Improving care for red blood cell alloimmunized pregnant women

Slootweg, Y.M.

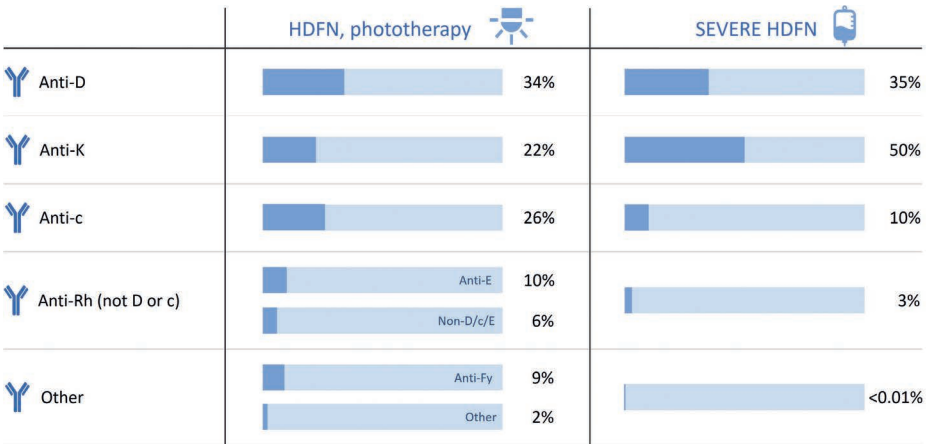
Chapter 1

Development of preventive measures to reduce occurrence of severe hemolytic disease of the fetus and newborn

General introduction

Hemolytic disease of the fetus and newborn (HDFN) is a serious complication in pregnancy, that may be life-threatening for the (unborn) child. (1-9) HDFN is caused by red blood cell (RBC) alloantibodies, developed by the mother and transferred to the fetus. Most severe cases are caused by RhD, Rhc and K antibodies (see figure 1).(1, 3-5, 10) Without treatment, HDFN may result in progressive fetal anemia, fetal hydrops, asphyxia and perinatal death (see figure 2). Even though the incidence of fetal alloimmune hydrops has declined in the last decades, this condition is still a well-known risk for adverse perinatal and long-term outcomes.(9, 11-14) After birth, neonatal hyperbilirubinemia may lead to 'kernicterus', a cause of neurodevelopmental impairment, including athetoid cerebral palsy, hearing problems and psychomotor handicaps.(12, 13, 15, 16) Primary prevention is one cornerstone in the reduction of the incidence of severe HDFN. When, despite prevention, RBC antibodies are present, early detection followed by identification of high-risk pregnancies is the second cornerstone in the prevention of adverse outcome. (17-21) If antibodies with the potency to cause HDFN are found, it is possible to genotype the fetus for the implicated blood group antigens with cell-free fetal DNA derived from maternal plasma.(22-24) If the fetus is (possibly) antigen-positive, the (relative) quantity of antibodies in the plasma and their potential hemolytic activity can be used to select pregnancies with a high risk of HDFN.(10, 18, 19, 25-27) Subsequent intermittent fetal monitoring is aimed to identify fetuses with (imminent) anemia, needing intrauterine transfusions (IUTs) and/or elective (preterm) delivery, possibly followed by phototherapy or (exchange) transfusions.(15, 28, 29)

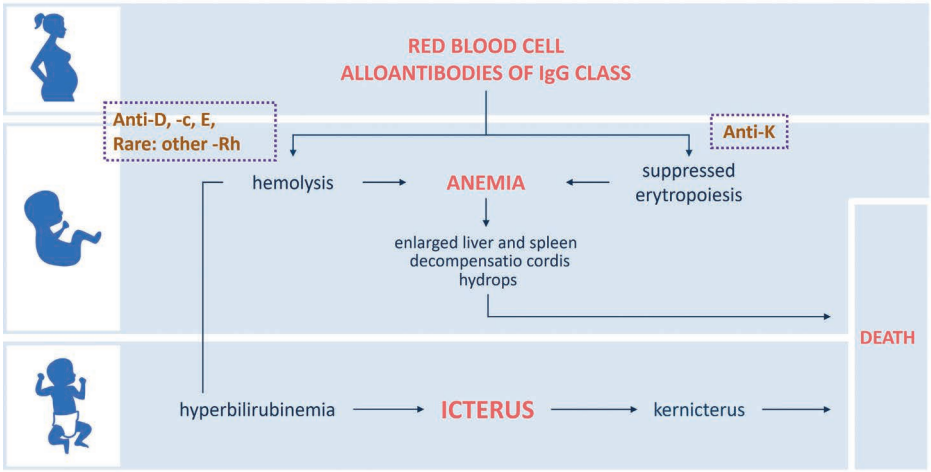
Figure 1: *Type of RBC antibody and course of disease severity.*



Koelewijn et al., Transfusion, 2008; Slootweg et al., AJOG 2018; de Winter et al., unpubished (RhD mild disease)

Primary and secondary preventive measures, such as: prenatal and postnatal RhD immunoglobulin prophylaxis (RhIg), matched blood transfusions for Rhc, RhD, RhE and K antigens to women of fertile age (<45 years) and a routine first trimester prenatal screening for RBC antibodies substantially reduced the risk on maternal alloimmunization. Also improvements in fetal monitoring and therapeutic possibilities have added significantly to a better perinatal outcome of HDFN over the past decades.(25, 30-32) However, even in countries with high standards of fetal and neonatal care, perinatal death due to HDFN still occurs. Therefore, improving the prevention of alloimmunization, the timely identification and referral of pregnancies at risk, as well as the logistic structure around the screening program, remain important keys in effectively reducing HDFN-related perinatal death.(28, 30)

Figure 2: *Pathophysiology of hemolytic disease of the fetus and newborn*



Historical scope of primary preventive measures: RhD immunization

It was already known in the 1960s that fetomaternal incompatibility in ABO blood groups affects the chance of maternal D-alloimmunization.(33) It was postulated that the mother's naturally occurring anti-A or anti-B may bind and destruct fetal RBCs, thus reducing the likelihood of D-immunization to occur in RhD-negative pregnant women with an RhD-positive fetus(34) In 1962, both a British research group led by Prof. Clarke and an American research group (Freda and co-workers) showed that male volunteers were protected against RhD immunization, if anti-D was administered prior to a test transfusion with RhD-positive blood.(35, 36) This so-called 'RhIg prophylaxis' was subsequently tested in England, America, Canada, Germany and the Netherlands, for the prevention of anti-D formation in RhD-negative women after giving birth to an RhD-positive child. The results were promising, as

it was concluded that immunization could thus be effectively prevented in 95% of cases.(33, 37-40)

In 1965, Dr Krijnen of the Central Laboratory of the Blood Transfusion Service of the Dutch Red Cross (later merged into Sanquin) produced the first batch of RhIg from the plasma of D-immunized donors.(41) Clinical studies were conducted using this batch, in order to determine the optimal dose of postnatal RhIg in the Dutch situation. Testing was performed, using different doses of RhIg (120, 180 and 250 µg), administered intramuscularly within 24 hours after delivery.(40) In this study with eighty centers participating, it was concluded that all doses were effective to prevent RhD immunization. Because RhIg was scarcely available, a dose of 200 µg RhIg was chosen. It was concluded that without RhIg prophylaxis, D-immunization occurs in 8% of RhD-negative pregnant women who gave birth to one RhD-positive child, increasing up to 17% after giving birth to two RhD-positive children. However, when using postnatal RhIg prophylaxis, only 1% of the pregnant women showed anti-D, if screened six months after giving birth.

Dr Borst-Eilers has conducted extensive and important research, in the period from 1958 to 1962, into the development and prevention of anti-D formation or “Rhesus Immunization”. Based on this research, in her thesis Dr Borst-Eilers states that, in order to implement an optimal prevention schedule with RhIg, one should first consider the following facts regarding the occurrence of RhD immunization:

1. RhD immunization can already occur before delivery;(40)
2. RhD immunization can occur during uncomplicated pregnancy and childbirth, because of not noticed fetomaternal bleedings.(42) Anti-D formation can be further prevented by performing a Kleihauer test to quantify fetal RBCs and then adjusting the RhIg dose;(43)
3. RhD immunization can occur as a result of loss of pregnancy in the first trimester;
4. A negative Kleihauer Betke test is no guarantee that immunization will not occur. (42)

Also in the current prevention program, using prenatal and postnatal administration of RhIg, these facts will still impact the outcome of this policy. Although the current prevalence of RhD alloimmunization is low (Chapter 2, this thesis),(44) it may well be that the above mentioned risk factors still lead to cases of RhD alloimmunization, preventable if only RhIg administration would be adjusted, for example by quantification of fetal RhD-positive RBCs in blood of the mother.

Developments in the prevention program: anti-D prophylaxis

In 1992, the Dutch Health Council reviewed the prevention program as it was first introduced in 1970, including the administration of RhIg to all RhD-negative women after childbirth. The Health Council concluded that significant health benefits for neonates could be achieved , by expanding the program by including the administration of RhIg prophylaxis during pregnancy.(45-47) Since RhIg is a scarce blood product, the Health Council initially proposed that only women without a living child should be given RhIg during pregnancy, because also antenatal RhIg prophylaxis predominantly contributes to prevention of severe HDFN in a next pregnancy and these women have the highest chance to have a next pregnancy. The committee assumed at that time, that "monoclonal anti-D preparations, prepared with biotechnological techniques and therefore not from the plasma of volunteers, will probably be widely available in a few years' time".(45) In the last decades, a few clinical studies have been performed with monoclonal antibody (MoAb) based RhIg prophylaxis. Still, there is no successful MoAb RhIg largely available.(48-50)

When antenatal RhIg prophylaxis was introduced, there were the following considerations of possible harm: RhIg administered antenatally can also reach the fetal circulation via active transport across the placenta and bind to the fetal red cells.(51) Indeed, after birth anti-D sensitized RBCs are observed, but the level of sensitization is too low to result in fetal hemolysis. Another adverse effect of antenatal administration of RhIg was the theoretical possibility that a very low concentration of RhIg in the mother could actually increase the chance of immunization.(52) Therefore, it was recommended in 1998 that the level should be at least 100 IU during the remaining pregnancy period after administration. In the UK, a low dose of 500 IU is given at two time points in pregnancy, week 28 and week 34, so that this lower limit is achieved over a longer period. For the Dutch situation, it has been calculated that after administration of 1000 IU in week 30, the lower limit of 100 IU is reached at a gestational age of 42 weeks. The Health Council recommended in 1992 that an extra dose of RhIg should be administered to pregnant women in week 42, and that in these cases the postnatal RhIg dose could be omitted.(44) However, this recommendation has never been incorporated into policy. In a nationwide study performed in 2004 in RhD-negative women who had one previous delivery (Parae 1), Koelewijn et al. found that post maturity is a risk factor for RhD immunization. (53) It was postulated that this finding indicated a failure of RhIg prophylaxis at advanced gestational age. It was recommended to administer an extra dose of RhIg when 42 weeks are reached or to split the prior single dose at 30 weeks into two gifts at 28 and 34 weeks. In current obstetrical practice in developed countries post maturity beyond 42 weeks has become rare, as most pregnancies are terminated before or around 41 weeks, for the benefit of fetal outcome.(54) In the group of RhD-immunized women who received complete antenatal and postnatal RhIg prophylaxis,

Koelewijn and coworkers further observed that a relatively young age at first delivery, non-spontaneous delivery (cesarean section or assisted vaginal delivery) and post maturity in the previous pregnancy emerged as independent risk factors for RhD immunization.(53) Koelewijn et al. reported that addition of antenatal RhIg to the RhD immunization prevention program halves the risk of anti-D formation in RhD-negative women and concluded that this was in line with the expected effect.(44)

Although initially, only women without a living child were eligible for administration of antenatal RhIg, in 2008 this was extended to all RhD-negative pregnant women, irrespective of their number of previous pregnancies.

Developments in the prevention program: RBC alloantibody screening.

RhD-negative women are already since the 1960's screened for the presence of anti-D (and other RBC alloantibodies) in the last trimester of pregnancy. This screening was first conducted in week 32 of pregnancy and with the introduction of antenatal RhIg prophylaxis, the blood sample was taken immediately prior to the administration of RhIg in week 30. In 1992, the Health Council stated that screening for RBC alloantibodies should become standard care during pregnancy, because besides anti-D also other type of RBC alloantibodies can cause severe HDFN.(55) An additional advice was that the laboratory testing for RBC alloantibody specificity and RBC alloantibody titers could best be centralized. Firstly, because of the required expertise in the interpretation of the results and secondly for the necessary epidemiological analysis. In 1998, these recommendations of the Health Council led to the introduction of the RBC alloantibody screening for all pregnant women before week 13 of pregnancy to be combined with the screening for infectious diseases, as part of the prevention program for screening infectious diseases and erythrocyte immunization (PSIE), nowadays being conducted by the Center for Population Research (CvB) of the National Institute for Health and Environment (in Dutch RIVM; Rijksinstituut van Volksgezondheid en Milieu). The laboratories of the University of Groningen and that of Sanquin in Amsterdam continued their role as reference laboratories for RBC antibody specification, antibody titration and determination of the hemolytic potential of the RBC alloantibodies with the antibody dependent cellular cytotoxicity assay (ADCC; only in Amsterdam).

Evaluation of RBC alloantibody screening introduced in 1998: the OPZI study

In the period 2004 to 2008, the in 1998 introduced measures (antenatal RhIg in women without living child and general RBC screening) were evaluated by the OPZI

(Investigation and Prevention Pregnancy Immunization) research group. The research team contained a mixture of expertise, with substantial immunohematological laboratory experience (prof. van der Schoot, prof. de Haas, associated with Sanquin), obstetric practical experience (dr. Koelewijn (midwife and researcher)) and epidemiological expertise (prof. Bonsel and dr. Vrijkotte). Evaluation of the effectiveness of antenatal RhIg by the OPZI team is discussed above (see under Developments in the (screening and) prevention program: anti-D prophylaxis). To evaluate the effectiveness of the screening of RBC alloantibodies before week 13 in pregnancy, a study was conducted by the OPZI team based on a nationwide sampled cohort of 400,000 pregnancies. Recommendations of this study were implemented in laboratory monitoring of RBC alloimmunized pregnancies. One of the observations was that anti-M antibodies hardly were of clinical significance and that laboratory follow-up was only necessary if anti-M was of IgG class.(10) Another recommendation was that laboratory monitoring could focus on Rh and K antibodies and that for all other type of RBC alloantibodies one additional test in last trimester would be sufficient to create sufficient awareness of the newborn being at risk to develop HDFN. These recommendations were implemented in the reference laboratories. An important observation was that 25% of severe cases of HDFN in RhD-positive women occurred unexpectedly, after a negative screening result in the first trimester. Some of the children of these unexpected cases suffered from HDFN-related handicaps caused by perinatal asphyxia or kernicterus, because fetal anemia and hyperbilirubinemia were not timely detected.(10) In contrast, all cases of alloimmunization already detected at first trimester screening were treated in a timely manner and children were healthy at the age of 1 year. All first trimester screen-negative cases of severe HDFN were caused by anti-c and/or anti-E. However, long-term sequelae were only found in anti-c cases.

The outcome of the studies performed by the OPZI team and international data formed the basis for a new advice by the Health Council in 2009 (56). The following additional measures were advised:

- Selective screening in primiparous women or women with a previous blood transfusion;
- Selective follow-up, only of women with clinically relevant antibodies (anti-D, -c, -E and -K);
- Rhc-antigen determination at 12 weeks and a second screening of Rhc-negative women around 30 weeks;
- Additional matched transfusion policy in women aged <45 years, concerning Rhc, E and K;
- Implementation of the determination of the fetal RhD-antigen at 27 weeks, followed by RhIg prophylaxis, only to women pregnant of an RhD positive fetus;

After a prospective risk analysis by the RIVM (57), in 2011, the advised selective screening in primiparous women and all women with a previous blood transfusion was rejected by the obstetric care providers because of reasons of impracticability. (56) Screening for RBC alloantibodies at the booking visit in all pregnant women continued as part of the program (see figure 3). The advice for an additional screening of all Rhc-negative women, comprising 18.7% of pregnancies, at week 27 was reviewed and implemented in order to increase the detection rate of severe HDFN with 25% (from 75% to 100%).(10) Also RIVM reviewed the feasibility and costs of fetal RHD typing in week 27 to target RhIg administration in week 30 and after delivery and it was decided by the Minster of Health to implement this measure in 2011.(24) Finally, in 2011, it was advised by the Transfusion Guideline to select Rhc, RhE and K-matched red blood cell units for girls and women if <45 years of age.(32)

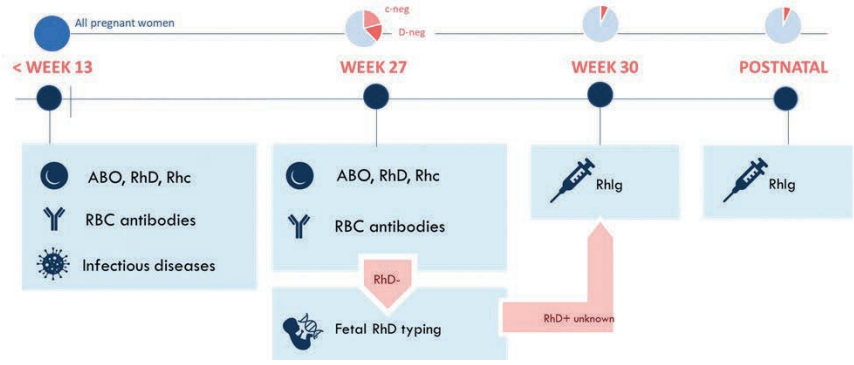


Figure 3: *Prenatal screening Infectious diseases and Erythrocyte immunization, program design since 2011.*

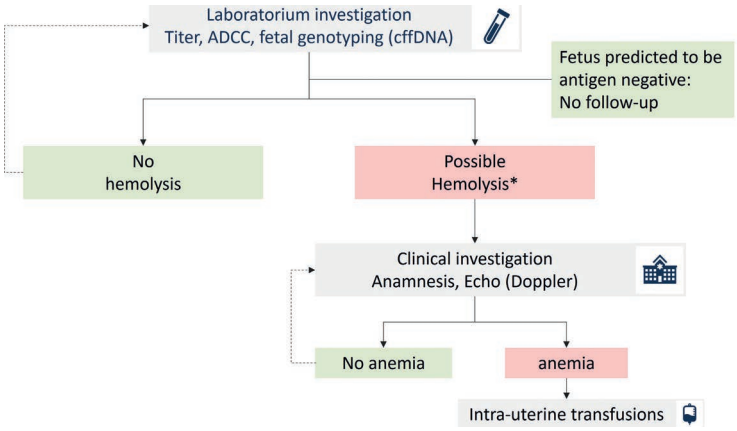
Laboratory monitoring of high-risk HDFN pregnancies

The prevention program enables timely detection of clinically relevant RBC antibodies. When detected, it is necessary to select those pregnancies at risk for severe HDFN (see figure 4). In most centers, to identify pregnancies at risk for severe HDFN, the concentration of RBC antibodies in blood plasma is quasi-quantified by determination of the RBC alloantibody titer. A titer reflects the number of times a plasma sample can be diluted before the RBC alloantibodies cannot be detected anymore, the higher the titer, the higher the concentration of RBC alloantibodies. If their RBC alloantibody titer is above a certain threshold, pregnant women are referred to a maternal-fetal medicine center for close fetal surveillance and, if needed, for fetal or neonatal treatment. In the Netherlands, the reference center is the Leiden University Medical Center. High-risk pregnancies are monitored with ultrasound and Doppler middle

cerebral artery (MCA) peak systolic velocity measurements, to predict the presence of fetal anemia.(18, 29)

In The Netherlands, fetal genotyping for the implicated RBC blood group antigen is performed with cell-free fetal DNA isolated from maternal plasma.(22) Alloimmunized pregnant women with an antigen-positive fetus are monitored by serial testing of the antibody titer and the antibody-dependent cellular cytotoxicity (ADCC) bioassay, a monocyte-based assessment of the destructive capacity of the antibodies.(58) For laboratory management of RhD-immunized pregnant women, Oepkes et al validated the cut-off values for titer and ADCC.(19) The 'critical titer' to identify pregnancies at risk for hemolysis was set on 16 for most (clinically relevant) antibodies in the Netherlands, the ADCC on 50% for anti-D and 30% for non-D antibodies.(26) An ADCC test result lower than 10% means that the pregnant woman can receive obstetric care in her own center without the necessity for referral to a specialized center. For anti-K it was concluded that a titer of 2 should already be regarded as a critical limit. In general, the guidelines in other countries have non-D/non-K alloantibodies cut-off levels of 32. (4, 21, 59, 60) In the Netherlands, pregnancies with anti-D, -c or -K are monitored every 2 weeks and other if Rh antibodies are present (anti-C/-E/-e) monitoring every 3 weeks is advised during the last trimester. In case of other antibodies (anti-Fy^a/-Fy^b, -Jk^a/-Jk^b, -S/-s and other) the laboratory testing is repeated only once in week 30. These intervals are based on the OPZI study.(10, 26, 61)

Figure 4: *Selection of high-risk pregnancies for HDFN after RBC antibody found during the screening in the 1st trimester or at 27 weeks of gestation*



**Hemolysis can be expected with a cut-off for the titer anti-D: ≥ 1:16; K: ≥ 1:2; other: ≥1:16 and a cut-off for the ADCC anti-D: ≥ 50%; K: ≥30%; other: ≥ 30%.*

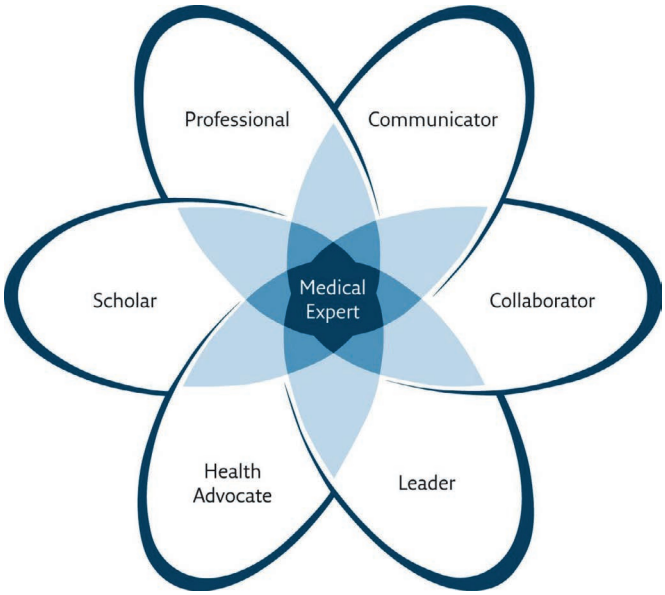
Contextual framework of the thesis

The care to pregnant women and their partners with an unborn child at risk for a rare, but potentially severe disease, such as HDFN, demands various skills from the obstetric care provider. One should not only have the knowledge of the pathology of the disease, of disease risk assessment and required laboratory and clinical tests at various moments in pregnancy, moreover one should inform and counsel the pregnant woman and her partner. Since alloimmunization in pregnancy is rare, most obstetric care providers (primary care midwives and even obstetricians) do not regularly take care of pregnant women with RBC alloantibodies. Every year, the performance of the prevention program is evaluated.(62) Currently, only 0.12-0.22% of all pregnant women in the Netherlands has clinically relevant RBC antibodies at the first trimester screening and 0.22% of RhD and Rhc negative pregnant women has clinically relevant RBC antibodies at 27th week of gestation.

Knowledge on which alloantibodies are more often associated with risks for HDFN may not easily be available. A Dutch questionnaire survey in 2004 showed that 50% to 70% of the women, particularly those with RBC antibodies, indicated that they needed more information, preferable verbally, about the consequences of the RBC alloantibodies for their child.(63) Supportive written information (e.g. folders/hand-outs) was lacking, both prenatally and postnatally.

In Canada, the Royal College of Physicians and Surgeons developed a framework that can be used for training and qualification in all these different professional roles: the so-called Can MEDS roles (Canadian Medical Education Directives for Specialists). Seven different Can Meds roles are discriminated (Figure 5). The Can MEDS framework aims to develop the competences that are of importance to improve the care system, and to provide insight in the current level of performance on the set of competences. This contextual framework may be useful to achieve high-level care to women with pregnancies complicated by the presence of RBC alloantibodies.

Figure 5: *Can Meds (Canadian Medical Education Directives for Specialists) framework for improving patient care.*



Formal adaptation by the Royal College of Physicians and Surgeons of Canada in 1996 and updated in 2005 and 2015.

Aim and outline of the thesis

The aim of this dissertation was to identify gaps in knowledge and room for improvements in certain aspects of the current system of prevention and care regarding RBC alloimmunization in pregnancy. The focus in this thesis was to evaluate the performance of new measures in the prevention program, the follow-up with laboratory monitoring in alloimmunized pregnancies and the counseling of pregnant women and their partners, in cases of RBC alloimmunization.

The need for the RBC alloantibody screening in Rhc-negative women in week 27 of pregnancy has been questioned by health care professionals in the field. Evaluation was planned to judge if the new measure led to improved care for and outcome of the expected number of 6-7 babies at risk for severe anti-c mediated HDFN (**Chapter 3**).

We postulated that the evaluation of risk factors for RhD immunization, as reported by the OPZI study group as published in 2009, created awareness for risk factors for D alloimmunization around delivery. We set out a study to investigate risk factors for D alloimmunization in the current setting with targeted RhIg administration, now based on fetal RHD typing in week 27 of pregnancy. We also planned to review the current rate of D alloimmunization (**Chapter 2**). In addition, a study has been set up to assess how women who are D immunized feel about the option of donating plasma for RhIg production (**Chapter 4**).

To further design a lean protocol for follow up laboratory testing in alloimmunized pregnancies, we conducted studies on laboratory testing in anti-K complicated pregnancies and in pregnancies with other type of RBC alloimmunization (**Chapters 5** and **6**).

Finally, since HDFN is a rare disease, we set out qualitative studies among obstetric care providers and (pregnant) women, with pregnancies at risk for HDFN, to evaluate if and how to improve the moments of contact between care providers and the women and their partners (**Chapters 7** and **8**).