcare providers with more information about alloimmunization and hemolytic disease of the newborn. However, these education tools are not used by all. In **chapter 8,** we showed that health care providers had little knowledge about items defined in the national guidelines, such as: when an extra dose of RhIg should be administered

or how a titer or ADCC result should be interpreted. These items are until now not clearly enough described in the guidelines, and possibly therefore multi-interpretable. We advise to increase the clarity of recommendations in an updated guideline. In **chapter 7,** we showed that most concern was caused during referral to a reference center and the interpretation of laboratory results. It is therefore important that the national guidelines are amended in such a way, that the healthcare provider has easy access to the necessary information. Attention must also be paid to the information that pregnant women need and must receive and the fact that an expert can always be consulted when there are questions or uncertainties. From the perspective of professionalism, this also means that a care provider knows the limits of own knowledge and skills, especially in the context of rare diseases. In **chapter 8** we report that immunized women indicate to have felt insecure because of too much or incorrect information by their primary obstetric care provider. It is important to create awareness, for instance through national scientific journals, of the impact of incorrect or too much not very concrete or contradictory information by health care providers, in case of a rare disease.

Finally, from the role of health advocacy, there are opportunities to make RhIg more widely available and thus also to reduce the morbidity and mortality of HDFN on a global level. If monoclonal antibody based RhIg is not available, we must rely on enough volunteer anti-D plasma donors, and we might join forces in international context, as is currently done by a working group led by Prof. van der Schoot and initiated by the European Directorate for the Quality of Medicines (EDQM). Sufficient RhIg is also of importance to increase the access to RhIg in low-income countries. This is of major importance for reducing global morbidity and mortality from anti-D mediated HDFN.

Conclusions and future perspectives

In general, we can conclude that the current Dutch screening and prevention program for alloimmunization is on a high standard level. Adjustments can be made to be strict in the policy of recognizing risk factors, determination of estimated FMH volume and adjustment of RhIg dosing, especially in pregnancies with complicated deliveries, including cases of major bleeding and surgical interventions, such as cesarean section and manual (surgical) removal of the placenta. Miscarriage and abortion can be considered as risk factors for alloimmunization, although further research is still necessary to determine the preventive effect of RhIg in all cases. For selection of pregnancies with a high risk of HDFN in K- and non-D immunized pregnancies, the RBC alloantibody titer can be used to make a first selection, preferably after fetal typing of the cognate antigen or after RBC antigen typing of the father. When the titer is equal or above the cut-off value of 4 in K-immunized pregnancies and 16 in other types of RBC immunizations, further clinical follow-up is required. The ADCC test

cannot be effectively used in the first selection of high-risk pregnancies, other than in cases of RhD immunization. Our studies can be used to reduce intensive laboratory management in pregnancies complicated by RBC alloimmunization. Due to the rarity of HDFN, it is of importance to keep the level of knowledge high in expert centers. An up-to-date guideline needs to be available that can be used by all obstetric care providers in primary care. These professionals need to be aware of the limits of their abilities and never refrain from consulting experts in the field for their opinion. This is especially of importance to improve the experience and wellbeing of the immunized pregnant woman and not to confuse and frighten her and her partner unnecessarily.