Detection and prevention of pregnancy immunisation : the OPZI study

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CHAPTER 9

General discussion and summary

History of maternal alloimmunisation

Until 1970 haemolytic disease of the fetus and newborn (HDFN) was a major specific cause of perinatal mortality and morbidity.[1] The condition is caused by maternal alloimmunisation directed against red blood cells (RBCs) of the fetus.[2;3] In most cases of severe HDFN maternal alloantibodies directed against the Rhesus D antigen are responsible for the disease; in a minority of cases alloantibodies against other blood group antigens than D, such as anti-K and anti-c, are responsible.[4] This is caused by the higher immunogenicity of the D antigen compared with other antigens, combined with the high potency of anti-D antibodies to destruct D-positive fetal RBCs.[5] Because of the high immunogenicity of the D antigen D-negative persons receive only D-negative RBC transfusions.[6] To prevent D immunisation resulting from fetomaternal haemorrhage (FMH) during pregnancy and delivery, since 1969 anti-D Ig prophylaxis with 200 µg anti-D Ig (1,000 IU) is administered to D-negative women after the birth of a D-positive child. Additional guidelines advise administering of anti-D Ig in conditions prone to fetomaternal transfusion (FMH), such as miscarriage, termination of pregnancy, invasive prenatal diagnostic procedures, external version, caesarean section, etcetera.[7-10] Since the implementation of this strategy the prevalence of new anti-D immunisations in pregnancy in the Netherlands declined from 3.5% in 1969 to 0.5% in 1990.[11]

Prevention

Given the low prevalence of HDFN caused by non-D maternal alloantibodies, primary prevention by matching of donor RBCs for other blood group antigens is still under discussion and the development of antigen-specific Ig prophylaxis so far has not been a serious option. To enable timely detection and treatment of relatively rare cases of severe HDFN caused by non-D antibodies, a national screening programme, comprising one screening test in the first trimester of pregnancy, has been implemented in the Netherlands since 1998.[8] Before July 1st 1998 only D-negative pregnant women were screened for anti-D and other RBC alloantibodies in the 32nd week of pregnancy.

The decision on this extension of the RBC alloantibody screening programme was mainly based on the fact that many other European countries did already screen for maternal non-D alloimmunisation.[12] However, the scientific evidence for the effectiveness of the new programme was unavailable, as was cost information. In formal terms, it was not known whether the Wilson & Jüngner (W&J) criteria were met. The objective of the OPZI study, as presented in this thesis, was therefore to provide this scientific evidence, and to develop evidence-based recommendations for improvement, if possible.

In July 1998, it was also decided to introduce antenatal anti-D Ig prophylaxis in the 30th week of pregnancy, to prevent anti-D immunisation during pregnancy, on top of the post-natal prevention programme. Here too, unequivocal data on the efficiency of this measure

were unavailable at that time, and no randomized trials were ongoing or anticipated. The second objective of the OPZI study was therefore to evaluate the effect of the Dutch scheme of antenatal prophylaxis on anti-D immunisation and HDFN, within an observational format.

The OPZI study

The Netherlands are the first country performing a nation-wide evaluation of these prevention programmes through the OPZI study. Thanks to the well-organised system of obstetric care, the public funding of the prevention programme and the centralisation of the laboratory and clinical monitoring of alloimmunised pregnancies, it was possible to perform this large-scale evaluation study. The outcomes of the OPZI study will also be relevant for other developed countries.

Hereafter we will discuss the two evaluation studies separately.

9.1 Screening for maternal non-D alloimmunisation

The main objective of the screening programme for maternal non-D alloimmunisation is timely detection of severe HDFN, caused by non-D RBC alloantibodies.

In this thesis severe HDFN was defined as perinatal death or the need for an intra uterine transfusion (IUT) and/or for an exchange or top-up transfusion in the first week of life, because of maternal RBC alloantibodies; 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.

To evaluate the first trimester screening for non-D alloantibodies we adhered as closely as possible to the widely used W&J criteria, developed in 1968 at the request of the World Health Organisation (WHO) for the evaluation of (new) screening programmes. These criteria are still upheld today as 'classics', and have stood well the test of time.[13]

Hereafter we will discuss whether the screening programme for maternal non-D alloimmunisation meets these criteria.

Criterion 1: The condition sought should represent an important health problem.

Before the start of the study it was clear that severe HDFN is a serious disease, which can - if untreated - result in fetal death, and - in surviving children - in fetal hydrops or asphyxia, caused by severe anaemia, or in kernicterus with life-long sequelae. However, the prevalence data about maternal non-D alloimmunisation and subsequent severe HDFN showed a wide variation. Table 9.1 presents data from an extended literature search. The prevalences of maternal non-D alloimmunisation vary from 0.15% to as high as 6.2% and of severe HDFN from 0 to 18/100,000. This variation can be explained by the heterogeneity

of the screening protocols, by the absence of an unequivocal definition of 'the presence of clinically relevant alloantibodies' and, most importantly, by the use of pre-selected high risk study populations with an unknown relation to the general population of pregnant women. The best existing estimates of population data are from three regional studies in Sweden which established a prevalence of maternal non-D alloimmunisation between 0.15% en 0.25%.[14-16] However, it should be kept in mind that the prevalences in these studies included women with an antigen-positive partner only. The prevalence of severe HDFN in these studies was between 5 and 10/100,000. In the Netherlands some small studies were available in selected populations, such as a university hospital [17;18] or in women with a history of RBC transfusion or prior delivery.[19] In these studies prevalences of maternal alloimmunisation between 0.62% and 2.1% were found. Another Dutch study in a primary care centre established a prevalence of 0.36% upon screening in week 28-32.[17] In one national study, reporting HDFN during two years, a prevalence of severe HDFN of 6/100,000 (pregnant population n= 386,000) was established.[20]

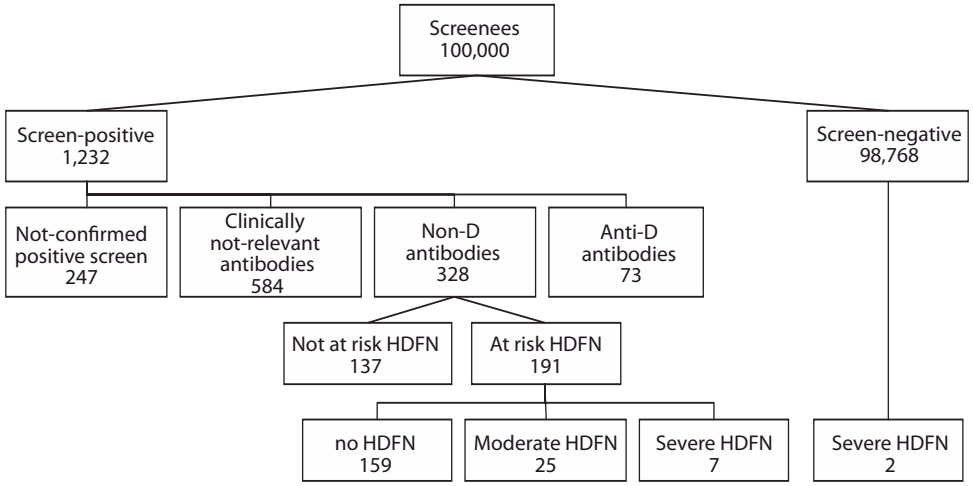
The nation-wide design of our study with full collaboration of all care workers and laboratories allowed for an unbiased estimate of all principal parameters. As described in **chapter 2**, we established a prevalence of maternal non-D alloimmunisation of 0.33% of which 0.19% were at risk for HDFN because the father carried the blood group antigen against which the maternal alloantibodies were directed. Anti-E was the most frequently observed antibody specificity, followed by anti-K and anti-c. Also in the group of pregnancies at risk for HDFN anti-E was the most frequent antibody, but was followed by anti-c, while the majority of women with anti-K antibodies had an antigen-negative partner. In 14% of the alloimmunised pregnancies more than one antibody was found; in 25% this concerned a combination of anti-c and anti-E.

Severe HDFN occurred in 2.1% of all immunised pregnancies and in 3.7% of the pregnancies at risk for HDFN. This reflects a prevalence of screen-detected severe HDFN of 7/100,000 pregnancies (See Figure 9.1) and a Number Needed to Screen (NNS) to detect one case of severe HDFN of 15,000.

Antenatal treatment with IUTs was necessary in 1.7/100,000 pregnancies, exchange transfusions were given in 3.3/100,000 pregnancies and an RBC top-up transfusion only in 2/100,000 pregnancies. All screen-detected cases were timely treated and survived without long-term sequelae. Of the cases at risk for HDFN (191/100,000) 17% was treated with phototherapy, compared to 4% in the control group, reflecting a prevalence of moderate HDFN, caused by non-D antibodies, of 25/100,000 pregnancies.

The established prevalence of screen-detected severe HDFN implies that yearly in the Netherlands about three children at risk for fetal death, caused by severe anaemia (hydrops, asphyxia), and about six children at risk of kernicterus, caused by profound postnatal hyperbiluribinaemia, are timely detected and treated. These numbers are lower than the

Figure 9.1 Flow diagram screening results first trimester antibody screening and occurrence of HDFN in screen-detected and screen-undetected cases (n/100,000)



estimations before introduction of the screening [21] (three cases of perinatal death and 17 children needing an exchange transfusion); for policy trade-offs it is relevant to consider that the order of magnitude of the prevalence of severe cases conforms prevalences of other diseases aimed at by existing prenatal and neonatal screening programmes. For example, the prevalence of other diseases for which each newborn is screened such as phenylketonuria or adrenogenital syndrome, are between 10 and 20 children per year respectively in the Netherlands.[22]

The first criterion of W&J is therefore fulfilled with the current programme.

Criterion 2: There should be an accepted treatment for patients with detected disease.
Antenatal treatment with IUTs and postnatal treatment with exchange transfusions, RBC transfusions and phototherapy are widely accepted treatment procedures for severe and moderate HDFN. Moreover, this therapy is highly effective. If the therapy is timely started, it can prevent irreversible long term effects in almost all cases.[36–37] The ability of the Dutch screening programme to detect pregnancies at risk for HDFN in time, was already suggested by the evaluation of the outcome of Kell-immunised pregnancies, referred to the Leiden University Medical Centre (LUMC), the Dutch national expert centre for maternal alloimmunisation. Compared to the time period before introduction of the screening programme, more timely referrals of Kell-immunised pregnancies were seen, resulting in a higher perinatal survival rate after implementation of the screening programme: all 25 screen-detected children survived, while the perinatal mortality in cases referred before screening was 39% (7/18).[38]

Table 9.1 Studies on the prevalence of non-D maternal alloimmunisation and subsequent HDFN

Study	Period	Study	Population/ setting	Screening moment(s)	Prevalence non-D alloimmunisation and subsequent very severe HDFN*				Antibodies causing HDFN	Remarks
					detected upon 1st screening immunisation	HDFN	detected after 1st screening† immunisation	HDFN		
Hardy Wales[23]	'48-'78	retro spective	D-positive women regional	unclear	0.19% 733/380,790	0.003% 11/380,790			NR‡	denominator women in stead of pregnancies
Bowell UK[24]	'83-'84	retro spective	all pregnant women regional	intake week 28 + after week 28	0.45% 315/70,000	NR	0.29% 201/70,000	NR		denominator = estimation
Van Dijk[17] Netherlands	'86-'87	pros pective	university hospital	intake	0.62% 8/1293	NR				screening test in enzyme
Van Dijk[17] Netherlands	'88-'89	pros pective	primary care centre	week 28-32	0.36% 8/2,250	NR				
Belfrage Sweden[25]	'83-'89	retro spective	referral university hospital	'initially'	NR	0.01% 17/147,068			E, c, K, etc C^w+Fy^a	
Heddle Canada[26]	'80-'90	retro spective	community hospital	1st trimester moment 2nd screening unclear	0.33% 58/17,568	NR	0.24% 42/17,510	NR		
	'87-'89		secondary care hospital							
Gottvall Sweden[14]	'83-'89	pros pective	all pregnant women regional	week 25 week 35	0.24% 188/78,300	0.01% 8/78,300	moment of detection unclear	NR		only pregnancies with an antigen-positive partner were included
Filbey Sweden[15]	'80-'91	retro pective	all pregnant women regional	week 10 week 35	0.15% 171/111,939	0.005% 6/111,939	moment of detection unclear	c, E, cE, K, Fy^a		
Wong[27] Hong Kong	'95-'96	pros pective	pregnant women regional	intake	0.25% 5/1,997	0 0/1,997	NR			
Heringa[18] Netherlands	'91-'95	pros pective	D-positive women university hospital	week 30	1.3% 37/2,852	0				
Heringa[18] Netherlands	'87-'95	pros pective	D-negative women university hospital	week 30	0.8% 8/1,023	0				
Howard Uk[28]	'93-'94	retro spective	all women 7 maternity units	intake	0.65% 144/22,264	0.018% 4/22,264			c, ce, E	
Rothenberg USA[29]	'88-'97	retro spective	D-positive women tertiary care referral centre + public hospital	1st trimester 3rd trimester	0.37% 35/9,348	0.011% 1/9,348	0.06% 6/9,313	0 0/9,313	Fy^a	

De Vrijer[19] Netherlands	'95-'98	pros pective	M-parae + women with history of RBC transfusion, regional	intake	2.1% 50/2,392	0.08% 2/2,392	0.3% 7/2,335	0 0/2,335	K	not clear whether all eligible women in the region were included
Van Dijk[20] Netherlands	'95-'96	pros pective	national study, reporting HDFN	no screening programme		0.006% 22/386,034			NR	denominator: national number of births
Andersen Denmark[30]	'96	pros pective	D-positive women, regional	1st trimester 3rd trimester	1.1% 34/3,046	NR	0.43% 13/3,012	0 0/3,012		1st trimester: including Lewis antibodies
Lee Hong Kong[31]	'97-'01	retro spective	national screening lab, ethnic Chinese women	intake	0.27% 57/21,327	0.005% 1/21,327	NR	NR	E	
Jovanovic Yugoslavia[32]	'95-'00	retro spective	national screening lab	unclear	0.58% 124/21,370	NR			Unclear whether number of antibodies or number of pregnancies is reported; unclear whether non-RhD ab's are combined with RhD ab's	
Lurie Israel[33]	'99-'02	retro spective	Women's Health Centre	1st trimester	0.16% 1/631	0 0/631				
Ameen Kuwait[34]	'92-'01	retro spective	National Blood Bank	unclear	6.2% 182/2,932	NR				
Adeniji UK[35]	'02-'04	retro spective	D-positive women, regional	1st trimester > week 28			0.24% 34/14,143	0 0/14,143		
Woiski[§] Netherlands	'95-'03	retro spective	D-positive women university hospital	1st trimester week 30			0.7% 12/1,820	0.11% 2/1,820	c, c+E	
Gottvall Sweden[16]	'92-'05	retro spective	all pregnant women, regional	week 10 week 35	0.25% 196/78,145	0.01% 8/78,145	moment of detection unclear		c, E, c+E, Fy[a]	only cases with antigen-negative father included

* Very severe HDFN: need for intra uterine transfusion and/or exchange transfusion, perinatal death or fetal hydrops because of maternal RBC alloantibodies

† Incidence of antibodies, newly detected during pregnancy, after a negative result of first trimester screening

‡ NR: not reported

§ Unpublished results, presented at Gynaecongres, 2007

However, it should be noted, that this form of antenatal therapy is not without risk and in our view should be performed in a specialized centre. Cordocentesis at midgestation carries a fetal loss rate of 1.4%.[39] But, a higher complication rate of IUT when compared with diagnostic cordocentesis might be expected. Schumacher and Moise reported a procedure-related fetal loss rate following intravascular transfusion of 2.0% (range 1.3%-2.5%) from a review of seven series.[40] The risk of IUT in the Dutch setting has been carefully evaluated by van Kamp et al. They reported that each IUT has a procedure-related loss rate of 1.6%. Procedure-related death of seven fetuses and five neonates was observed between 1988 and 2001 in 254 pregnancies treated with 740 IUTs (4.7%).[36;41] Because of this treatment-related mortality, there is still need for a better therapy. Inhibition of transport of alloantibodies across the placenta or inhibition of binding of destructive alloantibodies to the fetal red blood cells might in the future be viable alternatives in preventing HDFN without risk of fetal loss.[42]

However, even under current conditions, the second criterion of W&J seems to be fulfilled.

Criterion 3: Facilities for diagnosis and treatment should be available.
Facilities for laboratory and clinical monitoring and for treatment are available. The RBC antibody screening is performed by qualified regional laboratories, while the specificity testing and laboratory monitoring of cases at risk are performed by two national reference laboratories with standard protocols and relevant expertise.

In 2002 we sent a questionnaire to all screening laboratories in the Netherlands (± 90) to check whether the protocols concerning RBC antibody screening in pregnancy were adequately followed. We found that 7% of the screening laboratories performed the specificity testing after a positive screen result in their own laboratory, instead of sending a blood sample to one of the reference laboratories. (All cases identified by these laboratories were also included in our study). However, about 50% of the laboratories preferred to perform the specificity testing in their own laboratory. The main argument upheld by these laboratories was that the result of the specificity testing could then directly be registered in the laboratory information system, and not only after receiving the information from the reference laboratory. Information on the presence of RBC alloantibodies is important when the mother needs an RBC transfusion around delivery. This argument has become less relevant as since May 2007 a national registry of RBC alloantibodies, called TRIX (Transfusion Registry Irregular Antibodies and (X)Cross Match), has become available, on-line accessible for all transfusion laboratories connected to the system, which further guarantees optimal availability of information on RBC alloantibody typing results in pregnant women and patients needing an RBC transfusion.

All severe cases are referred to the national expert centre for maternal alloimmunisation

in the LUMC, which centre can also be asked for advice by obstetric care workers. This centre has excellent expertise in ultrasonography and Doppler flow measurements for clinical monitoring, and in intrauterine transfusions.[7]

The third W&J criterion is fulfilled.

Criterion 4: There should be a detectable latent or early symptomatic phase.
This criterion rests on two subcriteria: a. finding/detecting an early disease stage, and b. demonstrating that treatment of cases, detected in this early stage, generates a better outcome than later detection.

Fetuses who have an antibody-induced anaemia, but do not have developed yet irreversible disease, can, in principal, be detected by the combined approach of laboratory monitoring (surveillance) and clinical monitoring.

The aim of laboratory monitoring is to distinguish between cases at increased risk for severe HDFN, that need clinical monitoring, and cases with a negligible risk for severe HDFN, in which clinical monitoring can be safely omitted. Clinical monitoring is actually focused on the detection of early signs of fetal haemolysis and/or anaemia. Early detection of fetal anaemia and/or haemolysis is possible by Doppler flow measurement [43], combined with ultrasound measurements of the fetal spleen and liver.[44]

The 'the earlier the better' subcriterion is always difficult to test experimentally, but the Leiden group has demonstrated that the outcome of antenatal treatment of HDFN is determined by the fetal condition at start of the treatment. Preferably treatment should start before the development of hydrops, but also moderate hydrops is still reversible.[45] Irreversible damage is almost exclusively observed in children with perinatal asphyxia and a low haemoglobin level at birth, hence children who were not early detected.[37;45] Per definition hyperbilirubinaemia only develops after birth, and timely detection and treatment will prevent dangerously high bilirubin concentrations.[46] We therefore judge that the 'the earlier the better' assumption is true in this context.

Taken together the evidence we regard the fourth W&J criterion to be fulfilled.

Criterion 5: There should be a suitable test or examination.
Suitability of a test rests on the simplicity of execution and on the test performance. The screening test for maternal non-D RBC alloantibodies is a two-phased programme.

Phase 1 Screening performance
This phase establishes whether a pregnancy is potentially at risk for HDFN, by screening pregnant women for the presence of clinically relevant alloimmunisation and typing the partner for the antigen against which the maternal antibodies are directed.

The introduction of the RBC antibody screening test was simple as it is an add-on to

the standard blood tests, performed in the first trimester. It only requires an additional small vial of blood, hence, demands from the care worker and the patient are minute.

The test performance was unknown until this study. In particular the sensitivity of the first trimester non-D alloantibody screening was unclear in terms of detecting all cases of maternal alloimmunisation and - the main focus of the screening programme - of all cases of severe HDFN. A false-negative test can occur at the laboratory level by technical failures or by administrative errors. Another potential source of missing cases at risk for HDFN is an undetectable low titre of antibodies despite prior immunisation. It is known that over the course of time 25-30% of once developed antibodies become undetectable.[47] A new exposure to antigen-positive (fetal) RBCs induces a rapid boosting of these antibodies to detectable levels later in pregnancy. Finally, a less than 100% sensitivity of a single first trimester screening can be due to the development of new antibodies, induced by exposure to fetal RBCs during the current pregnancy. In our study we observed new antibodies, additional to non-D alloantibodies already present upon first trimester screening, in 7% of the non-D immunised pregnancies. In 30% of these pregnancies anti-c was the additional antibody, causing severe HDFN in one case. The risk for detecting additional anti-c in c-negative women, already alloimmunised against another antigen than c, was 14%. As it is known that the risk for further alloimmunisation in already alloimmunised patients is higher, the risk for antibodies emerging during pregnancy in screen-negative women will be substantially lower.[48]

To be sure that all clinically relevant alloimmunisations are detected, in many western countries screening programmes comprise two or even three screening moments: next to the first trimester also in the second and/or third trimester. However, as shown in table 9.1 the effectiveness of even a second screening for non-D RBC alloantibodies in all women is debatable because the observed incidence of newly detected antibodies is low (from 0.06% to 0.4%) and no cases of severe HDFN caused by antibodies detected later in pregnancy have been reported in most studies.[24;26;29;30;35] A third screening moment seems futile.

Two groups have studied the results of a second screening in screen-negative women in the Netherlands. De Vrijer established in a regional study an incidence of 0.3% new antibodies detected around delivery, but no cases of severe HDFN.[19] Another study in a university hospital reported an incidence of newly detected non-D antibodies of 0.7% (12/1,820) upon 30th-week screening, resulting in two children who were treated by exchange transfusion after birth, both because of anti-c (Woiski, Nijmegen, unpublished results). Remarkably, the authors judge this pick-up rate of the second screening as too low to justify a routine second screening, although this is higher than the population prevalence of maternal alloimmunisation, as we observed upon first trimester screening.

As part of the OPZI study our group collected data in another university hospital,

where we found a maximum incidence in screen-negative women of newly detected non-D antibodies of 0.2% (10/5,800 pregnancies) at screening around delivery, not resulting in severe HDFN (unpublished results).

As outlined in **chapter 2** we have thoroughly investigated how many cases of very severe HDFN had occurred in pregnancies which were not identified as at risk for HDFN by screening (missed cases), as high sensitivity is the cornerstone of every screening programme. We identified in retrospect in two years nation-wide seven cases of very severe HDFN, treated by exchange transfusions after birth, all with a documented negative first trimester screen result. This reflects a sensitivity of the screening programme to detect very severe HDFN of about 75%. As the retrospective case-finding strategies might not have identified all 'missed' cases of severe HDFN, especially cases of fetal death, the sensitivity may be even lower than 75% (Figure 9.1). This is a serious problem as one of the 'missed' cases was not timely detected after birth and suffered from kernicterus and showed permanent severe brain damage at one year. Another child (anti-c) had an intracerebral bleeding, caused by asphyxia, probably related to the severe prenatal anaemia (Hb 2.4 mmol/L). At one year, the prognosis was still unclear. Similar to the screen-detected group, the majority of missed HDFN cases was caused by anti-c antibodies (5 out of 7). Moreover, as stated above, one of the cases of severe HDFN in the screen-detected group was caused by additional anti-c antibodies, detected during pregnancy. Although we cannot exclude administrative or technical errors on the laboratory level, the most likely explanation is that in these seven cases the titres of the antibodies were too low to be detected at first trimester screening, rather than the development of new antibodies, as in only one out of seven missed cases no risk factors, such as a history of RBC transfusion and/or prior parity, were present. The fact that most other studies did not detect cases of severe HDFN, detected upon a second screening, can be explained by lack of power, as the largest study population included 14,000 pregnant women[35], compared to 400,000 women in our study.

The sensitivity of the screening programme can increase with about 30% by introduction of a second screening. It can be considered to restrict the second screening only to the subgroup of c-negative women since our data indicated that especially these women are at risk of HDFN caused by antibodies not detected in the first trimester. This will greatly reduce the costs of a second screening since only 19% of Caucasians is c-negative.[49] Such a second screening programme implicates extension of the ABO and D typing with c typing at the moment of the first trimester screen. This typing strategy has the additional advantage to make the implementation of c-matched transfusions to women < 45 years (discussed hereafter) easier.

The optimal timing for a second screening in D-positive women is still unclear. For practical reasons we propose the same moment as the second screening in D-negatives: by

now the 30th week of pregnancy, but after introduction of routine fetal D typing (see § 9.2) the 28th week. It is assumable that antibodies emerging after week 28-30 will not give rise to severe HDFN. In addition, it is very unlikely that pregnant women with new antibodies detected after a negative first trimester screening, will need IUT treatment before this moment. In our study, in screen-detected cases with anti-c antibodies IUT treatment was necessary in only one pregnancy during 1.5 year; the first IUT in this pregnancy was given in week 31.

Apart from the sensitivity of the first trimester screening test, also the specificity and the positive predictive value to predict severe HDFN were unknown before implementation of the screening programme. Obstetric care workers in the Netherlands objected to the introduction of the screening programme because of this unknown predictive value and the expected negative psychological impact of false-positive screening results.[50;51] We found a specificity of the screening test of 99%. The predictive value of a positive screening (PPV) test depends on the definition of a positive test. Because of the low prevalence of severe HDFN, the predictive value of initial screen-positivity is only 0.6%. If the presence of clinically relevant antibodies, (= antibody of the IgG class directed against an antigen expressed on fetal RBCs), established by specificity testing in the reference laboratories, is taken as starting point, the PPV is 2.1%. The PPV is increased to 3.7% when only paternal antigen-positive cases are taken into account, and to 5.6% if the fetus is actually antigen-positive. The PPV depends strongly on the antibody specificity. In our prospective study we have established the risk for developing HDFN for each antibody specificity. (See Table 9.2).

Table 9.2 Positive predictive value to detect severe HDFN according to positive test results

Positive test result	Positive predictive value	
	all test- positives	fetal antigen positive
Screening test positive	0.6%	
Clinically relevant antibodies (any)	2.1%	5.6%
Only anti-K or anti-Rh (non D)	2.6%	7.7%
Anti-K	2.1%	26.3%
Anti-c	8.2%	10.2%
Anti-Rh (non c or D)	1.0%	3.0%
Clinically relevant, other than Rhesus antibodies or anti-K	0%	0%

The PPV is highest in pregnancies at risk because of anti-K antibodies. For many other specificities (i.e. Duffy, Kidd, S, M) case reports have been published showing that HDFN is theoretically possible. However, we have shown that the incidence is extremely low. No HDFN was observed in pregnancies with any of the other antibodies (Duffy: 42 antigen-

positive children, others 59 antigen-positive children).

Improvement of the PPV of initial screen-positivity might be possible by direct determination of the specificity of the antibody detected by the screening, since in about 70% of all screen-positive cases no antibodies or only clinically irrelevant antibodies are present. At present, not always enough blood is available to complete the serological analysis in the reference laboratory with the material drawn for the initial screening test, hence a new sample is requested. This could be circumvented by drawing routinely two blood samples. Another option is to perform the screening and the specificity testing in the same laboratory, either a regional laboratory or one of the reference laboratories. The PPV can also be improved by revision of the definition of clinically relevant antibodies to only anti-K and Rhesus antibodies. Even the introduction of a screening assay in which only anti-K and anti-Rhesus antibodies can be detected might therefore be considered, but at present such an assay is not available yet. On the other hand knowledge on the presence of antibodies which are not relevant for developing HDFN is useful in case the pregnant woman needs a blood transfusion around delivery.

Phase 2 Laboratory monitoring

In this phase pregnancies at risk are monitored to establish the risk for severe HDFN. If the fetal antigen is unknown because the father is heterozygous, it is relevant to determine the fetal antigen status with non-invasive fetal DNA typing, which is at present possible for c, C, D, E and K.[52;53] If the fetus is antigen-negative, no further monitoring is necessary. The accuracy of the tests in use (in the Netherlands titre and ADCC test) was unknown: the recommendations about the laboratory cut-offs, were based on data about D-immunised pregnancies [54]; lack of evidence also is suggested by the observed variation of the cut-offs of the tests used (in most countries only antibody titres), both between and within countries (i.e. laboratories).[4] Our study as described in **chapter 4** empirically established cut-off points for laboratory follow-up testing: clinical monitoring is indicated when the antibody titre is ≥16 and/or the ADCC test result is ≥30%. The sensitivity of these cut-offs was 94% and the specificity 77%. The only case of severe HDFN that should have been missed by using these cut-offs, was most likely not caused by the anti-c antibodies, detected upon screening, but by an ABO antagonism, which is beyond the scope of the RBC alloantibody screening programme. However, as only 16 cases of severe HDFN were included in this study, it was not possible to differentiate between the various antibody specificities, especially not for anti-K antibodies, which not only induce extravascular haemolysis of fetal RBCs, but also suppress the erythropoiesis by binding to K-positive erythroid progenitor cells.[55-57] It has been reported that severe K-mediated HDFN can already occur at low antibody titres.[58;59] Further research on this topic is needed.

Evaluation of criterion 5

Phase 1. The population screening to select cases of clinically relevant maternal alloimmunisation is simple and straightforward, with a moderate sensitivity and a relatively low PPV. To fulfil the fifth criterion the sensitivity can be improved by introduction of a second screening in c-negatives, while the PPV can be improved by revision of the definition of clinically relevant antibodies and by performing screening and specificity testing in the same laboratory.

Phase 2. The test performance of the laboratory monitoring fulfils the fifth criterion by introduction of the new established cut-offs.

Criterion 6: The test (procedure) should be acceptable to the population.

Our study is the first to explore the attitude towards the screening programme for maternal alloimmunisation.

All women, screen-negative controls and screen-positives with and without clinically relevant antibodies, showed a strongly positive balance between perceived utility and burden of the screening programme. So, the originally by the obstetric care workers feared, in most cases unnecessary anxiety caused by a positive screening result was limited.

The satisfaction about the provided information about the antibody screening was moderate in screen-positives, as well in screen-negatives. All screen-positives, including the women with an initial positive screening result that was not confirmed in the reference laboratories, and the women with clinically non-relevant RBC antibodies, desired more supportive information, especially about the consequences of maternal alloimmunisation for mother and child and for the next pregnancy, as well as about the blood tests. Dedicated written information materials and Internet information are needed.

Anxiety increased in screen-positives during the screening process, but decreased to basic levels postnatally. Anxiety two weeks after birth was not related to the result of the antibody screening test.

We also evaluated the acceptance of the screening programme by obstetric care workers and laboratories (unpublished results). Despite the initial objections of especially obstetric care workers to the screening programme, obstetric care workers and laboratories were strongly positive about the screening programme and wanted to maintain it. Most care workers stated that the programme and the policy after a positive screening were clear to them. However, the moderate satisfaction of pregnant women about the verbal and written information, seems contradictory.

Evaluation of criterion 6

The acceptance of the first trimester RBC screening programme by pregnant women, obstetric care workers and laboratories is good. The sixth W&J criterion is fulfilled.

Further improvements can be achieved by providing supportive information to, especially, screen-positive women.

Criterion 7: The natural history of the condition, including the development from latent to manifest disease, should be adequately understood.

The real natural course of the HDFN caused by non-D antibodies has never been studied. The best way to study the natural course of disease would be a prospective study in which women are tested for the presence of any (clinically relevant) RBC non-D alloantibody and the test result is not made available to the obstetric care worker, nor any treatment is instituted upon chance detection or postnatal diagnosis. It is obvious that given the known risk of detrimental outcome in case of untreated anti-c and anti-K antibodies[4;60-65], and the benefits of postnatal treatment this is not ethically justified. In this respect, it is meaningful that as discussed above (criterion 5) in two of the cases in which the antibody was missed during first trimester screening serious long term effects occurred. The life-time consequences of surviving children with kernicterus or perinatal asphyxia however are unknown, as in the time that these conditions were seen frequently, no postnatal treatment was available and most children died shortly after birth. Moreover, in that time there was also no insight in the various antibody specificities that could be responsible for the disease.[66;67]

Our observational prospective study described **in chapter 2** enabled us to study the natural history of all cases in which no treatment was indicated, since we investigated cord blood for haemolysis parameters and documented from all screen-positive the children the clinical follow-up.

As shown in table 9.3, in which the antenatally treated newborns are excluded, we could show that the group of fetuses at risk for HDFN had a significantly lower haemoglobin level (K and anti-Rh) and signs of haemolysis, resulting in icterus after birth (all specificities) and a higher frequency of phototherapy (K, Rhesus and Duffy). However, in the majority of cases no clinical signs of antibody-induced anaemia were observed. Presumably, the haemolytic effect of most antibodies was low and if present, compensated by increased erythropoiesis as reflected by higher reticulocyte counts in some of the newborns, except for anti-K antibodies which are known to suppress haematopoiesis.

From other studies it was already known that anti-K and anti-c were the most dangerous non-D antibodies, for which antenatal treatment may be necessary.[4;60-65] Rarely, the presence of other Rhesus antibodies, such as anti-E, anti-C, anti-e or anti-C^w has been reported to require intra uterine treatment as in almost all cases of severe HDFN by these antibodies, postnatal treatment is sufficient.[68-71] Also in our study, in none of the cases with these Rhesus antibodies and antigen-positive children (n=135) antenatal treatment was

Table 9.3 Outcomes, according to antigen-status of the child

Specificity	Haemoglobin		Haematocrit		Reticulocytes		Icterus	
	Hb (sd) mmol/L	<μ-2sd (%)	Ht (sd)	<μ-2sd (%)	10^{9}/1000 ery's (sd)	>μ+2sd (%)	Recog-nized (%)	Photo-therapy (%)
Controls/antigen-negatives (n=964)	9.7 (1.1)	2.4	0.52 (0.07)	2.5	3.8 (1.1)	1.5	11	4
Anti-K (n=19)	9.3 (1.7)	8.3*	0.47* (0.11)	18.2*	4.2 (0.7)	0	37*	42*
Anti-c (n=118)	9.3* (1.2)	11.4*	0.49* (0.08)	8.5*	3.9 (1.2)	7.6*	39*	33*
Anti-E (n= 95)	9.5 (1.1)	2.7	0.50* (0.06)	1.5	4.4* (2.0)	11.4*	27*	19*
Anti-Rhesus (non-D,E,c)(n= 40)	9.2* (0.9)	2.9	0.49* (0.07)	9.7	4.0 (0.7)	0	30*	20*
Anti-Fy (n=42)	9.7 (1.3)	0.0	0.52 (0.08)	4.5	4.1 (1.3)	13.0*	26*	14*
Other (n= 59)	9.5 (1.1)	4.2	0.51 (0.06)	0.0	4.0 (1.1)	13.3*	19*	7

* $p<0.05$ compared with control group

indicated, although in three cases an exchange transfusion shortly after birth was given. In the LUMC in eleven years two fetuses (out of 210) received IUTs because of other Rhesus antibodies than anti-D or anti-c. In one of these cases, other pathology contributed to the fetal anaemia.[41]

Incidental cases of HDFN by Duffy and anti-M have been reported too, not requiring intra uterine treatment.[72-74] In the LUMC study, mentioned above, in eleven years one pregnancy with Duffy antibodies was treated with IUTs. Most of the studies on specificities other than D, K and c are case reports or include a selected group of cases at higher risk of severe HDFN, e.g. with titres above 16 or 32 or women with a seriously affected prior child. Therefore such studies do not provide information about the unbiased (population) risk of such antigen-positive fetuses to progress to HDFN, which anyway must be very small.[65;75]

Evalution of criterion 7

While it was not possible to study the natural course in presence of the various non-D antibody specificities and the long term consequences of the very rare cases of irreversible damage, our study has provided reliable information about the actual risk for HDFN in the presence of the various antibody specificities. Antenatal treatment is almost exclusively indicated in anti-K or anti-c antibodies. Of the other specificities only anti-Rhesus antibodies led to haemolytic anaemia and in only a few cases.

The seventh criterion is therefore in our view fulfilled to the degree required for screening evaluation, although economic evaluation of screening may suffer from the lack of long term data on the rare cases with permanent sequelae.

Criterion 8: There should be an agreed policy on whom to treat as patients, and how.
Before the study it was clear which fetuses and newborns should be treated as patients.[43] See also criterion 4. The 8th criterion is fulfilled.

Criterion 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.
Prior to the OPZI-study no information was available on this issue. While our research provided detailed estimates on the majority of economically relevant parameters, from an international perspective it is relevant to acknowledge the dependence of the programme on a centralized highly structured prenatal programme with universal access, and strong support by the obstetric care workers.

To calculate the cost-effectiveness of different screening scenario's, we had to handle with several uncertainties: 1) The risk of too late detection of profound hyperbiluribinaemia, resulting in kernicterus, in cases, undetected by the screening programme. We made calculations assuming a minimum risk of 5% and a maximum risk of 10%. 2)The life expectancy of surviving children with severe antenatal anaemia, hydrops or kernicterus, which we assumed to be 60 years; 3) The life-time costs of these surviving children.

The costs of the current non-D screening programme, including diagnosis and treatment of detected cases, and following the established cut-off points for laboratory monitoring, were calculated as € 2.6 million per 100,000 screenees. The costs per detected case at risk were € 7,900, per timely detected case of severe HDFN € 378,500 and per prevented case with long-term sequelae between € 3.5 and € 4.5 million, dependent on the assumed risk for missing kernicterus. The costs per QALY are between € 20,000 and € 67,000.

Based on the clinical and epidemiological information the costs of alternative scenario's were calculated: a. limitation of laboratory monitoring; b. subgroup screening; c. second screening in c-negatives.
Ad a. We can change the current approach of clinically relevant antibodies (= all RBC alloantibodies that can theoretically cause HDFN) into a differentiated one: dangerous are anti-K and anti-c (and anti-D), which antibodies require intensive laboratory monitoring; potentially dangerous are all other Rhesus antibodies, requiring a single laboratory test in the third trimester to establish the risk for neonatal disease, the remaining antibodies are harmless requiring no laboratory monitoring at all. This will slightly reduce the costs to € 2.5 million per 100,000 screenees, the costs per detected case at risk are increased to € 8.900, as less pregnancies are considered as at risk, per timely detected case of severe HDFN reduced to € 359,000, per prevented case with long-term sequelae between € 3.3 and € 4.3 million and the costs per QALY are between € 11,000 and € 55,000.

Ad b. To investigate whether a substantial cost reduction could be achieved by subgroup screening without loss of effectiveness, we studied risk factors for maternal non-D immunisation in a large-scale case control study (**chapter 3**). Independent multivariate risk factors for the clinically most important antibodies anti-K, anti-c and Rhesus antibodies, other than anti-D or anti-c, were a history of RBC transfusion, of prior delivery, haematological disease and major surgery. RBC transfusion was the most important risk factor, especially for anti-K. No risk factors at all were present in 31% of pregnant women. Subgroup screening, with exclusion of D-positive parae-0 without a history of RBC transfusion, major surgery or haematological disease, reduces the costs with about 25% to € 1.9 million per 100,000 screenees, without missing cases with severe HDFN. The costs per detected case at risk are € 7,000, per timely detected case of severe HDFN € 275,500, per prevented case with long-term sequelae between € 2.5 and € 3.3 million, which makes this scenario cost-effective.

Ad c. Introduction of a second screening in c-negatives to enlarge the sensitivity of the screening programme, generates additional costs of € 0.7 million and increases the detection of severe HDFN with about 30%. A scenario of subgroup screening combined with a second screening of c-negatives and limited laboratory monitoring costs € 2.6 million, € 8,100 per detected case at risk, € 301,600 per prevented case with long-term sequelae. Assuming that the risk of too late detection of kernicterus is 10%, this scenario is cost-effective; if the risk is 5%, the costs per QALY are € 37,000.

However, introduction of subgroup screening, although efficient, requires careful documentation of risk factors and full compliance of obstetric care workers. A scenario with a second screening of c-negatives without introduction of subgroup screening, costs € 3.2 million per 100,000 screenee's, € 9,900 per detected case at risk, € 377,000 per prevented case with long-term sequelae and between € 20,000 and € 84,000 per QALY. We judge even this scenario to be acceptable, because: 1) The detection rate of a second screening may be higher than we found in our retrospective study; 2) While thorough professional observation of newborns during the first days of life theoretically should detect profound hyperbiluribinaemia in time and prevent kernicterus, in fact an increasing incidence of kernicterus in term children is reported in several countries, due to under-recognition and inadequate investigation of severe hyperbiluribinaemia.[76;77] Also in the Netherlands timely detection may not always be realised, as continuous care during 24 hours a day is not standard, especially since the unique Dutch system of maternity care has been limited to 3-6 hours per day.

Evaluation of criterion 9

The current programme costs between € 20,000 and € 67,000 per QALY, which is beyond the accepted threshold of € 80,000.[78]

If improvements are introduced, the screening programme is cost-effective or the costs per QALY are beyond the accepted threshold, even if a second screening in c-negatives is introduced. The ninth criterion is fulfilled.

Criterion 10: Case-finding should be a continuing process and not a 'once and for all' project. The ongoing Dutch screening programme fulfils the 10th W&J criterion, as the prenatal programmes all are well-established professional, state-supported programmes with a permanent status including process evaluation and quality control.

In conclusion, given the evidence which in part was developed in the OPZI study, the present screening programme fulfils the ten W&J criteria in the Dutch context of prenatal care**: *Routine first trimester screening for maternal RBC alloimmunisation should be maintained.*** The screening programme facilitates timely detection of three pregnancies at risk for fetal death, hydrops or severe anaemia, and six children at risk for profound hyperbiluribinaemia. The costs per QALY of the current screening programme are below the accepted threshold of € 80,000.

Beyond the evaluation context of W&J two more benefits of this screening programme can be observed: 1) Detection of D antibodies earlier in pregnancy than upon 3rd trimester screening and 2) Knowledge about the presence of RBC antibodies when the mother needs an RBC transfusion around delivery.

We have not evaluated the effect on first trimester detection of D antibodies. However, it is likely that some fetuses with severe anaemia in the second trimester of pregnancy, will benefit from earlier detection. IUTs because of D immunisation are performed from the 16th week of pregnancy onwards.[41] Further research on this topic is recommended.

In our study 6.1% (55/987) of the non-D immunised mothers received an RBC transfusion around delivery, versus 1.7% of the controls. In 66% of these cases the presence of the RBC alloantibodies was unknown before the first trimester screening in the current pregnancy. Knowledge of the presence of RBC antibodies might have contributed to transfusion safety and might have saved time, needed for the identification of antibody specificities. However, in 13% of the transfused cases the presence of the RBC alloantibodies was not known by the transfusion laboratory of the hospital were the transfusion was given. This underlines the importance of the introduction of the national registry of RBC antibodies (TRIX) in 2007.

Recommendations for improvement of the screening programme for maternal RBC alloimmunisation

The aim of our study was primarily to reveal evidence for the present programme, but our analysis also provided scientific evidence for possible adaptations of the program, which we have summarized below. Some of these recommendations were already discussed while evaluating along the W&J criteria.

Recommendation 1:

Women below 45 years of age should receive K-negative and c-matched RBC transfusions.

In **chapter 3** it was demonstrated that RBC transfusion is the most important risk factor for non-D immunisation (OR 16.7; 95%-CI: 11.4-24.6), also in women with a prior parity. More than 50% of women with clinically relevant RBC antibodies had a prior history of blood transfusion In the group of K-sensititized women this was even more than 80%. The introduction of matched blood transfusion to women under the age of 45 years will over time contribute to a major reduction of immunisations and cases of severe HDFN. The use of K-negative RBCs in transfusions to women younger than 45 is already prescribed by the current Dutch guidelines since 2004.[6] Because the largest proportion of cases of severe non-D HDFN is caused by anti-c antibodies, transfusion of c-matched RBCs to Rhc-negative girls and women of child bearing age would also be highly effective in preventing HDFN. This might prevent in the Netherlands more than 50% of anti-c immunisations. In 57% of the women with anti-c the fetus will be c-positive and hence at risk of HDFN because of an antigen-positive father. As in the Netherlands all donor RBCs are already typed for Rhesus CcDEe, preventive matching for c can relatively easily be implemented. Indeed, an economic evaluation revealed that matching blood transfusion for cE in fertile women is a cost-effective intervention. Only in the first four years the costs will exceed the benefits (Final report ZON-MW Doelmatigheidsonderzoek Dossier number : 94504608). It will be even more cost-effective when c typing in the first trimester of pregnancy (as discussed below) is introduced. Since 70% of RBC transfusions to women < 45 years of age are administered around delivery, pre-transfusion c typing of women < 45 years will be indicated in less then 30% of the blood transfusions in this patient group.

Recommendation 2:

A second screening of c-negative women should be introduced.

A second screening of c-negatives (19% of the population) enlarges the sensitivity of the screening with about 30%. The costs per QALY of such a programme are below the accepted threshold of € 80,000. Implementation of this new policy should be monitored by a thorough registration of the outcomes of these pregnancies as well as a set of quality indicators, such as the numbers of laboratory tests and of clinical diagnostics.(see

discussion on 5th and 9th criterion)

Recommendation 3:
Subgroup screening might be introduced, but only if careful documentation of risk factors is guaranteed and full compliance of obstetric care workers is obtained.
In **chapter 3** we established risk factors for non-D immunisation. Significant independent risk factors were: history of RBC transfusion, parity, history of major surgery, haematological disease. Introduction of subgroup screening based on these risk factors is theoretically possible without loss of effectiveness. About 30% of women will be excluded from screening and 25% of the costs will be saved. In our view subgroup screening can only be implemented if careful documentation of risk factors, for example by means of an electronic patients record, is guaranteed, and full compliance of the obstetric care workers is obtained.

Recommendation 4:
Monitoring of pregnancies at risk can be limited to intensive monitoring of pregnancies at risk with anti-K, anti-D or anti-c antibodies, and performance of one repeated laboratory test in week 34-36 in pregnancies at risk because of other Rhesus antibodies.
The risk of severe antenatal anaemia in pregnancies at risk because of other Rhesus antibodies is very low; one additional laboratory test between week 34 en 36 is recommended to establish the risk of hyperbiluribinaemia after birth and to plan hospital birth if test results are above the cut-offs.The risk for severe HDFN caused by other antibodies than anti-K and Rhesus antibodies is negligible. Only in case of Duffy antibodies, the obstetric care worker should be aware of a higher risk for hyperbiluribinaemia. The reference laboratory can draw attention to this by adding information to the report concerning the result of the specificity testing, which is sent to the care worker.

Recommendation 5:
Clinical monitoring of pregnancies with anti-K and anti-c alloantibodies is recommended if titres are ≥16 and/or the ADCC test result is ≥30%. In case of other Rhesus antibodies above these cut-offs a hospital delivery is advised and thorough observation of the child.
In our study all severe cases except one with probably an ABO antagonism had laboratory test results above these cut-offs (**chapter 4**). For selection of cases that should intensively clinically monitored, we propose a cost-saving measure, to perform only an ADCC-test if the titre is below the cut-off level. However, more research is needed about the optimal scheme of lab monitoring during pregnancy. Since the high risk of HDFN in case of an antigen-positive child for anti-c and anti-K antibodies, we recommend non-invasive fetal genotyping for Rhc and K in case of a heterozygous father; this can be also considered for

RhC, RhE and Rhe to select fetuses at risk.

Recommendation 6:
Centralisation of first trimester antibody screening, inclusive ABO, RhD and Rhc typing, and specificity testing, as well as 30th week screening and specificity testing in D- and c- negative women, in the reference laboratories should be considered.
The advantages of centralisation are: 1) Less false-positive screen results, as screening and specificity testing are performed by the same laboratory; 2) More expertise about the interpretation of test results in pregnancy and the consequences for the pregnancy; 3) Monitoring of the programme, especially of the proposed second screening in c-negatives, can easily be performed; 4) Costs can be saved because of economies of scale and the efficiency of the combination of screening and specificity testing.

Recommendation 7:
The existing guidelines concerning laboratory and clinical monitoring of pregnancies at risk, including the moment of referral to the obstetrician and to the LUMC, and the diagnostics of the newborn, should be revised, based on the evidence provided by the OPZI study, by representatives of all professional organisations: midwives, general practitioners, obstetricians, paediatricians and laboratories.
By now, only one guideline from the organisation of obstetricians is available.[7] A guideline written by all involved professionals, in which the implications of the evidence provided by the OPZI study are discussed, can also pay attention to requirements of a programme using laboratory monitoring for case-selection for clinical monitoring and to the responsibilities of all care workers. The revised guidelines should be communicated to all care workers involved.

Recommendation 8:
The existing leaflets with patient information should be revised.
More supportive information is needed for screen-positive women, including women with clinically not-relevant antibodies. Information about blood testing, about the consequences of maternal alloimmunisation for mother and child of the various antibody specificities and for the next pregnancy is necessary. The information should be available on Internet, but also written, and be understandable for Dutch and non-Dutch speaking pregnant women. The involved professional organisations should revise the existing materials and actively distribute these materials to professional care workers.

9.2 Antenatal anti-D prophylaxis

The introduction of postnatal anti-D Ig prophylaxis in 1969 was very successful in reducing the prevalence of anti-D immunisations in the Netherlands. The observed decrease in the prevalence of new anti-D immunisations, detected upon 32nd-week screening, from 3.5% to 0.5%[79], reflects a number needed to vaccinate (NNV) of 20 to prevent one anti-D immunisation and of about 80 to prevent one case of severe HDFN. Despite the effectiveness of postnatal prevention, the 0.5% prevalence still represented about 80 new detected cases per 100,000 pregnant women (15.3% D-negative mothers).

Several studies showed that routine antenatal anti-D prophylaxis (RAADP) could further reduce the immunisation prevalence. The dosage and timing of the anti-D Ig administration in these studies varied from 2 x 100 µg (500 IU) in week 28 and 34 [80-83] until 2 x 300 µg (1,500 IU).[84] Antenatal prophylaxis has been introduced in the Netherlands in July 1998. An administration schedule was chosen of one single dose of 200 µg (1,000 IU) in week 30, an administration schedule with a high procedural feasibility: only one extra administration of anti-D Ig during routine prenatal care of the same dose as was used postnatally, which would prevent any mistake between dosages.[85] However, the choice of one dose of 200 µg anti-D Ig carried a risk, since a single dose will result in lower circulating concentrations of anti-D Ig as term approaches, than the concentrations induced by the split dose scheme of most prior studies.[86] It was argued that in the studies of Bowman et al., a single dose of 300 µg, administered in week 28, effectively prevented anti-D immunisation.[84;87] Moreover, it was calculated that administration of 200 µg in week 30, instead of week 28, was sufficient to provide adequate anti-D Ig levels until the end of pregnancy.[85] However, protagonists may have overlooked that in the study of Bowman maternal anti-D Ig concentrations were monitored and additional anti-D Ig was given at around 36 weeks to those women in whom passive anti-D Ig was no longer detectable.[86;87]

Beside this variation in dosage and timing, supportive studies for antenatal prophylaxis also showed considerable heterogeneity in patient selection, outcome measures (predominantly proxy outcomes are used i.e. immunisations after birth or in the next pregnancy rather than the occurrence of HDFN), and results. From these studies, including one quasi-experimental study [81], it can be concluded that a dosage of at least 2 x 100 µg (2x 500 IU) anti-D Ig (in week 28 and week 34) reduces the remnant risk of anti-D immunisation by 50-80%.[80-84;87-90] However, in all studies reported in the literature the number of included women was small. Therefore, accurate data regarding the population prevalence of immunisations after the introduction of RAADP were not available at the start of our study, as well as data about which dosage is sufficient to achieve a substantial reduction of anti-D immunisations.[91] Moreover, the effect of antenatal anti-D prophylaxis on the occurrence of subsequent HDFN was unknown. For this reasons it was decided to

perform a nation-wide evaluation of the effect of the introduction of RAADP in the Dutch prenatal care programme, not only in terms of immunisations but also in terms of the true outcome, HDFN.

Effects of the Dutch RAADP programme

We established the effect of antenatal anti-D prophylaxis in a nation-wide study during three years, including 21,000 pregnant parae-1, who gave birth to a D-positive child in their first pregnancy (**chapter 7**). We thoroughly collected data about postnatal and antenatal anti-D Ig prophylaxis in the previous pregnancy to be sure that the antenatal anti-D Ig administration was the only difference between the groups. We studied both immunisation and HDFN in the next pregnancy as outcome.

Effect on prevalence of anti-D immunisation

The prevalence of anti-D immunisation upon first trimester screening in the next pregnancy decreased from 671/100,000 (95%-CI: 499-843) in women with only postnatal anti-D after the first delivery to 310/100,000 (95%-CI: 213-407) in women who received postnatal and antenatal anti-D, showing that the immunisation risk is halved. Remarkably no difference was observed in late immunisations detected at the 30th week of pregnancy.

The reduction in immunisations after antenatal prophylaxis is lower than observed in the three meta-analyses conducted by NICE (National Institute for Clinical Excellence) in the UK, concerning different dosages of anti-D, study populations and outcome measures.[92] This can almost completely be attributed to the lower immunisation rate we observed in the group receiving only postnatal prophylaxis. This, in turn, can be explained from the fact that we carefully collected all data of the individual patients on previous immunoprophylaxis via the obstetric care workers rather than relying on registry data, and only included women who received postnatal prophylaxis after their first delivery. When we would have included D-immunised parae-1 who probably did not receive postnatal prophylaxis after their previous delivery, this would have resulted in a prevalence of 1.0% in the group receiving only postnatal prophylaxis, which is comparable with the prevalences in the control group in other studies with the immunisation rate in the next pregnancy as outcome measure.[82;90]

The observed sensitisation risk in women receiving full prophylaxis (0.31%) is comparable to the risks (0.30%, 0.31% and 0.35%) observed in the three meta-analyses This might suggest that the single dose of 200 µg at 30 weeks is equally effective as the schemes analysed in these meta-analyses (2x100 µg at 28 and 34 weeks or a single dose of 300 µg at 28th week). However, it should be emphasized that the two studies using the same outcome measure as our study (immunisations in the next pregnancy), were community-based studies, in which also women were included in the intervention group

who actually did not receive complete antenatal and postnatal prophylaxis. Mackenzie [90] reports a 'true' sensitisation rate in women who actually received antenatal prophylaxis of 0.21% (6/2,822) and Mayne [82] a true rate of 0% (0/1,421). However, these studies are small and in the Mackenzie study it is not clear whether the denominator of women 'at risk' also includes the proportion of women (about 40%) who delivered a first D-negative child. If that is indeed the case, the 'true' sensitisation rate would be 0.29%, comparable to our findings. While it can be concluded that the Dutch single dosage of 200 µg halves the sensitisation risk as established in the next pregnancy, it is not possible to compare this effect with the effect of the split dosage of 2 x 100 µg in other studies.

Effect on prevalence of HDFN

The introduction of RAADP halved the incidence of subsequent severe HDFN from 230/100,000 to 104/100,000 (not significant, due to small numbers). No data about the effect on HDFN were available until now. Most recent prevalence data on HDFN come from the UK, and these are difficult to compare. Before the introduction of antenatal prophylaxis in the UK 500 fetuses developed HDFN and 25-30 babies died from HDFN caused by anti-D each year.[92] For the study described in **chapter 7** we inventoried all D-immunisations recognized in the Netherlands during three years. In this period, in which a similar number of confinements (600,000) occurred as in England and Wales (620,000) we observed in total 682 anti-D immunisations, of which 432 were newly detected. Since the risk for severe HDFN in pregnancies with anti-D antibodies is less than 1/3 (see below), we expect that maximally 230 cases of severe HDFN have occurred in three years, which is clearly lower than the numbers of HDFN cases in the UK. We have no explanation for these differences, possibly the compliance with the anti-D prophylaxis programme in the Netherlands is better, because of the well organized antenatal care. We observed that in only 25% of the immunisations probably no anti-D prophylaxis had been administered, whereas in the UK studies more logistic failures are reported.[92;93]. Also the reported perinatal mortality in the UK appeared to be higher, possibly explained by the almost 100% coverage of the first and third trimester antibody screening in D-negative women in the Netherlands, which facilitates timely treatment.[94] An interesting observation was that while RAADP did not influence the incidence of late immunisations (detected at the 30th week of pregnancy), the risk for severe HDFN caused by these 'late' anti-D antibodies was significant lower after administration of antenatal anti-D and postnatal anti-D prophylaxis in the previous pregnancy. It needs further study to explain the immunological mechanism. These data suggest that in some women who encountered the D antigen for the first time in the presence of anti-D, the immune response is not completely prevented but yet attenuated. Also Tovey et al. have reported in 1975 that after the introduction of postnatal prophylaxis the level of anti- D antibodies and the risk for severe HDFN was

in most alloimmunised women lower, when compared to alloimmmunised women who never received prophylaxis.[95] More recently, MacKenzie et al. also described that in the far majority of alloimmunised women who received full antenatal and postnatal prophylaxis, the level of anti-D in the second pregnancy was low.[96]

Table 9.4 shows the NNVs of the Dutch programme of restricted anti-D prophylaxis, as practised in the Netherlands until May 2008. The programme prevents yearly (numbers for 2004) 36 immunisations and 11 cases of severe HDFN, which translates in a NNV of 357 to prevent one immunisation and of 1,255 to prevent one case of severe HDFN.

It should be kept in mind that a substantial part of new anti-D immunisations is detected in women who probably or for sure did not receive postnatal anti-D prophylaxis, especially after a first delivery in countries without a national anti-D prevention programme (Figure 9.2). This was the case in 17% of new anti-D immunisations in parae-1. This problem is beyond the scope of this thesis, but it raises the question what developed countries can do to help less developed countries to organise a prevention programme, comprising of in any case adequate postnatal anti-D prophylaxis.

At this stage (2008), the implementation of routine fetal D typing in maternal plasma can decrease all NNVs with about 40%, the proportion of fetuses that will be typed as D-negative, in which pregnancies RAADP is unnecessary and can be omitted.[97]

Table 9.4 Numbers needed to vaccinate in different scenario's of RAADP

Numbers Needed to vaccinate to prevent:	Scenario 1 RAADP restricted to women without a living child	Scenario 2 RAADP to all pregnant women	
			Additional effect of RAADP to parae->=1
one anti-D immunisation in week 12 of later pregnancies	357	570	1,101
one case of subsequent severe HDFN	1,255	2,339	7,856

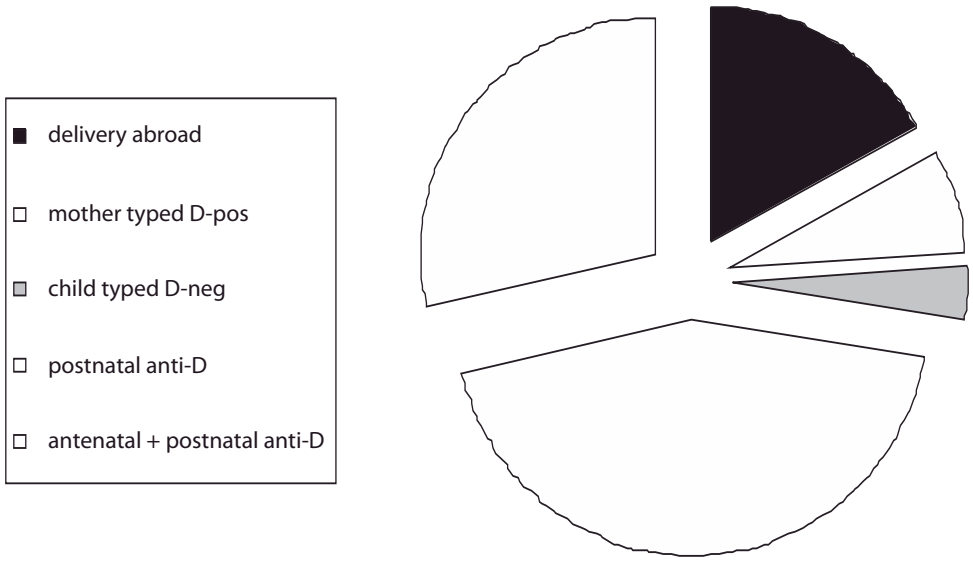
Effect of extending the RAADP programme

Extending to all pregnant women

In a scenario in which RAADP is not restricted to women without a living child but extended to all pregnancies, in the Netherlands only 11 additional immunisations would be prevented and two more cases of severe HDFN, with as shown in Table 9.4 considerably higher NNVs for this additional effects: 1,101 and 7,856 to prevent one immunisation and case of severe HDFN, respectively.

The carry-on effects to all subsequent pregnancies are based on calculations, assuming that the immunisation risk in all future pregnancies, following a pregnancy

Figure 9.2 Anti-D prophylaxis in first pregnancy of parae-1 with newly detected D immunisation at first screening (1999, 2002, 2004)



without antenatal prophylaxis, is similar to the immunisation risk before the introduction of RAADP. However, there are some preliminary findings that the protection against immunisation provided by antenatal prophylaxis in primigravidae extends beyond the first pregnancy. However, this hypothesis is based on studies, which lack the power to draw this conclusion. Thornton et al. originally postulated this idea because no immunisations were seen in 200 D-negative women in their second or third pregnancies.[83] More recently Mackenzie et al. made a similar suggestion, as they found under a restricted RAADP policy (only in the first pregnancy) the same prevalence of new anti-D antibodies in parae-1, who delivered a previous D-positive child (0.46%) as in parae-2, who delivered a second D-positive child (5/1071=0.47%).[96] However, in the same study the prevalence of new immunisations in parae-III, who delivered a D-positive third child, was 0.62% (2/549), and another 1.29% of parae-III (7/549) was already immunised from a prior pregnancy, which was not discussed by the authors. Moreover, the study population was small.[96] However, there are some experimental data which are in favour of the hypothesis of protracted effect. Mollison et al. already described in 1970 that the response rate to repeated RBC injections was decreased in subjects who had previously been administered anti-D coated RBCs compared with those who only received RBCs.[98] A similar observation has been done by Kumpel et al. She investigated the protective effect of monoclonal anti-D antibodies in 24 healthy volunteers. Only six of these volunteers had accelerated clearance of RBCs after repeated subsequent unprotected challenging, which is lower than expected. Moreover,

in one of these responders the level of anti-D was too low to detect serologically and in the other five responders the levels of anti-D tended to decrease rapidly, unlike responses in individuals immunised by pregnancy or transfusion.[99] This observation might also explain our finding that we did see after the introduction of antenatal prophylaxis relatively more women with anti-D antibodies detected only in the 30th week screening. Possibly, women who encounter the RhD antigen for the first time in the presence of anti-D (which occurs during their first pregnancy), but become immunised by FMH during their first delivery, have a more rapid decline of anti-D titer and are therefore not detected at the 12th week of the subsequent pregnancy. So, in conclusion there are no strong data available supporting the hypothesis that antenatal prophylaxis provides continuing protection for all subsequent pregnancies, but it might very well be possible that it decreases the immunisation risk in all subsequent pregnancies and that in some women in which the immune response is not prevented, the response is attenuated. However, much more research is needed to confirm or disprove this hypothesis.

Extending by focussed prevention

As stated above, the additional effect of extending RAADP to all pregnant women is small. Additional to or instead of increasing the intensity of general RAADP by extending coverage to all pregnancies, one can aim at intensification of focussed prevention. Focussed prevention implies the administration of extra anti-D Ig in pregnancies and deliveries complicated by an event known to increase the risk for immunisation. The common denominator of such events is FMH, and although guidelines exist on focussed prevention in selected conditions guided by the Kleihauer-Betke test (KHB-test),[7;8] the adherence appears limited. This situation offered the opportunity to establish risk factors for anti-D immunisation in a case control study. Independent risk factors for anti-D immunisation in parae-1 despite adequate antenatal and postnatal anti-D Ig prophylaxis in the first pregnancy, appeared a previous caesarean section or assisted vaginal delivery, postmaturity, a pregnancy-related RBC transfusion and younger age. Assisted vaginal delivery and pregnancy-related RBC transfusion, most likely indicators for a prolonged and more traumatic second and third stage of labour with a concomitant higher risk for FMH, should be added to the set of risk factors for D immunisation in the current guidelines. Our study provides targets to reduce the risk for anti-D immunisation in the presence of risk factors by focussed prevention. Assisted delivery was present in almost 50% of all immunised women (OR 2.2); a pregnancy-related RBC transfusion was given to 14% of the cases (OR 3.5) and postmaturity occurred in 19% of the cases (OR 3.1).Two policies are to be considered: administration of a standard extra dosage of anti-D Ig ór testing for FMH, followed by adjusted anti-D Ig prophylaxis. Current guidelines advise (and not *prescribe*) this last policy after a caesarean section, but we observed unanimous non-adherence

to this guideline in any of the anti-D immunised cases in our study. It is reassuring that clinical conditions where standard *additional* anti-D Ig is universally *prescribed*, such as spontaneous miscarriage, termination of pregnancy, invasive procedures during pregnancy and external version, did not emerge as a risk factor in our analysis. This again indicates that Dutch obstetric care workers strictly adhere to guidelines for the administration of anti-D following potentially sensitising events in pregnancy. This seems to be in contrast to the UK, in which the guideline recommendations were not recorded as being followed in upto 39% of the pregnancies.[100;101] It might be expected that additional anti-D Ig also will effectively prevent anti-D immunisation after a traumatic delivery. We calculated that if administration of a standard or KHB test-guided extra dosage of anti-D Ig would prevent all immunisations in the next pregnancy after a non spontaneous first delivery and/ or pregnancy-related RBC transfusion (one third of all first deliveries), the NNV would be maximally ±110, which actually is lower than the NNV of standard antenatal anti-D prophylaxis to women without a living child and considerably lower than the effect of routine antenatal prophylaxis to all D-negative pregnant women. In short: easy to grasp preventive benefits with no apparent disadvantages, at a low price.

Administration of a standard extra dosage of anti-D Ig in the presence of risk factors may be more effective than the performance of the Kleihauer-Betke test, as not all hospital laboratories are experienced in the performance and interpretation of this test. In fact, our own data show that it appears to be rather difficult for most obstetricians to apply this test routinely. Moreover, several studies showed no correlation between assisted delivery and the results of testing for FMH [102-107], so it is unclear whether the Kleihauer-Betke test, applied shortly after delivery, detects all FMHs relevant for immunisation.

In our view, extending of the RAADP programme to all pregnant women is not the most efficient way to use a relative scarce product as anti-D Ig. Also the burden of anti-D manufacturing to anti-D donors who are the human source for this plasma product: monthly plasmapheresis and once or even twice a year the administration of a small amount of D-positive RBCs to increase the level of antibodies. By now, there is no anti-D scarcity in the Netherlands, but this may a future inevitable consequence of a nearly perfect prevention programme. The NNVs and the costs of expansion of the programme to all pregnancies are considerable, while only two more cases of severe HDFN are prevented per annum in the Netherlands (in 30,000 D-negative pregnant women). The risk for long-term sequelae in these children is very low, as the anti-D screening programme will detect these cases in time. Our study on risk factors shows that focussed prevention might be more efficient, although it has to be demonstrated whether extra dosages indeed will prevent all immunisations.

However, there can be some injustice in the withdrawal of antenatal prophylaxis to women in their second pregnancy who consider to become pregnant another time. It can

be argued that the actual risk of developing anti-D antibodies in general is lower during the second pregnancy and delivery than the risk of 0.67% we established in parae-1 who received only postnatal prophylaxis, as the risk for a traumatic delivery is smaller than in parae-1. Also the - sofar unexplained - protective effect of increasing age can be taken into consideration. The actual immunisation risk in parous women without antenatal anti-D prophylaxis may be comparable with the immunisation risk despite antenatal prophylaxis in women pregnant of their first child.

A better way is, in my view, to offer also multiparous women an additional dosage of anti-D Ig in the presence of risk factors during delivery, especially if the woman is not sure that this was her last child.

One or two dosages?

While our observations allowed for, as we judge, a historical valid comparison of the added value of the Dutch RAADP programme, no further information could be collected on the questions of the best dosage / administration schedule. In absence of unequivocal evidence, one can speculate on dosage variants. Splitting of the single dose of 200 µg or 300 µg (1,000 or 1,500 IU respectively) of anti-D Ig in two gifts, in week 28 and in week 34 respectively, theoretically could have a cumulative effect on anti-D Ig plasma levels, which might contribute to sufficient levels of anti-D Ig in postmature pregnancies, hence to a decreased immunisation risk. In 7% (3/42) of the cases in our study, postmaturity was the only risk factor, while in another five cases postmaturity was combined with an assisted or surgical delivery. So, the effect of administration of a split dosage on immunisations in postmature pregnancies, will be relatively small. Furthermore, there is evidence that compliance with a two-dose regimen is less than ideal.[108;109] However, more research is needed concerning the anti-D Ig levels after administration of antenatal prophylaxis, especially after the 40th week of pregnancy, and the predictive value of too low anti-D Ig levels on immunisation in a subsequent pregnancy.

Economic aspects

We did not perform an economic analysis of the prevention programme for anti-D immunisation. The cost of one ampoule of anti-D Ig are about € 50,--, so the costs per avoided anti-D immunisation in a future pregnancy if anti-D is given only to women without a living child, are € 25,000 and the costs to prevent one case of severe HDFN € 75,000. The total costs of the programme in 2004 were € 670,000. On the other hand, costs of monitoring and treatment of immunised cases are saved. In our economic analysis of the non-D screening programme we calculated the costs of one screen-detected case of severe HDFN as € 6,000, and the costs of one case of moderate HDFN to be € 2,500. The costs of anti-D immunised cases may be higher, as we expect that these cases, especially

the moderate cases, will undergo more laboratory tests and will be referred more frequently to the LUMC than cases with non-D immunisation. We have no data about the proportion of moderate HDFN cases, caused by anti-D antibodies, but it is assumable that this proportion will be higher than in the total group of non-D alloantibodies. Moreover, all anti-D immunised pregnancies are at risk for HDFN as immunisation is always triggered by a prior pregnancy from a D-positive father. We assume that timely detection and treatment will prevent fetal death and long-term sequelae in almost all cases and that only costs are saved during pregnancy and the first months after birth. The NICE report has calculated the saved costs per affected pregnancy (all severities) to be £ 1,442 (price level 2002).[92] A future economic evaluation of the D prevention programme also should take into account the fetal D typing in maternal plasma.[110] The implementation of routine fetal D typing in maternal plasma can decrease all NNVs with about 40%, the proportion of fetuses that will be typed as D-negative, in which pregnancies RAADP is unnecessary and can be omitted.[97]

Recommendations for improvement of the prevention programme for maternal anti-D alloimmunisation

Recommendation 1:
Antenatal anti-D prophylaxis with one single dose of 200 µg of anti-D Ig to women without a living child should be maintained, while expansion of antenatal prophylaxis to all pregnant women is debatable.
The prevention programme halves the risk for anti-D immunisation and subsequent severe HDFN in future pregnancies. The NNVs to prevent one D immunisation during pregnancy and one case of subsequent severe HDFN are 357 and 1,255 respectively.

In my view, extending of the RAADP programme to all pregnant women is not the most efficient way to use a relative scarce product as anti-D Ig. The NNVs and the costs of expansion of the programme to all pregnancies are considerable, while only two more cases of severe HDFN are prevented per annum in the Netherlands (in 30,000 D-negative pregnant women). The risk for long-term sequelae in these children is very low, as the anti-D screening programme will detect these cases in time.

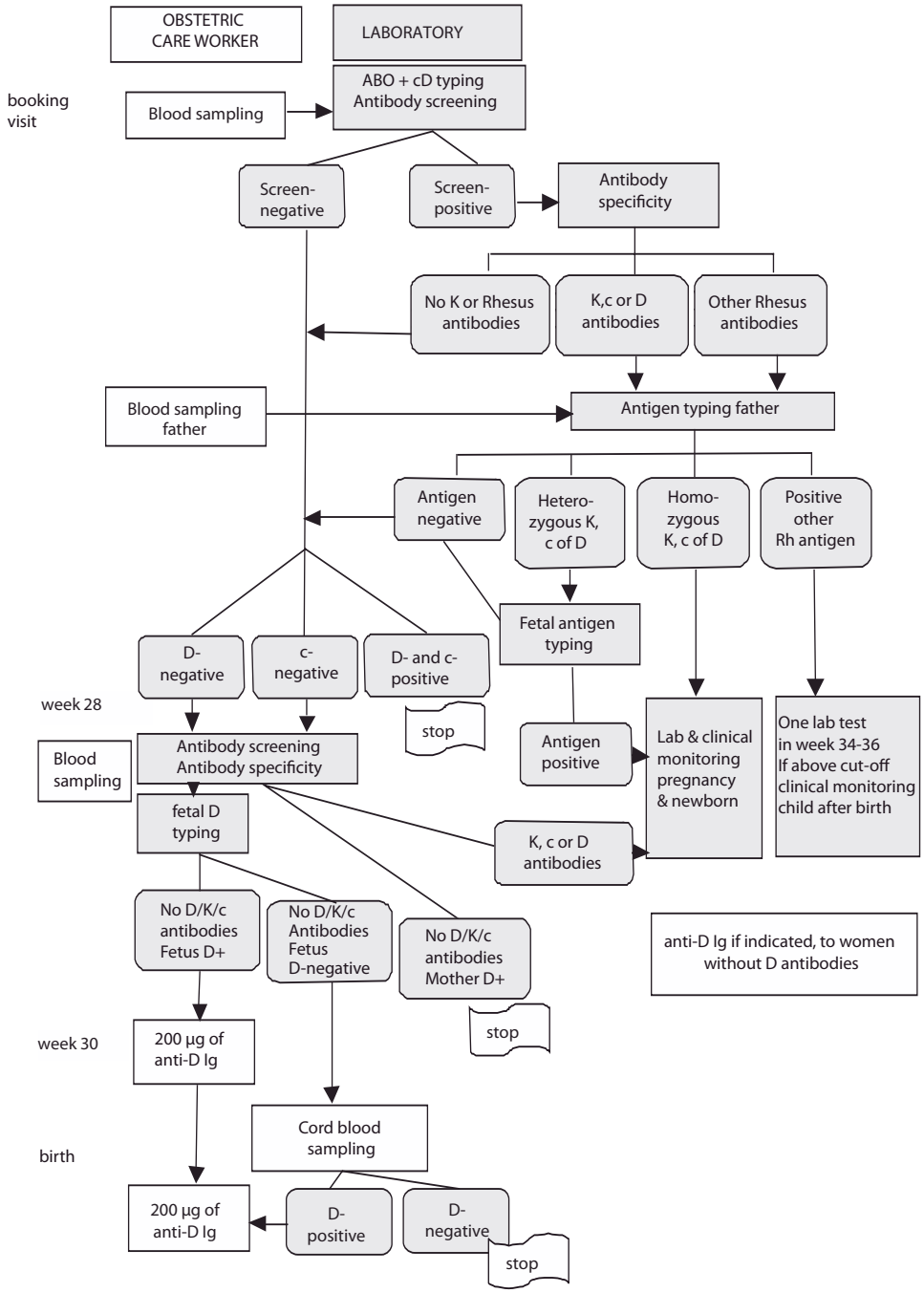
Recommendation 2:
Assisted vaginal delivery and a pregnancy-related RBC transfusion should be added to the list of risk factors for anti-D immunisation. Moreover existing guidelines on focussed prevention should be prescriptive.
In presence of one or more of these risk factors, a standard extra dosage of anti-D Ig should be administered ór testing for FMH has to be performed, followed by adjusted anti-D Ig

prophylaxis. Consistent focussed prevention has superior effectiveness and efficiency, if e.g. compared to the current extension of the programme to all pregnant D-negative women.

Recommendation 3:
Administration of a split dose of anti-D Ig in week 28 and week 34 to prevent immunisation in postmature pregnancies, is not recommended yet.
Although theoretically this measure can reduce the immunisation risk in postmature pregnancies, its effect is still unknown and might be small while implementation would be demanding.

Recommendation 4:
Screening for fetal D-antigen by PCR tests in maternal plasma should be implemented unless costs are prohibitive
This technique was not investigated in the OPZI study, but the implementation of fetal D screening is an indissoluble part of a revised prevention programme for maternal alloimmunisation.
Facilities for routine screening of all D-negative pregnant women for the fetal D antigen in maternal plasma are available.This screening saves 40% of anti-D Ig in women who are pregnant of a D-negative child. The costs of this screening test are in balance with the saved costs of anti-D Ig.[111]

Figure 9.3 Proposal revised national prevention programme maternal alloimmunisation



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