Detection and prevention of pregnancy immunisation: the OPZI study
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CHAPTER 1

General Introduction
Haemolytic disease of the fetus and newborn (HDFN) is a disease which - if untreated - can cause perinatal mortality and morbidity with a substantial risk for long-term sequelae. HDFN was for long the major specific cause of perinatal mortality and morbidity, while many mothers-to-be with a risk of recurrence were facing a difficult reproductive decision in view of the limited therapeutic possibilities.

Since the first description of the disease, the treatment possibilities have substantially improved the prognosis of affected fetuses, provided that the onset of the disease is detected in time, for example by introduction of a screening programme. Also primary prevention measures have been developed.

1.1 Historical background
The first description of what, in retrospect, was a case of severe HDFN was in 1609. Louise Bourgeois, a French midwife, described a twin birth of which the first child died immediately after birth and the other a few days later. The first child was oedematous, a condition called hydrops fetalis, the second suffered from severe jaundice, also termed as icterus gravis, a condition reflecting high levels of bilirubin in the blood of the newborn. In 1932 Diamond et al. realised that hydrops fetalis, icterus gravis and congenital anaemia were manifestations of the same disease, which they named erythroblastosis fetalis. This term refers to the finding of extramedullary (i.e. outside the bone marrow) production of red blood cells (RBCs), also called erythrocytes, and the presence of many young RBCs, called erythroblasts. In 1938 Darrow hypothesized that the disease was caused by placental transmission of a destructive influence from the mother to the fetus. In 1941 Levine elucidated the process of fetal and neonatal red blood cell (RBC) destruction (haemolysis) due to maternal alloantibodies against blood group antigens of the unborn child, in most cases directed against the RhD antigen, which blood group antigen was described firstly by Landsteiner and Weiner in 1940. Not until 1954 it was reported that mothers were sensitised by a fetomaternal haemorrhage (FMH), mainly occurring at delivery. Nowadays, the term erythroblastosis fetalis has been replaced by haemolytic disease of the newborn (HDN) or haemolytic disease of the fetus and newborn (HDFN). The last term better reflects the antenatal onset of the condition.

The first important step in neonatal treatment of severe HDFN was made in 1946 by Wallerstein, who described that neonatal exchange transfusion was an effective method to lower high neonatal bilirubin levels. Hyperbilirubinaemia can cause kernicterus, the most serious and feared neonatal complication of HDFN. Antenatal diagnosis of severe HDFN was not possible until Liley introduced in 1961 bilirubin measurements in amniotic fluid, the first tool to acquire antenatal information on the presence and severity of HDFN. In 1963 Liley introduced intra uterine intraperitoneal fetal transfusions, a technique that created the possibility to improve a detoriating fetal condition. In 1981 intravascular intra
uterine fetal transfusions were introduced, using fetoscopy.\textsuperscript{10} Since 1983 the less invasive technique of ultrasound-guided percutaneous cordocentesis is used for intrauterine fetal transfusions.\textsuperscript{11} Nowadays, more than 90\% of the antenatally transfused fetuses survive without long-term sequelae.\textsuperscript{12}

\section*{1.2 Maternal alloimmunisation}
Maternal alloimmunisation, also called pregnancy immunisation, is defined as the presence in the blood of a pregnant woman of irregular red cell alloantibodies that can theoretically cause HDFN. This is the case when the maternal antibodies are of the ImmunoglobulinG (IgG) class and thus can be transported across the placenta, and are directed against red cell antigens that are expressed on fetal RBCs. Such RBC alloantibodies are considered as potentially clinically relevant antibodies in respect to risk for HDFN.\textsuperscript{13} The adverb ‘potentially’ refers to the observation that these antibodies can theoretically result in clinical disease of the fetus or newborn, but that this is certainly not always and, for certain antibody specificities, even only rarely the case.

\subsection*{1.2.1 Blood groups and alloantibodies}
A blood group specificity is determined by the chemical structure of the antigenic determinants located on a protein or a carbohydrate linked to a protein or lipid on the red cell membrane, the so-called carrier molecule. A blood group antigen can induce an immune response in people who miss this antigen, leading to development of antigen specific alloantibodies.\textsuperscript{13} Once antibodies have been developed, they may become undetectable over the course of time, but in case of a new exposure to the antigen, the immune system will respond with a rapid rise of the antibody level.\textsuperscript{19} By now, over 250 blood group antigens with their corresponding alloantibodies have been identified, and are grouped into 30 systems: e.g. Kell (with for example the antigens K and k\textsuperscript{I}), Rhesus (most important antigens C, c, C\textsuperscript{w}, D, E, e), Duffy (most important antigens Fy\textsuperscript{a} and Fy\textsuperscript{b}), Kidd (antigens Jk\textsuperscript{a} and Jk\textsuperscript{b}) etcetera.\textsuperscript{15,16} The presence or absence of blood groups is entirely genetically determined. Each blood group system is encoded by a specific gen or set of linked genes, which can have different variants, alleles, resulting in the expression of different blood group antigens on the red cell membrane. In case of heterozygosity, the presence of for example the RhC allele as well as the c allele, results in the expression on the red cell membrane of both corresponding blood group antigens (RhC and Rhc). For RhD, RhD positivity and RhD negativity are discriminated; RhD negativity (Rhd) implies that the RhD protein is completely missing.\textsuperscript{15,16} In short, in the remainder of this chapter the letters D, c, etc refer to RhD, Rhc etcetera.
1.2.2 Regular and irregular red cell alloantibodies

Regular red cell alloantibodies are antibodies directed against the A antigen or the B antigen. These antibodies are naturally occurring in individuals lacking the corresponding antigen. Anti-A and anti-B antibodies are mostly of the IgM class which antibodies cannot cross the placenta.

Red cell antibodies other than anti-A and anti-B are called irregular antibodies. Most of these antibodies are immune antibodies, developed when individuals who themselves lack the antigen, are exposed to the corresponding antigen. Immune antibodies are mostly of the IgG class and can cross the placenta.

Some of the irregular IgG antibodies can also, like anti-A and anti-B antibodies, occur naturally (for example anti-E, anti-Cw and anti-Wr*), and are found in individuals who have never been exposed to red cells containing the relevant antigen. If an individual has been exposed to red cells before, it is not possible anymore to make a distinction between immune-induced and naturally occurring IgG alloantibodies.  

1.2.3 Exposure to allogeneic RBCs and subsequent immunisation

Major causes of exposure and subsequent immunisation are RBC transfusion and FMH during pregnancy and delivery, as neither the transfused RBCs nor the fetal RBCs perfectly match those of the woman. A therapeutic RBC transfusion usually involves the administration of 280 ml (one unit) to 560 ml (in case of two units) of RBC concentrate. FMH involves smaller amounts: in 3% of the women fetal RBCs are detectable in the maternal circulation during the first trimester of pregnancy, in 12% during the second trimester, in 45% during the third trimester and in 64% of the women after delivery, usually in amounts less than 20 ml of fetal blood. Several conditions are thought to increase the risk for a larger amount of FMH, such as spontaneous miscarriage, termination of pregnancy, invasive diagnostic procedures, external version, caesarean section, vaginal assisted delivery, and surgical removal of the placenta. An organ or tissue transplantation can also induce an immune response against blood group antigens.

Whether actual immunisation occurs after exposure to allogeneic RBCs depends on the so-called immunogenicity of the antigen and on the individual immune response. The D antigen is the most immunogenic, followed by the K antigen and other Rhesus blood groups. Characteristics determining the individual immune response are hardly understood. These risk factors will be partly genetic, such as HLA class II antigens but also environmental factors apparently are of relevance. It has been demonstrated in a murine model that viral inflammation enhances alloimmunisation to transfused red cells.
1.2.4 Prevalence of maternal alloimmunisation

In the literature the prevalence of maternal alloimmunisation varies from 0.15% in a Swedish study to as high as 6.2% in Kuwait. The prevalence depends on:

- The definition of clinically relevant alloantibodies. In many studies, it is unclear which antibody specificities were included and/or whether also pregnancies from an antigen-negative father were included (in these cases immunisation has no consequences for the current pregnancy).
- The time of screening. Screening in the first trimester shows a lower prevalence than screening later in pregnancy, because new antibodies can develop during pregnancy if fetal RBCs enter the maternal circulation during pregnancy. In many studies it is unclear at which moment in pregnancy the alloantibodies were detected.
- The sensitivity of the screening test. For example: The indirect antiglobulin test, discovered by Coombs in 1945, is the most widely applied screening test. Accepted screening tests are those which at least match the sensitivity of the bovine albumin IAT to detect clinically relevant antibodies. A screening test is in general performed with so-called untreated red cells. Testing with enzyme-treated cells enhances sensitivity for some alloantibodies, but will not detect others.
- The study population. In populations at high obstetric risk, the prevalence of immunisation also will be higher, as most obstetric risk factors directly or indirectly are related to FMH in the past. Also the antigen distribution differs between populations. For example in the Chinese population D immunisation is rare since the frequency of D-negativity is low (<1%). Also the parity distribution is important, as each pregnancy and delivery increases the cumulative risk for FMH. For obvious reasons the prevalence is lower in countries with a low fertility index.
- The national or regional transfusion policy and the prenatal prevention programme for maternal alloimmunisation.

1.3 Haemolytic Disease of the Fetus and Newborn

1.3.1 Pathophysiology

In case of maternal alloimmunisation, maternal IgG red cell antibodies cross the placenta. The Fab part of the antibody will attach to the corresponding fetal red cell antigen, if present. The Fc part of the antibody will subsequently adhere to the Fc receptors of macrophages, which generally results in fetal red cell destruction via a process termed extravascular haemolysis. This process comprises of phagocytosis in the reticuloendothelial system and of cytotoxic lysis. Anti-K antibodies not only induce extravascular haemolysis of fetal RBCs, but also suppress the erythropoiesis by binding to K-positive erythroid progenitor cells. Both chronic haemolysis and chronic suppression of the erythropoiesis finally
result in fetal anaemia.

Fetal anaemia can cause enlargement of the fetal spleen and liver (hepatosplenomegaly) resulting from compensatory erythropoiesis in these organs, in a decrease of fetal movements, and - in case of progressing anaemia - in cardiomegaly, a hyperdynamic circulation, cardiac decompensation and finally a condition called fetal hydrops, consisting of oedema in the fetal skin and in serous cavities, which can cause fetal death or asphyxia.

Fetal haemolysis causes high levels of bilirubin. During pregnancy, the excess of bilirubin is cleared via the maternal circulation as bilirubin passes the placenta. After birth, the immature liver of the neonate cannot sufficiently conjugate the excess of bilirubin while the haemolytic process does not stop at the very moment of delivery; severe hyperbilirubinaemia may result in brain damage known as ‘kernicterus’. In the past, many hydropic children and children with kernicterus died in the neonatal period. Surviving children with kernicterus develop a severe form of athetoid cerebral palsy, hearing problems and psychomotor handicaps. It is unknown to what extent these severe neurological handicaps translate into mortality under present care conditions.

It is clear that not all pregnancies complicated with non-D RBC alloantibodies, in which no adequate treatment is given, will result in serious morbidity or mortality. However, no data are available to which extent RBC alloantibodies will lead to serious disease without intervention. This also depends on the specificity of the antibody. D antibodies are known to generate the greatest risk for severe HDFN. Anti-K antibodies are also risky, they can induce severe fetal anaemia in early pregnancy (from 16 weeks onwards), while hyperbiluribinaemia from K antibodies is less common. Anti-c and to a lesser extent other Rhesus antibodies (ant-C, -E, -e, -Cw etcetera) occasionally cause serious HDFN. Severe HDFN caused by other antibodies, like Duffy, Kidd or S antibodies, is very rare.

1.3.2 Diagnosis of HDFN
If the presence of maternal red cell alloantibodies is unknown, the only, unspecific and unpredictable, clinical symptoms of HDFN are a decrease of fetal movements or sudden fetal death. Sometimes an ultrasound examination, spuriously reveals fetal hydrops. However, these symptoms are seen at a late stage of the disease, hence prognosis is unfavourable.
When the presence of maternal red cell alloantibodies is known from screening or from a previous pregnancy, the pregnancy can be monitored by repeated laboratory tests and - if necessary - clinical diagnostics.

Laboratory testing
If maternal alloimmunisation is diagnosed and the father of the fetus is known, the RBCs
of the father can be typed for the presence of the antigen against which the maternal antibodies are directed. If the father is antigen-negative, there is no chance that the fetus will be antigen-positive and therefore there is no risk for HDFN caused by this maternal alloantibody.

If the father is heterozygously antigen-positive, it is at present possible in case of D, C, c, E and K antibodies, to determine the fetal antigen status with a DNA test performed with maternal plasma. Other fetal antigens can, at this moment, only be typed with DNA tests performed with amniotic fluid, collected upon amniocentesis. However, this procedure has a risk of fetal loss and increases the risk for FMH, which can boost the antibody production.55-58

For laboratory monitoring of pregnancies with a possibly or for certain antigen-positive child numerous techniques have been developed. Generally the HDFN risk increases with the antibody titre, but this indicator is not accurate. Hence surveillance policies differ between countries. In the USA, a ‘critical titre’ of 16 is accepted for D antibodies as an indicator of the need for clinical monitoring of the fetus, but the sensitivity of this cut-off point is < 100%.59 Critical titres for non-D antibodies have not been formally established; anti-K, for example, may cause fetal anaemia at lower titres and a critical titre of 8 has been proposed.54 In the Netherlands, the Antibody Dependent Cellular Cytotoxicity test (ADCC test) is widely used. This test measures the lytic activity of the antibodies in vitro, which is similar to the cytotoxic lysis occurring in the fetus. The ADCC assay has been validated for D antibodies, but the cut-off for non-D antibodies was unknown.60,61

All surveillance policies institute clinical monitoring if titres or ADCC values go beyond the threshold selected.

Clinical monitoring

Clinical monitoring is focused on detection of symptoms of fetal haemolysis and/or anaemia. In the past the clinical monitoring was entirely based on the so-called Liley index, a measurement of the amount of bilirubin in the amniotic fluid. Presently, additional non-invasive diagnostics have become available. Ultrasound examination can currently detect hepatosplenomegaly and signs of fetal hydrops. The sensitivity and specificity of ultrasound measurement of the fetal spleen and liver to detect fetal anaemia, however, are far below 100%; a relatively small Canadian study showed a sensitivity of the spleen and liver measurement of 66% respectively 33%, and a specificity of 64% respectively 90%.62 Doppler flow measurement can measure the flow in the arteria cerebri media, an indicator of a hyperdynamic circulation, typical for anaemia (and other conditions). A multicentre study, comparing Doppler flow measurement with measurement of the Liley index, showed that Doppler flow measurement has the best diagnostic performance. The sensitivity and specificity in expert centres to detect fetal anaemia, defined as a fetal
haemoglobin level in umbilical cord blood (sample taken before the first IUT or after birth) of 5 SD or more below the mean for gestational age, were 88% and 82% respectively, compared to 76% sensitivity and 77% specificity of the Liley index. Most centres have gradually implemented the non-invasive Doppler flow measurement instead of the invasive policy of amniocentesis and measurement of the Liley index, for the management of alloimmunised pregnancies. Note that one may still add Liley index measurement in uncertain cases, as it is an independent indicator.

1.3.3 Treatment of HDFN

Treatment prior to delivery was quite hazardous when it was introduced. The current option is intra uterine transfusion (IUT) through cordocentesis which is given to an anaemic fetus at a stage that primary section or induction of labour is too early. The IUT procedure can be performed with a good prognosis for the child if no fetal hydrops is seen upon the first IUT. In the Netherlands, IUTs are only performed in the national expert centre, the Leiden University Medical Centre (LUMC). After birth, severe cases of HDFN can safely be treated by exchange transfusion, which is necessary if hyperbilirubinaemia is profound. Less severe cases only receive an RBC transfusion. Moderate HDFN is treated by phototherapy alone. Needless to say that postnatal treatment depends on timely detection of hyperbilirubinaemia, which in turn requires professional care after birth.

1.3.4 Prevalence of HDFN

The prevalence of HDFN depends on:
- The definition of HDFN. We defined severe HDFN as perinatal death or the need for an IUT and/or for an exchange or top-up transfusion in the first week of life, because of maternal RBC antibodies; within this group very severe HDFN are the most severe cases, excluding those needing only top-up transfusions; moderate HDFN was defined as the need for treatment only by phototherapy, because of maternal RBC antibodies.
- Whether a national or regional screening programme is implemented.
- The population in which the prevalence is established. The denominator of the prevalence should be clearly defined to facilitate a comparison between studies.
- The antibody specificity. It should be clear whether HDFN was caused by anti-D alone or whether HDFN caused by non-D antibodies was also included.

The reported prevalences of severe HDFN vary from 3/100,000 to 80/100,000 pregnancies. Prevalence data of moderate HDFN in an unselected population are unavailable.
1.4 Prevention of HDFN

In public health literature often the terms primary, secondary and tertiary prevention are used. Primary prevention is regarded as prevention in the true sense of the word: i.e. intervention undertaken prior to any manifestation of the disease. The beneficiaries of primary prevention are never individually known. The meaning of the terms secondary and tertiary prevention has become less obvious over time. The objective of secondary prevention is described as to improve the prognosis of the patient by early detection, while the objective of tertiary prevention is to prevent complications and long-term sequelae in situations of manifest disease. In particular, preventive actions in genetic late onset diseases, which can be diagnosed at the DNA level, and therapeutic ‘preventive’ actions which (also) decrease recurrence, have blurred the distinctions. Because of the inconsistent usage Kue Young advises avoiding the terms secondary and tertiary prevention and to distinguish, instead, five major types of health care interventions, based on various points in the natural history of disease where they act: primary prevention, early detection, clinical treatment, rehabilitation and palliation. In the context of maternal alloimmunisation two additional features complicate the standard use of these terms: actually the mother is ‘diseased’, but the fetus is affected, and in utero fetal disease (HDFN) is essentially asymptomatic regardless the severity.

Primary prevention is focussed on reduction of the level of risk factors in a population or on prevention of the exposure to risk factors. In a rare case it aims at decreasing vulnerability (vaccination) or increasing resistance (physical exercise). Early detection, also called screening when it is applied on a large organized scale, takes place at the early stages of the disease process, usually before the onset of signs and symptoms. A special case is screening in families with a proven genetic trait. Clinical treatment can be offered to an individual when signs and symptoms of the disease prompt the individual to seek help from a health care professional.

Hereafter we will apply the terms primary prevention and early detection on HDFN. Clinical treatment of HDFN has been described in 1.3.3. Rehabilitation and palliation are beyond the scope of this chapter.

1.4.1 Focus of prevention

Which disease should be prevented by a prevention programme of alloimmunisation in pregnancy can be debated: maternal alloimmunisation, moderate HDFN, severe HDFN or mainly long term sequelae of HDFN. Of course, prevention of maternal alloimmunisation also prevents moderate and severe HDFN and long-term sequelae.

Maternal alloimmunisation does not harm the mother. Only when the mother is pregnant of an antigen-positive child or when she needs an RBC transfusion can the RBC
antibodies cause HDFN or a haemolytic transfusion reaction respectively. To prevent the latter, all guidelines prescribe administering donor blood that does not carry the blood group antigens against which the maternal alloantibodies are directed.°71

Moderate HDFN, here defined as hyperbilirubinaemia, only requiring treatment by phototherapy, reflects a condition also frequently seen in children without RBC alloantibody-induced HDFN. Its prevention is welcomed, but no substantial health benefit is gained.

What truly matters is the prevention of severe HDFN, which in our view should be the main objective of a prevention programme, and, only as second best option, timely clinical treatment to prevent perinatal mortality and kernicterus with the long-term sequelae of this condition.

1.4.2 Primary prevention
Once maternal alloimmunisation has been established, primary prevention of HDFN is no longer possible. So, primary prevention of HDFN translates to primary prevention of RBC immunisation in potentially reproductive women, that is women below, say, 45 years of age.

Primary prevention can be directed against immunisation by incompatible RBC transfusions and against incompatible FMH during pregnancy and delivery. Incompatibility refers to the administration of allogeneic RBCs carrying an antigen which the recipient is lacking.

RBC transfusions
In developed countries RBC transfusions are always matched for D. This policy prevents transfusion-induced D immunisation. A further primary decrease in alloimmunisation could be reached by extended matching of RBC transfusions in women below 45 years of age. Since 2004 Dutch guidelines recommend additional K-matching in women aged younger than 45 years, but in other countries this is mostly not the case. This policy prevents K immunisation in K-negative women (91% of the population) and could in the Dutch case easily be implemented, as ,in the Netherlands, donor RBCs are routinely typed for the K antigen.71 Further extension can of course be considered, as will be discussed in this thesis.

It is obvious that the prevalence of transfusion induced alloimmunisation can also be reduced by a more restricted transfusion policy in general, in particular in obstetrics. Admittedly, the impressive variation in blood use across hospitals suggests substantial practice variation regarding the indication.°275
Introduction

Prevention of incompatible FMH during pregnancy and delivery

Assuming that invasive or otherwise traumatic diagnostic and therapeutic procedures during pregnancy and delivery are only performed upon a valid indication, primary prevention of FMH is hardly possible.

Preventive matching of spouses for some blood group specificities, known to cause severe HDFN, to reduce the risk for incompatibility between mother and fetus is only a theoretical possibility.

Anti-D prophylaxis

Anti-D immunisation is responsible for the majority of cases of severe HDFN. Primary prevention of HDFN due to D immunisation in prior pregnancy was found to be possible by administration of anti-D immunoglobulin (anti-D Ig). In the Netherlands Borst-Eilers et al. demonstrated that postnatal administration of 250 µg anti-D Ig to D-negative women who gave birth to a D-positive child, reduced the immunisation rate as established 4-6 months after the birth of a D-positive child, from 5 to 0.9% Passively administered anti-D Ig prevents the anti-D immune response of the mother upon exposure to D-positive fetal RBCs; the precise responsible mechanism is still unclear. Postnatal anti-D prophylaxis to D-negative women who gave birth to a D-positive child is common practice in developed countries since the 1960s. In the Netherlands a postnatal dosage of 200 µg (1,000 IU) is used. Additional guidelines advise to administer anti-D Ig in conditions prone to FMH, such as miscarriage, termination of pregnancy, invasive prenatal diagnostic procedures, external version, caesarean section, etcetera. Since the implementation of this new strategy in 1969, in the Netherlands the prevalence of new D immunisations in pregnancy declined from 3.5% in 1969 to 0.5% in 1990. This primary prevention option is only available for D immunisation. It might also be possible to develop similar approaches for other RBC antigens, but given the low prevalence of HDFN caused by RBC alloantibodies with other specificities it has never been an option to investigate the effectiveness of and to invest in the development of other antigen-specific postnatal Ig prophylaxis.

New opportunities in prevention of D immunisation

A further decrease in prevalence of D immunisation was observed in several studies investigating routine antenatal administration of anti-D Ig, primarily to prevent immunisation from undetected FMHs, especially during the last trimester of pregnancy. In the Netherlands antenatal anti-D prophylaxis has been implemented since July 1st 1998, comprising one single dosage of 200 µg (1,000 IU) in week 30, and, because of the relatively scarcity of anti-D Ig, restricted to women without a living child until May 2008.
1.4.3 Early detection ('secondary prevention')

Early detection of maternal alloimmunisation creates the possibility of laboratory and – if indicated – of clinical monitoring and treatment of pregnancies at risk of HDFN as described in 1.3.2 and 1.3.3. Severe HDFN cannot be prevented, but it is possible to prevent the development of severe anaemia causing fetal hydrops and/or asphyxia, by timely antenatal IUT treatment, and of kernicterus by postnatal treatment with exchange transfusions.

Routine antibody screening of all pregnant women for D and non-D antibodies has been common practice in most developed countries for many years. In the Netherlands, third trimester screening of D-negative pregnant women for D antibodies has been implemented since the 1960s. Routine first trimester screening of all pregnant women for D and non-D RBC antibodies has been implemented since July 1st 1998. D-negative women are screened again in the 30th week of pregnancy before the administration of the antenatal anti-D prophylaxis.

Screening for the presence of RBC alloantibodies and subsequent antigen typing of the father is the first step in the screening process, which identifies pregnancies at risk for HDFN. The second step is timely detection of HDFN by laboratory and – if the test results are above the cut-off level – clinical monitoring.

1.5 Evaluation national prevention programme maternal alloimmunisation

As stated before, two major changes were implemented in the Dutch national prevention programme of maternal alloimmunisation as of July 1st 1998: 1) Routine RBC antibody screening of D-negative and D-positive women in the first trimester of pregnancy, and 2) Antenatal anti-D Ig prophylaxis with one single dose of 200 μg (1,000 IU) anti-D Ig in the 30th week of pregnancy, until 2008 restricted to women without a living child.

1.5.1 National prevention programme maternal alloimmunisation

The national prevention programme is outlined in Figure 1.1. The obstetric care worker is responsible for blood sampling from mother and child, for administration of antenatal and postnatal anti-D Ig and for clinical monitoring and treatment, if indicated. The first trimester screening is performed by regional screening laboratories (5 per million inhabitants). A positive screen result only points out that one or more RBC antibody specificities may be present, but not whether there is a potentially clinically relevant antibody. If the screening result is positive, confirmation of the positive screening result, establishment of the potential clinical relevance of the antibodies and paternal antigen typing is performed by two national reference laboratories: Sanquin Diagnostic Services in Amsterdam, and the BIBO (Special Institute for Blood Group
**Figure 1.1** National prevention programme maternal alloimmunisation since 1-7-1998

**OBSTETRIC CARE WORKER**

- Booking visit
  - Blood sampling

**SCREENING LABORATORY**

- ABO + D typing
- Antibody screen

**REFERENCE LABORATORY**

- Antibody specificity
  - No clinically relevant antibodies
  - Clinically relevant antibodies

- Antigen typing
  - Father

- D-negative
- D-positive

- Blood sampling

**week 30**

- 1,000 IU of anti-D Ig *

**birth**

- Blood sampling newborn
  - D-positive
  - D-negative
  - Stop

**Lab & clinical monitoring**

- During pregnancy
- Of the child after birth

* Anti-D Ig if indicated to RhD-negative women without D antibodies

* Until 2008 antenatal anti-D prophylaxis was restricted to RhD-negative women without a living child
Investigation) in Groningen. These reference laboratories also perform laboratory monitoring during pregnancy by determination of antibody titres and ADCC tests (ADCC Sanquin only). Because the risk for severe HDFN of the various antibody specificities is unknown, all pregnancies with theoretically clinically relevant RBC antibodies are currently considered as at risk for HDFN and monitored by the reference laboratories.

1.5.2 Scientific evidence for the screening programme
Admittedly, the two new prevention measures were not evidence-based, and no economic considerations were apparently at stake at the time of these decisions. Representatives of the Dutch organisations of obstetric care workers (midwives, general practitioners and obstetricians) objected to the implementation of these new measures because of the lack of scientific evidence, the expected negative psychological impact of false-positive screening results on pregnant women, and because of the increased, uncompensated workload of obstetric caregivers.\textsuperscript{87,88}

All of these arguments appeared valid, at least to some extent.

Although routine antibody screening of all pregnant women for D and non-D antibodies is common practice in most developed countries since many years, there was and still is no clear evidence for the effectiveness and costs of screening for non-D RBC antibodies. Lack of formal evidence most likely is responsible for the observed wide geographical variation of screening schemes even within countries. The effect of antenatal anti-D Ig prophylaxis has been shown in several studies,\textsuperscript{82,83,89-92} however, the Dutch administration scheme of one single dosage of 200 μg (1,000 IU) in week 30 was never put into practice and never studied; also the restriction to women without a living child was unique. The existing studies showed considerable heterogeneity in intervention (timing, dosage), patient selection, outcome measures (predominantly proxy outcomes are used i.e. immunisations after birth or in the next pregnancy and not the occurrence of HDFN), and results.

The second argument is relevant too. No impact has ever been collected from false-positive tests. A false-positive test may happen in all phases of the screening process, with a different impact on the setting of care during pregnancy and delivery, and therefore with different psychological impact. Five instances of false-positive test results are possible: 1) A positive screening result is established in the screening laboratory, but this is not confirmed by the reference laboratory or followed by identification of only clinically not-relevant RBC antibodies; 2) Potentially clinically relevant antibodies are found, but the paternal antigen points out to be negative; 3) Laboratory monitoring is performed during pregnancy, but the test results do not prompt the obstetric care worker to perform clinical diagnostic procedures; 4) Clinical diagnostics are performed, but no symptoms of severe HDFN are found 5) Clinical and laboratory test results give rise to a suspicion of severe
HDFN, but a non-affected child is born.

Finally, the economic impact on the care setting apparently was ignored, most likely because - true or not - it was assumed that the additional work was negligible.

In view of the objections and professional reluctance, the Ministry of Health decided to perform a nation-wide evaluation of the non-D red cell antibody screening programme. Also the effect of the Dutch programme of antenatal anti-D prophylaxis should be studied. Both evaluations were observational; a vigorous plea by the project leaders of the study reported here, to allow for a limited experimental design, was not followed.

1.5.3 Wilson & Jüngner criteria
During the preparation of the study proposals underlying the investigations reported here, it was decided at onset to stick as closely as possible to the so-called Wilson & Jüngner (W&J) criteria for the evaluation of the non-D RBC screening programme. For the antenatal anti-D programme, a more limited approach seemed justified.

At the request of the World Health Organisation (WHO) in 1968, Wilson & Jüngner developed ten criteria to evaluate a (new) screening programme. See Box 1.1. Since then, the original criteria and some variations have been widely used all over the world.

**BOX 1.1 Wilson & Jüngner criteria**

1. The condition sought should represent an important health problem.
2. There should be an accepted treatment for patients with detected disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a detectable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test (procedure) should be acceptable to the population.
7. The natural history of the condition, including the development from latent to manifest disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients, and how.
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not a ‘once and for all’ project.
1.6 Research questions and outline of the thesis

This thesis provides results of a series of studies, collectively designated the ‘OPZI’-study, which were dedicated to the collection of missing evidence on the two extensions of the prenatal prevention programmes of maternal alloimmunisation in the Netherlands.

Table 1.1 provides an evaluation of the available evidence at the time of institution of the non-D screening programme, which could support this programme, following the W&J criteria. The table shows the remaining questions which in turn guided our evaluation of the non-D RBC antibody screening; the last column provides the finding place of our answers.

Note that the non-D programme is actually a two-phased programme, first screening women for relevant immunisations, next screening/monitoring of fetuses in women with relevant immunisations, for the presence of severe HDFN. Some of the W&J questions are relevant to both screen stages.

The research questions for the evaluation of the antenatal anti-D prophylaxis programme are presented after this table: here a substantial number of the W&J criteria were already fulfilled: improvement and efficiency of the extension of the standard postnatal programme was at stake rather than the principal question of screening per se.
## Table 1.1 Research questions and outline of the thesis

**Screening maternal non-D alloimmunisation: evaluation of W&J criteria versus information provided by this thesis**

<table>
<thead>
<tr>
<th>W&amp;J Topic</th>
<th>Research questions</th>
<th>chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe disease? Prevalence maternal non-D immunisation? Prevalence HDFN?</td>
<td>Yes ($§1.3.1$) No ($§1.2.4$) No ($§1.3.4$) Prevalence of non-D immunisation, in general terms and for each non-D antibody specificity? Presence of severe and moderate HDFN totally and by non-D antibody specificity?</td>
<td>2</td>
</tr>
<tr>
<td>Accepted treatment?</td>
<td>Yes ($§1.3.3$)</td>
<td>2</td>
</tr>
<tr>
<td>Facilities diagnosis and treatment: . lab diagnosis . clinical diagnosis . treatment</td>
<td>Yes ($§1.5.1$) ($§1.3.2$) ($§1.3.3$)</td>
<td>4</td>
</tr>
<tr>
<td>Detectable early stage?</td>
<td>Yes ($§1.3.2$)</td>
<td>4</td>
</tr>
<tr>
<td>Suitable test programme? . population screening . laboratory monitoring</td>
<td>No ($§1.3.1$) ($§1.3.2$) ($§1.5.1$) ($§1.5.2$) Sensitivity, predictive value of the screening programme to detect severe HDFN? Cut-offs laboratory tests to select pregnancies at high risk HDFN?</td>
<td>2</td>
</tr>
<tr>
<td>Acceptability test programme . to women . to care workers . to laboratories</td>
<td>($§1.5.2$) No No No Acceptance of the non-D screening programme by women? Acceptance by care workers and laboratories?</td>
<td>5</td>
</tr>
<tr>
<td>Natural history immunisation Natural history HDFN in immunised women, including postnatal follow-up</td>
<td>No ($§1.3.1$) Risk for severe and moderate HDFN in utero, according to non-D antibody specificity? Risk for postnatal complications following HDFN?</td>
<td>2</td>
</tr>
<tr>
<td>Who to treat as patients?</td>
<td>Yes ($§1.3.2$)</td>
<td>2</td>
</tr>
<tr>
<td>Costs of the programme</td>
<td>No ($§1.5.1$) Costs of the non-D screening programme, compared to the null scenario (D prevention only) and to other hypothetical non-D programmes based on specificity specific approaches and/or subgroup screening?</td>
<td>6</td>
</tr>
<tr>
<td>Continuity of screening process</td>
<td>Yes ($§1.5.2$)</td>
<td>3</td>
</tr>
</tbody>
</table>

### Evaluation antenatal anti-D prophylaxis in the 30th week of pregnancy

<table>
<thead>
<tr>
<th>Research questions</th>
<th>chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of antenatal anti-D prophylaxis on the prevalence of new D immunisations and subsequent HDFN in the next pregnancy?</td>
<td>7</td>
</tr>
<tr>
<td>Risk factors for D immunisation in the next pregnancy despite adequate antenatal and postnatal prophylaxis in the foregoing pregnancy?</td>
<td>8</td>
</tr>
</tbody>
</table>

### Summary and general discussion of the two evaluation studies

|                                                                      | 9       |
Reference List


24 Hendrickson JE, Desmarets M, Deshpande SS, Chadwick TE, Hillyer CD, Roback JD et al. Recipient inflammation affects the frequency and magnitude of immunization to transfused red blood cells. Transfusion 2006; 46(9):1526-1536.


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58 Finning K, Martin P, Summers J, Daniels G. Fetal genotyping for the K (Kell) and Rh C, c, and E blood groups on cell-free fetal DNA in maternal plasma. Transfusion 2007; 47(11):2126-2133.
69 Kue Young T. Planning Population Health Interventions. In: Kue Young T., editor. Population
Chapter 1


77 Borst-Eilers E. [Rhesus immunisation: origin and prevention]. Amsterdam: Thesis, Centraal Laboratorium voor de Bloedtransfusiedienst, 1972.[Dutch]

78 Kumpel BM. On the immunologic basis of Rh immune globulin (anti-D) prophylaxis. Transfusion 2006; 46(9):1652-1656.


