Detection and prevention of pregnancy immunisation : the OPZI study
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One single dose of 200 μg of antenatal RhIG halves the risk of anti-D immunisation and haemolytic disease of the fetus and newborn in the next pregnancy

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Chapter 7

ABSTRACT

Background: Objective was the evaluation of the effect of the Dutch national routine antenatal RhIG (anti-D) immunisation prevention (RAADP) programme comprising one single dose of 200 μg (1,000 IU) of RhIG in the 30th week of pregnancy, restricted to women without a living child.

Study design and methods: A nation-wide historical control study was performed. All newly detected anti-D-immunised parae-1 in 1999, 2002 and 2004 were included and classified on the basis of received prophylaxis during the first pregnancy: antenatal and postnatal versus only postnatal RhIG. The numbers of D-negative parae-1 who delivered a D-positive first child before the introduction (control group), or after the introduction (intervention group) of the RAADP were calculated from Vital Birth Statistics (8,700 and 12,000, respectively).

Results: Fifty-eight newly detected anti-D immunisations in the first trimester were observed in the control group and 39 in the intervention group, which resulted in a significant reduction of the prevalence of new anti-D immunisations from 0.67% (95%-CI: 0.50-0.84%) to 0.31% (95%-CI: 0.21-0.41%). No reduction was observed in anti-D immunisations newly detected at the 30th-week screening (0.25%). A non-significant risk reduction of the risk of severe haemolytic disease of the fetus and newborn (HDFN) was found (0.23% versus 0.10%). The numbers needed to treat to prevent one anti-D-immunised pregnancy and one case of subsequent severe HDFN were 357 and 1,255, respectively.

Conclusions: RAADP of one single dose of 200 μg of RhIG in addition to postnatal RhIG (200 μg) halves the risk of anti-D immunisation and subsequent severe HDFN.
INTRODUCTION

Haemolytic disease of the fetus and newborn (HDFN) has for long been a major specific cause of perinatal mortality and morbidity.\(^1\) In 1941, Levine elucidated the process of fetal and neonatal red cell destruction by maternal red cell antibodies (RBC), that bind to blood group antigens expressed by the RBCs of the fetus or neonate.\(^2\) In most cases of severe HDFN such antibodies are directed against the D antigen.\(^3,4\) Immunisation is triggered by fetomaternal haemorrhage (FMH), occurring during a prior pregnancy and/or upon the delivery of a D-positive child. Passively administered RhIG prevents the anti-D immune response of the mother upon exposure to D-positive fetal cells; the precise responsible mechanism is still unclear.\(^5\) In developed countries, routine postnatal anti-D prophylaxis was introduced since the 1960s, combined with additional RhIG administration in high-risk situations during pregnancy and delivery. A substantial decrease in the anti-D immunisation-rate and anti-D-related perinatal mortality was observed.\(^6\) In the Netherlands, the prevalence of new anti-D immunisations in pregnancy declined from 3.5% in 1969, at the start of this new strategy, to recently 0.5%.\(^7\) A further decrease of anti-D immunisations was anticipated by routine antenatal RhIG (anti-D) prophylaxis (RAADP), which was subject of several studies.\(^8-18\) These studies, however, 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. From these studies, including one quasi-experimental study,\(^15\) it can be concluded that a dosage of at least 2 x 100 μg (2x 500 IU) RhIG (in week 28 and week 34) reduces the risk of anti-D immunisation by 50-80%.\(^19,20\) RAADP is practised in several countries, nationally or in some parts of the country. However, reliable data regarding the percentage of immunisations after the introduction of RAADP, are not available, as well as data about which dosage is sufficient to achieve a substantial reduction of anti-D immunisations.\(^21\) Moreover, the effect of RAADP on the occurrence of HDFN is unknown.

The available evidence prompted the Dutch health care authorities to introduce antenatal prophylaxis in 1998 as a single dose of 200 μg (1,000 IU) in week 30, an administration schedule with a high procedural feasibility: only one extra administration of the same dose as was used postnatally, which would prevent mistakes between dosages.\(^22\) The choice of one dose of 200 μg RhIG carried a risk, since a single dose will result in lower circulating concentrations of RhIG as term approaches, than will the split dose as used in most previous studies.\(^23\) It was argued that in the studies of Bowman and coworkers, a single dose of 300 μg, administered in week 28, effectively prevented D immunisation.\(^8,9\) Moreover, it was calculated that administration of 200 μg in week 30, instead of week 28, was sufficient to provide adequate RhIG levels until the end of pregnancy.\(^22\) However, in
the study by Bowman and colleagues maternal RhIG concentrations were monitored and additional RhIG was given at around 36 weeks to those women in whom passive RhIG was no longer detectable. Monitoring of RhIG levels during pregnancy was not performed in the Netherlands.

In the Netherlands, prophylaxis is restricted to women without a living child, due to RhIG scarcity. In this article, this antenatal RhIG strategy is evaluated. To our knowledge, this is the first nation-wide study on the prevalence of anti-D immunisation after the introduction of antenatal immunoprophylaxis. The main objective of our study was to estimate the effect of the programme in terms of prevented immunisations and cases of severe HDFN.

**MATERIALS AND METHODS**

**National prevention programme**
The free of charge Dutch programme for prevention of pregnancy immunisation consists of a) ABO and D typing and screening for RBC antibodies as part of the booking visit protocol around the 12th week of pregnancy, b) repeat of this procedure around week 30 in D-negative women only, c) at week 30 antenatal administration of 1,000 IU of RhIG, restricted to non-immunised D-negative women without a living child and d) postnatal administration of 1,000 IU of RhIG to all D-negative women who delivered a D-positive child. The obstetric care worker (in the Netherlands: independent midwife, general practitioner or obstetrician) is responsible for the collection of maternal and cord blood and, if indicated, administration of RhIG. Certified Dutch laboratories (N=±90) perform the 12th-week blood testing and the D typing of cord blood red cells, according to existing national guidelines. In two reference laboratories, the antibody-specificity is determined after a positive 12th-week screening; the father is typed for the D antigen if D antibodies are detected; and a 30th-week screening is performed in non-immunised D-negative women. The programme is monitored by the National Vaccination Offices, unless the pregnant woman objects to registration (rarely the case).

The coverage of the prevention programme is almost 100%. Anti-D-immunised pregnancies are monitored by repeated laboratory testing (i.e. antibody titration and ADCC test) in two assigned reference laboratories. If the tests suggest a major risk for HDFN, women are referred to secondary care for clinical monitoring by ultrasound, Doppler flow measurement, non-invasive fetal RH D typing with maternal blood and to a lesser extent by amniocentesis (Liley-index). All intra uterine transfusions (IUTs) are given in one national tertiary care facility (Leiden University Medical Centre, Leiden).
Study design
We performed a historical control study, in which the effect of RAADP was established in pregnant parae-1 (± 37 % of all pregnancies \(^{39}\)), who gave birth to their first child shortly before (historical control group) or after (intervention group) the introduction of the RAADP. A comparison was made between parae-1 who received only postnatal prophylaxis after the birth of their first (D-positive) child and parae-1 who received antenatal and postnatal prophylaxis. The entire study was discussed with and approved by all relevant professional organisations (obstetricians, midwives, general practitioners, paediatricians, clinical laboratories). Representatives of these organisations monitored the study process. The professional organizations guided full implementation of these procedures during the study period.

Registration data are legally available for scientific research. Primary study data (routine laboratory results) were retrieved from existing registries; these data were completed by additional routine care data, obtained from the obstetric care workers. These study procedures did not require individual consent.

Participants and definitions
Cases were all D-negative pregnant parae-1 in the Netherlands with anti-D, newly detected in 1999, 2002 and 2004. (In the initial study design, we expected that cases identified in 1999 would all belong to the control group and cases identified in 2002 to the intervention group. Approximately half of the cases in 2002, however, turned out to have given birth to their first child before introduction of RAADP, hence to belong to the control group. To increase the intervention group, we expanded our intervention group and also included all cases detected in 2004). A newly detected case was defined as a pregnant woman who previously delivered a D-positive child under Dutch care after the 30th week of pregnancy and received postnatal RhoG, with an anti-D immunisation detected not earlier than after the (negative) 30th-week screening in the previous pregnancy and not later than the 30th-week screening in the current pregnancy. The number of pregnancies at risk of D immunisation (denominator) consisted of all pregnant D-negative parae-1 in 1999, 2002 and 2004 who previously gave birth to a D-positive child after the 30th week of pregnancy (N = ± 21,000).

Inclusion of a pregnant para-1 in the intervention group was defined by the administration of antenatal RhoG, additional to postnatal RhoG, in the previous pregnancy. RAADP was introduced in July 1998; taking into account the interval between the birth of a first and a second child, it could be estimated that of the total group at risk of D immunisation in 1999, 2002 and 2004 respectively 93%, 20% and 10% fell in the historical control group (calculations available on request). Severe HDFN was defined as perinatal mortality, the need for intra uterine transfusion and/or for exchange transfusion because
of anti-D immunisation.

**Data collection**

Primary data about all new anti-D immunisations were retrieved from the two reference laboratories. Based on follow-up test results, it was excluded that the detected antibodies were from administered RhIG. Additional data about the administration of postnatal and antenatal RhIG in the previous pregnancy were collected via the obstetric care workers by means of a questionnaire, as well as data on subsequent severe HDFN in newly immunised parae-1.

**Data analysis**

If no reliable data on postnatal prophylaxis could be obtained, for the calculation of prevalences, these women (N=13) were counted as having been pregnant of a D-positive child and having received postnatal prophylaxis only if: 1) the anti-D immunisation was detected at the 12th-week screening in the ongoing pregnancy (N=6) or 2) the anti-D immunisation was detected later in pregnancy, while the woman had been pregnant by an homozygously D-positive man (rhesus phenotype-based; N=4). Of the other cases (N=3) with unknown status of postnatal anti-D-prophylaxis 50% was counted as having received postnatal prophylaxis. The anti-D-immunised women with unknown status of antenatal prophylaxis (N=6), who delivered their first child after 15-9-1998 (hence, pregnancy duration ≥ 30 weeks at introduction of antenatal prophylaxis on July 1st 1998), were counted, for the calculation of prevalences, as having received antenatal prophylaxis. See Figure 7.1.

If the care worker could not provide data on HDFN (N=3) and the maximum ADCC test result was <= 20%, it was assumed for the prevalence calculation that no severe HDFN had occurred.\(^{28}\)

The denominators for the calculation of the prevalence of new immunisations and subsequent severe HDFN in the control (± 8,700) and the intervention group (± 12,000) were calculated from data from the Office of Vital Statistics (in Dutch: CBS \(^{31}\)); the CBS provides the number of deliveries and the birth interval between a first and a second child. The numbers were adjusted for miscarriages between screening and 24 weeks, the start of the birth statistics (3.2%), and unregistered pregnancies (2.0 %).\(^{32}\) The estimated D genotype frequencies in the Netherlands used were 38.5% DD, 46.2% Dd and 15.3% dd, implicating that 61.5% of D-negative women will carry a D-positive child.\(^{33}\) Calculations are available on request.

The following Numbers Needed to Treat (NNT) were calculated:

1) to prevent one anti-D immunisation and one case of subsequent severe HDFN in the next (second) pregnancy;
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**Figure 7.1** Numbers of Para-1 at risk of anti-D immunisation in screening in week 12 and week 30, and numbers of newly detected anti-D immunisations and subsequent occurrence of haemolytic disease of the fetus and newborn (1999, 2002 and 2004 combined), according to anti-D prophylaxis in the previous pregnancy.

<table>
<thead>
<tr>
<th>Anti-D prophylaxis in previous pregnancy</th>
<th>Para-1 to be screened in week 12</th>
<th>Para-1 to be screened in week 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postnatal prophylaxis</td>
<td>N = 8,645*</td>
<td>Postnatal prophylaxis</td>
</tr>
<tr>
<td>Post- &amp; antenatal prophylaxis</td>
<td>N = 12,576*</td>
<td>Post- &amp; antenatal prophylaxis</td>
</tr>
</tbody>
</table>

#### Detected anti-D immunisations in screening week 12

- N = 134

#### Detected anti-D immunisations in screening week 30†

- N = 64

<table>
<thead>
<tr>
<th>Valid new immunisations</th>
<th>Estimated for prevalence</th>
<th></th>
<th></th>
<th>At risk HDFN¶</th>
<th>HDFN**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postnatal prophylaxis</td>
<td>N = 14††</td>
<td>Postnatal prophylaxis</td>
<td>N = 12</td>
<td>Postnatal prophylaxis</td>
<td>N = 6</td>
</tr>
<tr>
<td>Post- &amp; antenatal prophylaxis</td>
<td>N = 39</td>
<td>Post- &amp; antenatal prophylaxis</td>
<td>N = 21.5</td>
<td>Post- &amp; antenatal prophylaxis</td>
<td>N = 29</td>
</tr>
<tr>
<td>N = 53+3§</td>
<td>N = 37+1+1§</td>
<td>N = 19+3§</td>
<td>N = 25+1+1+2§</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Prevalence

- **Postnatal prophylaxis**: N = 58
- **Post- & antenatal prophylaxis**: N = 39

#### Previous pregnancy/ delivery not in the Netherlands

- Mother erroneously typed D-positive in previous pregnancy
- Previous child typed D-negative
- First delivery <= 30 weeks

#### 2nd child abortion < 24 weeks

- 2nd child RhD-negative

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* Calculations available on request. The higher proportion of women with only postnatal prophylaxis in week 30, is because the first pregnancy in this group was six months earlier than in the group with ante- & postnatal prophylaxis.

† Detected screening week 30: negative screen result available earlier in pregnancy.

‡ In 3 of these 5 cases the D typing was unknown; the mothers did not receive postnatal RhIG for unknown reasons.

§ Bold: sure data; underlined: postnatal prophylaxis unknown; italic: antenatal prophylaxis unknown; italic/underlined: post- + antenatal prophylaxis unknown.

|| Women with unknown postnatal prophylaxis are regarded to have received prophylaxis if the anti-D antibodies were detected at 12th-week screening or if the husband was typed as homozygously D-positive; other patients are estimated as 0.5 in the prevalence calculation.

¶ The D-factor and therefore the risk status for HDFN was unknown in 3 cases, detected in week 12 (2 only postnatal, 1 post- and antenatal prophylaxis) and in 2 cases, detected in week 30 (both post- & antenatal prophylaxis).

** HDFN: perinatal mortality, intra uterine transfusion or exchange transfusion because of anti-D antibodies.

†† In one pregnancy with an ADCC test > 55%, the outcome of the child was unknown; counted as HDFN case.
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2) to prevent one anti-D immunisation and one case of subsequent severe HDFN in all later pregnancies, under the Dutch policy of RAADP only in the first pregnancy;

3) to prevent one anti-D immunisation and one case of subsequent severe HDFN in all later pregnancies, in a scenario of RAADP in all pregnancies.

The NNTs were calculated by dividing for (1) and (2) the number of D-negative parae-0 (45% of all pregnant women), and for (3) the number of all D-negative pregnant women, through the number of prevented cases of anti-D immunisation and of severe HDFN.

For the calculation of the numbers of prevented cases of anti-D immunisation and severe HDFN in all later pregnancies, it was assumed that the risk of anti-D immunisation and severe HDFN in later pregnancies after RAADP or after only postnatal anti-D prophylaxis is the same as we found in the pregnancy of the second child. Fifth and higher ranked children were included in the group of fourth children. According to Dutch birth statistics of 2006, 83.4% of women who have given birth to a first child will get a second child, 28.6% of them will get a third child and 11.3% of them will have a fourth child.

**RESULTS**

**Prevalence of new anti-D immunisations and severe HDFN**

To investigate the effect of the introduction of the RAADP programme on anti-D immunisation we performed a nation-wide study in three 1-year cohorts, shortly after this introduction in the Netherlands. Because of our centralized prevention programme it was possible to detect all anti-D immunisations that occurred in a total group of around 600,000 pregnancies in those three years. To precisely determine the effect on the immunisation risk only D-negative parae-1 that had been pregnant of a D-positive child in a previous pregnancy were included. This group consisted of approximately 21,000 women, 59% of these women belonged to the intervention group who received both postnatal and antenatal prophylaxis in their first pregnancy, and they were compared to the other 41% belonging to the control group, who received only postnatal prophylaxis. The numbers of new anti-D immunisations and subsequent severe HDFN, related to the numbers of pregnancies at risk of D immunisation, are shown in Figure 7.1.

New anti-D immunisations were detected in 198 parae-1. All women with a previous pregnancy outside the Netherlands were excluded, as well as those women who did not receive postnatal RhIG in their previous pregnancy because they were erroneously typed as D-positive or their child was typed as D-negative (see Figure 7.1). In total, new anti-D immunisations were detected in 149 women who previously gave birth to a D-positive child after the 30th week of pregnancy; 80 of them received only postnatal prophylaxis (control group) and 69 both postnatal and antenatal prophylaxis (intervention group).

The reduction of 12th-week anti-D immunisations in the intervention group was 54%
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(decrease from 0.67% to 0.31%; see Table 7.1). The prevalence of new anti-D immunisations, first detected upon routine second screening in week 30, did not differ between the intervention and the control group (0.25% and 0.24% respectively).

The prevalence of severe HDFN in the intervention group was 0.10% versus 0.23% in the control group, a non-significant risk reduction of 55% (see Table 7.1). No HDFN-related perinatal mortality was reported in either group; two pregnancies ended in a spontaneous abortion, one child in the control group died because of congenital malformations of the fetus (no signs of HDFN).

Once anti-D immunisation had occurred, the risk of developing severe HDFN was the same in the intervention and the control group (19% and 25% per immunisation respectively). However, in the subgroup in which immunisation was detected only in the 30th-week screening, the prevalence of severe HDFN was significantly decreased in the intervention group (1 out of 29) versus the control group (6 out of 21.5); p=0.020.

In the control group, nine of the 83 (11%) D-immunised women delivered their first child after 15-9-1998, hence, should have received antenatal RhIG prophylaxis. This indicates that the coverage of the antenatal prophylaxis programme was less than 100%.

Under the assumption of a 95% coverage of the RAADP during the study period, the risk reduction of first trimester anti-D immunisations was still significant: 0.33% in the intervention group versus 0.63% in controls (relative risk [RR] 0.52; 95% confidence interval [CI] 0.10 – 0.95); the prevalence of severe HDFN decreased from 0.22% to 0.11%. The minimum risk reduction was calculated after exclusion of the cases in which it was unknown whether postnatal prophylaxis was applied (N=13) and assignment of those in which antenatal prophylaxis was not ascertained, to the intervention group (N=6); the risk reduction was then still significant (RR 0.51; 95%-CI 0.09-0.92).

**Numbers Needed to Treat**
As stated above we have determined that RAADP decreases the risk of anti-D immunisation with 0.36% and the risk of severe HDFN with 0.13%. Because 61.5% of the women receive antenatal anti-D while pregnant from a D-positive child and 83.4% of the women will give birth to a second child, the NNT to prevent one anti-D immunisation in the 12th week of the next pregnancy is 523. The NNT to prevent one case of subsequent severe HDFN is 1,526. (see Table 7.2).

The effect of RAADP in the first pregnancy, however, is greater, because not only the second pregnancy but also possible subsequent pregnancies of a D-positive child will benefit from the prevented anti-D immunisation. If also the effect in later pregnancies was taken into account, the NNT to prevent one anti-D immunisation in week 12 is 357 (95%-CI: 196-1,978); the NNT to prevent one case of subsequent severe HDFN is 1,255 (95%-CI: 517-∞). If RAADP would be extended to all pregnant women, the NNTs of the programme
**Table 7.1 Prevalence of New anti-D Immunisations and Severe HDFN in Parae-1, According to anti-D-Phylaxis During First Pregnancy/Delivery (1999, 2002 and 2004)**

<table>
<thead>
<tr>
<th>Parae-1</th>
<th>Anti-D-prophylaxis in first pregnancy/delivery</th>
<th>New anti-D immunisation</th>
<th></th>
<th></th>
<th>Severe HDFN*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence per 100,000 D-negative parae-1, 1st child D-positive</td>
<td></td>
<td></td>
<td></td>
<td>Prevalence per 100 parae-1, new anti-D immunisation</td>
</tr>
<tr>
<td></td>
<td>Moment of detection anti-D antibodies</td>
<td></td>
<td></td>
<td></td>
<td>Moment of detection anti-D antibodies</td>
</tr>
<tr>
<td></td>
<td>1st screening 2nd screening 1st + 2nd screening 1st screening 2nd screening 1st + 2nd screening 1st screening 2nd screening 1st + 2nd screening</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>no (95%-CI) no (95%-CI) no (95%-CI) no (95%-CI) no (95%-CI) no (95%-CI) no (95%-CI) no (95%-CI) no (95%-CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only postnatal RhiG</td>
<td>671 (499-843) 244 (141-347) 915 (714-1,116) 162 (77-247) 68 (14-123) 230 (129-331) 24 (13-35) 28 (9-47) 25 (16-35)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ante- + postnatal RhiG</td>
<td>310 (213-407) 252 (160-343) 562 (431-692) 95 (41-149) 9 (0-26) 104 (48-160) 31 (16-45) 3 (0-10) 19 (10-28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative Risk ante-+ postnatal RhiG versus only postnatal</td>
<td>0.46 (0.09-0.84) 1.03 (0-2.18) 0.61 (0.22-1.01) 0.59 (0-1.50) 0.13 (0-0.68) 0.45 (0-1.08)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Perinatal mortality, intra uterine transfusion or exchange transfusion because of anti-D antibodies
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Table 7.2 Numbers needed to treat in different scenario’s of antenatal RhIG prophylaxis

<table>
<thead>
<tr>
<th>Numbers Needed to treat to prevent:</th>
<th>Scenario 1*</th>
<th>Scenario 2*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect only in next pregnancy</td>
<td>Effect in all later pregnancies</td>
</tr>
<tr>
<td>One anti-D immunisation in week 12 of later pregnancies</td>
<td>523†</td>
<td>357</td>
</tr>
<tr>
<td>One case of subsequent severe HDFN</td>
<td>1,526†</td>
<td>1,255</td>
</tr>
</tbody>
</table>

* Scenario 1: Antenatal anti-D prophylaxis restricted to D–negative women without a living child
Scenario 2: Antenatal anti-D prophylaxis to all D–negative pregnant women
† calculation (one immunisation): 100%/(83.4%/(1-3.2%) * 61.5% * 0.36%)
severe HDFN: 100%/(83.4%/(1-3.2%) * 61.5% * 0.13%)
Other calculations available on request

will increase, as is shown in Table 7.2.

Discussion

Until now reliable data about the effectiveness of RAADP at a population level were not available. In a large-scale population study we investigated whether the Dutch programme, comprising one single dose of 200 μg RhIG in week 30, leads to a substantial reduction of anti-D immunisation and of HDFN in later pregnancies. We established a significant reduction of anti-D immunisation, as detected at the first screening in the subsequent pregnancy. In D-negative women who previously gave birth to a D-positive child and received postnatal RhIG, the prevalence of new anti-D immunisations decreased from 1:150 to 1:330 if antenatal RhIG had been routinely administered in the previous pregnancy. The prevalence of severe HDFN declined from 1:450 to 1:1,000, a decrease which did not reach statistical significance (RR 0.45, 95%-CI 0-1.08).

The effectiveness of RAADP in our study is comparable with results from a much smaller-sized prospective study in two regions of the United Kingdom after introduction in one of these regions of antenatal prophylaxis to women without a living child of 2 x 100 μg of RhIG in week 28 and 34.18 This study observed a significant decline of new anti-D immunisations in the next pregnancy from 1:125 (26/3,146) to 1:250 (12/3,320), but did not provide data on the occurrence of subsequent HDFN. Furthermore, it is unclear whether only immunisations detected in the first trimester were included or also new immunisations later in pregnancy.

Other published studies, with different dosages of antenatal RhIG, observed risk reductions (defined shortly after delivery, six months after delivery or in the next pregnancy) in the range of 0.10 to 0.55. Higher risk reductions compared to our study


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(seven studies) were all due to a higher anti-D immunisation rate in the (historic) control group \(^8-12,14,17\) (ranging from 1.1 to 1.9%). These higher immunisation rates can be due to inclusion of controls who received possibly or surely \(^a\) no postnatal prophylaxis. In our study, we carefully collected all data of the individual patients on previous immunoprophylaxis via the obstetric care workers rather than relying on registry data. When we would have included anti-D-immunised parae-1 who probably did not receive postnatal prophylaxis after their previous delivery, this also would have resulted in a prevalence of 1.0% in the control group and a risk reduction to 29%. In the United Kingdom also the postnatal administration of only 100 μg of RhIG, compared to 200 μg in the Netherlands, might have contributed to a higher prevalence of anti-D immunisation in the group with only postnatal prophylaxis. Possibly the Kleihauer test, which is routinely done to adjust the dosage of RhIG if necessary, is not able to recognize all relevant FMH or not always performed.\(^21\)

The failures of RAADP might partly be due to failure of prophylaxis in case of large FMH as we have shown that women with assisted delivery have a higher risk to develop anti-D immunisation (submitted manuscript). On the other hand, we also found postmaturity to be a risk factor. It can therefore not be excluded that a further risk reduction might be accomplished with schedules in which higher titres of RhIG are reached. The immunisation rate in the intervention group is lower (0.1%), compared to our results, in one study using 2 x 300 μg or 1 x 300 μg of RhIG \(^8\) and in two much smaller studies, using 2 x 100 μg (1/599) \(^13\) and 1 x 300 μg RhIG (0/291).\(^14\) Overall, the prevalence of anti-D immunisation in all studies, using at least 2 x 100 μg of RhIG, ranged from 0.0 to 0.9%, that is comparable to our results. Although not significant, the parallel decrease of severe HDFN most likely suggests an equally-sized benefit in terms of the most relevant clinical outcome measure.

In our study, we observed that in cases in which antenatal and postnatal prophylaxis has failed, the anti-D antibodies were more often (\(p=0.053\)) detected later in the next pregnancy (intervention group: 29/68=43% in 30th-week screening) than in cases in which only postnatal prophylaxis was given (control group: 21.5/79.5=28%). Furthermore, the risk of severe HDFN in newborns from anti-D-immunised women when the antibodies were detected in the 30th-week screening, was significantly lower in the intervention group than in the control group (3% versus 28%; \(p=0.020\)). These data suggests that, at least in a subgroup of patients, the addition of antenatal prophylaxis has a long lasting suppressive effect on the strength of the immune response. A similar observation has been made after the introduction of postnatal prophylaxis. In women in whom this prophylaxis failed, the levels of anti-D antibodies and the severity of HDFN in subsequent pregnancies were less than in cases in which no prophylaxis was applied.\(^14\)

The nation-wide design with full collaboration of obstetric care workers and laboratories and the centralization of the screening programme, allowed for an unbiased
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estimate of the principal parameters. It should be noted that, because all women were followed from the 12th week of pregnancy also fetal loss due to HDFN would have been recognized. Some caveats can be recognized, however: 1) Because of the study design, the second and first pregnancies in the control group occurred at a date some years earlier than those in the intervention group, which could have introduced time-related bias. There is no indication, however, that in this relatively short time period there were major changes in prevention policy (e.g. guidelines for use of RhIG in high-risk situations, in techniques of laboratory testing or in obstetric policy, especially with regard to the number of assisted deliveries), or in the population of pregnant women in the Netherlands. We excluded anti-D-immunised women who had their first pregnancy/delivery not under Dutch care because of uncertainty about the administration of postnatal prophylaxis. 2) It could not always be ascertained whether RhIG was administered in the previous pregnancy. A recent data-based procedural evaluation of the Dutch prevention programme, however, showed a very high compliance, which can also be concluded from the rise in delivered ampoules of RhIG (figures based on deliveries by Sanquin Products, until 2004 the only provider of RhIG). If cases with unknown antenatal RhIG status who delivered after Sept 15 1998, were misclassified as having received antenatal prophylaxis, the effectiveness of RAADP was under-estimated. On the other hand, if the actual number at risk of D immunisation in the intervention group was lower than calculated, the effect was over-estimated. We calculated that even with a minimal coverage of 95%, the risk reduction was significant.

The NNTs we calculated for a scenario with RAADP in all pregnancies, show that the numbers of ‘extra’ prevented anti-D immunisations and severe HDFN are much smaller than in the Dutch scenario with RAADP restricted to women without a living child. For example, in the Netherlands, in one year two or three ‘extra’ cases of severe HDFN would be prevented by administration of 15,000 extra ampoules of RhIG. RhIG is a relatively scarce blood product, therefore the surplus value of routine administration to all D-negative pregnant women is disputable. However, in this discussion it should also be taken into account that the NNTs can be decreased by 40% by implementation of routine fetal D typing in maternal plasma.

In conclusion, antenatal RhIG prophylaxis with one single dose of 200 μg RhIG halves the risk of anti-D immunisation and probably also severe HDFN in the next pregnancy.

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Chapter 7

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