# Future options to reduce RhD immunization in addition to a high coverage prevention program of antenatal and postnatal RhIg: a nationwide cohort study.

##### Future options to further reduce RhD immunization

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## Abstract

**Objective:** To evaluate which risk factors for RhD immunization remain, despite adequate routine antenatal and postnatal RhIg prophylaxis (1000 IU RhIg) and additional administration of RhIg. Assessment of the prevalence of RhD immunizations.

**Design:** Prospective cohort

**Setting:** The Netherlands.

Population: Two-year nationwide cohort.

**Methods:** RhD-negative women in their first RhD immunized pregnancy and their foregoing non-immunized pregnancy. Risk factors for RhD immunization were compared with population data.

Main outcomes measures: Risk factors for FMH and subsequently RhD immunization, prevalence of RhD immunizations.

**Results:** The prevalence of newly detected RhD immunizations was 0.31% (79/25,170) of all RhD-negative pregnant women in the Netherlands. After exclusion, 193 women remained. Significant risk factors found in the group of 113 parous women (previous pregnancy >16 weeks, RhD positive child) were; caesarean section (CS) (OR 1.7, 95% CI 1.1-2.6), perinatal death (OR 3.5, 95% CI 1.1-10.9), gestational age over 42 weeks (OR 6.1, 95% CI 2.2-16.6), postnatal bleeding (>1000mL) (OR 2.0 95% CI 1.1-3.6), surgical removal of the placenta (SRP) (OR 4.3, 95% CI 2.0-9.3). The miscarriage rate in the group of women without a previous RhD positive child was significantly higher than in the Dutch population (35% vs 12.5% p<0.001).

**Conclusion:** Complicated deliveries, including cases of major bleeding and surgical interventions (CS, SRP) need to be recognized as risk factor, requiring determination of FMH volume and adjustment of RhIg dosing. Miscarriage may be an additional risk factor for RhD immunization, requiring further studies.

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In high-income countries, the incidence of RhD immunization has decreased after implementing routine antenatal and postnatal Rh immunoglobulin prophylaxis (RhIg), combined with administration of RhIg in conditions at risk for foetomaternal haemorrhage (FMH).1-3 Subsequently, fewer babies suffer from haemolytic disease of the foetus and newborn (HDFN).4, 5 Still, RhD immunization occurs in RhD-negative women pregnant of an RhD-positive child with a risk of 0.3 to 1.3% , depending on the used prevention program and the adherence to this. 6-9 RhD immunization has a 30% risk of severe disease of the (unborn) child. 10, 11

Since blood transfusions are routinely RhD matched for decades, the main cause of RhD immunization is exposure to RhD-positive red blood cells (RBC) from the foetus, due to FMH during pregnancy or around delivery. 12 Small amounts of FMH can already lead to alloimmunization.13 FMH in small amounts occurs frequently during pregnancy (44% in third trimester and 64% after delivery).14 A major FMH (> 5 ml of foetal cells) occurs less frequently, with an estimated range of 0.1-6% of pregnancies.14-18 If there is a risk for a major FMH, administration of extra RhIg is often indicated in guidelines.1-3 However, the significance of possible risk factors for a major FMH, such as mode of delivery, abortion/miscarriage (spontaneous or instrumental), invasive prenatal diagnosis, external cephalic version, abdominal trauma and antenatal bleeding, is still controversial. 15, 16, 19, 20 In our previous study, non-spontaneous delivery (caesarean section or assisted delivery), post-maturity and a younger age at the previous delivery emerged as risk factors for alloimmunization. 20

In this study, we evaluated in a prospectively collected cohort which risk factors for RhD immunization remain, despite adequate routine antenatal and postnatal RhIg prophylaxis (1000 IU RhIg) and, if indicated, additional administration of RhIg, as based on a guideline from the Dutch organization of obstetricians. Since 2011, routine RhIg administration is based on foetal RhD typing.

## Methods

### Setting

In the Netherlands, all pregnant women are typed for ABO, RhD and Rhc blood group antigens and they are screened for the presence of alloantibodies against RBCs in the first trimester of pregnancy. RhD- and Rhc-negative women are screened again in week 27. The coverage of this screening program, monitored annually, is almost 100%.21 Following Dutch guidelines, RhIg is given at 30 weeks of gestation and after birth in case of an RhD-positive foetus and also after spontaneous abortion (>10 weeks) or when curettage is necessary. An extra dose of RhIg is given, after invasive prenatal testing or external cephalic version and, after estimating FMH with a Kleihauer Betke test (KBT), in case of abdominal trauma or antenatal bleeding after 16 weeks. At delivery, a KBT test should be performed, in order to adjust the RhIg dosage when a large FMH is suspected. All maternal blood samples with a positive screening result, identified at routine screening or at any other moment in pregnancy, are sent to one of the two national reference laboratories (Sanquin Diagnostic Services (90% of all tests) or, for the north-eastern part of the Netherlands, to the laboratory of the University Medical Center Groningen (UMCG).22, 23 The specialized testing of the foetal RhD type and the antibody-dependent cell-mediated cytotoxicity (ADCC), to determine the biological activity of RBC antibodies, is centralized at Sanquin Diagnostic Services.24

#### Study design and population

This study was part of the OPZI 2.0 study, a nationwide cohort study on RhD immunization in pregnancy. 25 All pregnant women with a positive screening for anti-D, identified at Sanquin Diagnostic Services during our study period, were eligible for inclusion. Positive screenings as a result of RhIg administration (information of obstetric care provider (OCP)) were not included. Women were identified during 28 months from two time periods (for practical reasons): from July 1, 2014 to March 31, 2015 and from August 1, 2015 to February 28, 2017.

The local OCP of eligible pregnant women was contacted, in order to obtain patient’s informed consent. If written consent was obtained, clinical data were collected from the OCP by means of a detailed questionnaire. If data were incomplete, the researchers made up to three attempts to contact OCP or study participants directly, in order to complete the data set. Furthermore, if it was unclear whether women received RhIg in a previous pregnancy, this information was obtained from the Department for Vaccine Supply and Prevention Programs (RIVM-DVP).

#### Data collection and outcome definitions

Maternal characteristics (age, weight, moment of antibody detection, pre-pregnancy blood transfusions) and relevant clinical data from allprevious non-immunized and immunized pregnancies were collected in the OPZI 2.0 database. Data on all RhIg administrations and possible sensitizing or boosting events during pregnancy (antenatal bleeding, abdominal trauma, invasive prenatal diagnosis, external cephalic version, twins, post-maturity) and delivery (twins, post-maturity, postnatal bleeding>1000 ml, perinatal death, caesarean section, surgical removal of placenta, assisted birth and pregnancy related RBC transfusion), were collected. Miscarriages preceding the current ongoing pregnancy were considered as possible sensitizing events.

To test the potential risk factors for RhD immunization, occurring despite antenatal and postnatal RhIg administration, we selected all women in their first RhD-immunized pregnancy. We excluded women with a prior delivery of an RhD-positive child who did not receive the complete RhIg prophylaxis at 30 weeks gestation and/or after giving birth. Women who received the complete RhIg prophylaxis (if delivered before 30 weeks only postnatally) in a previous pregnancy, while the RhD type of the child was unknown, were considered as exposed to the RhD antigen. We tested the potential risk factors in the following groups: the first group ‘exposed to the RhD antigen’ contains the women with a previous pregnancy (> 16 weeks) of an RhD-positive child; the second group ‘possibly exposed to the RhD antigen’ contains the women who had a previous miscarriage (< 16 weeks) without a prior pregnancy of an RhD-positive child; the third group ‘non-exposed to the RhD-antigen’ contains the women who had neither a previous pregnancy of an RhD-positive child nor a miscarriage. Risk factors related to the previous pregnancy and delivery were only analyzed in the ‘RhD exposed’ group of multiparous women. Risk factors related to the current pregnancy were analyzed in the combined group of ‘possibly’ and ‘non-exposed’ pregnancies. The prevalence of potential risk factors for RhD immunization were compared with the best available population data. These data were derived from the Dutch perinatal registration (Perined) or, when data were not available, from other nationwide studies performed in the same period. If data concerned potential risk factors occurring in previous pregnancies, only population data from women who had a previous pregnancy (>16 weeks) were used for comparison.

To assess the current prevalence of both newly detected and already existing RhD immunizations, we used data from the year 2016, that were collected in the OPZI 2.0 cohort. The denominators to assess the prevalence of RhD immunizations were derived from the monitor of the National Institute of Public Health and Environment of 2016. 26

#### Statistical analysis

The associations between potential risk factors and the occurrence of RhD-alloimmunization were described as Odds Ratios and 95% confidence intervals (categorical variables) or as mean difference and 95% confidence intervals around the mean difference (normally distributed continuous variables) according to Altman, 1991.27 All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) 26.0 and medcalc.org (https://www.medcalc.org/calc/odds\_ratio.php).

## Results

#### Prevalence of RhD immunization

The prevalence of newly detected RhD immunizations in 2016 was 0.31% (79/25,170) of all RhD-negative pregnant women in the Netherlands. Pregnancies likely immunized before immigration to the Netherlands were not included (N=15). The prevalence of all RhD immunizations (including immigrants) in 2016 was 0.09% of all pregnant women (158/171,727) and 0.63% of RhD-negative pregnant women in the Netherlands. For both the occurrence of new and of all RhD immunizations, approximately two-thirds was first detected at first trimester screening (0.18% and 0.06% respectively) and one third at third trimester screening (0.13% and 0.03% respectively). Only two cases were already detected before pregnancy.

#### Selection of the study population

During the study period, 304 RhD-immunized pregnant women were eligible for inclusion in the OPZI 2.0 study. Figure 1 shows the selection and the composition of our study population, used for the analysis of risk factors for RhD-immunization despite RhIg prophylaxis. After exclusion, 193 women remained, 65 of whom were nulliparous (34%) and 128 multiparous (66%). In the group ’exposed to the RhD antigen’, there were 113 multiparous women with a preceding pregnancy >16 weeks of an RhD-positive child. In the group ‘non-exposed to the RhD-antigen’, there were 40 primigravid women and 12 multiparous women with a previous pregnancy of an RhD-negative child. Three multiparous women with a miscarriage in between the current RhD-immunized pregnancy and a foregoing pregnancy of an RhD-negative child and 25 nulliparous women with a prior miscarriage were classified as ‘possibly exposed’. Only one woman carried an RhD variant (in the ‘possibly exposed group’) and she had not received previous transfusions Additional RBC antibodies were found in 53 (27.5%) women; the most common antibodies were anti-RhC (19.7%) and anti-RhE (3.1%) (Table S1).

### General risk factors for RhD immunization

When compared with the Dutch pregnant population, multiparous women were significantly overrepresented in our study group (66% vs 55.3% P=0.002), but still a large number of women were in their first ongoing pregnancy (Table 1, details population rates Table S2). The overall miscarriage rate in RhD-immunized women appeared to be significantly higher than in the general Dutch population (21% vs 12.5% p<0.001). A total of 40 women had a miscarriage preceding the RhD-immunized pregnancy (25 nulliparous and 15 multiparous women). Compliance to the national RhD prophylaxis program appeared to be poor, as 11/16 (69%) women who had a miscarriage past 10 weeks gestation or a curettage, incorrectly received no RhIg (Table S3).

More than half of the RhD immunizations (n=100) were detected in the first trimester of pregnancy and 91 cases (47%) were detected after 20 weeks or around delivery, most of these at the routine 27 weeks screening (table 1). First detection of anti-D at 27 weeks screening concerned 31 women from the ‘non exposed group’, 41 women from the ‘exposed group’ and 12 women from the ‘possibly exposed’ group. Two cases were first detected before the current pregnancy at a pre-operative screening or before blood transfusion.

### Risk factors for RhD immunization in previously RhD-exposed women

As shown in table 2, caesarean section, surgical removal of the placenta, post-partum bleeding >1000 mL, gestational age over 42 weeks and perinatal death in history were significant risk factors for RhD immunization in the ‘exposed’ group, when compared with the reference population (p<0.05). One third (37/113, 33%) of all ‘exposed’ women experienced none of the analyzed risk factors in the previous pregnancy. In 61% of these cases, anti-D was already detected in the first trimester of pregnancy. Apart from a previous pregnancy with an RhD-positive foetus, 12 women of the RhD- exposed group (10,6%) had an additional miscarriage in between the previous and the current pregnancy (details in supplemental table 3). The miscarriage rate in the exposed group is not different from that of the population rate of 12.5%.28

For comparing data on the incidence of antenatal bleeding before 16 weeks, we only found a prospective cohort study, including all women who received prenatal care in two US general hospitals.29 Data on lifestyle habits and complications, including antenatal bleeding, were obtained by structured questionnaire. Early antenatal bleeding was reported by over 20% of the pregnant women. Using this study as a reference, antenatal bleeding before 16 weeks appeared to be no additional risk factor for RhD immunization. For antenatal bleeding after 16 weeks, we used the Dutch perinatal registration data.30 None of the risk factors that are currently regarded as indication to administer (extra) RhIg prophylaxis, e.g. abdominal trauma, antenatal bleeding after 16 weeks and cephalic version, occurred more frequently in women of the ‘exposed group’.

Combined parturition-related risk factors

Some of the parturition-related risk factors appeared to overlap, suggesting a cascade of events, of which one, or several, are enhancing the immunization risk. Figure 2 shows how the observed and univariate significant risk factors interrelate. Post-partum bleeding > 1000 mL occurred in 8 out of 12 (67%) pregnancies in combination with other risk factors, most often with surgical removal of the placenta. Delivery from 42 weeks onwards was only once an isolated risk factor. Caesarean section was an isolated risk factor in 30 out of 32 (94%) pregnancies. Perinatal death (n=3) was in one case combined with postnatal large bleeding (not shown in figure 2).

#### Risk factors for RhD immunization in ‘non-exposed’ or ‘possibly RhD-exposed’ women

In the combined group of ‘non-exposed’ and ‘possibly exposed’ women (n=80) we analyzed possible sensitizing moments, occurring before or during the current pregnancy, but before anti-D was first detected (Table 3). Twenty-eight women (35%) had a miscarriage preceding the current pregnancy in which anti-D was first detected, whereas the population rate of miscarriage is only 10-15% (OR 4.3; 95% CI 2.7-6.8). In half of the women with a miscarriage in their history, anti-D was not identified until the third trimester of the subsequent pregnancy with an RhD-positive child (supplemental table 3). There was only one woman with a miscarriage in her history who had an additional incident (antenatal bleeding <16 weeks) during the current pregnancy, before anti-D was detected in the third trimester. Antenatal bleeding before 16 weeks appeared to be no risk factor for RhD immunization, this is comparable with the “exposed” group. Twenty percent of women (16/80) reported blood transfusion in history not related to the pregnancy . There are no comparable population data on incidence of non-pregnancy related blood transfusions in the history of women of fertile age. The women with a blood transfusion in history in our study did not have additional RBC alloantibodies.

## Discussion

### Main findings

This study describes significant risk factors for RhD immunization, that remain despite the use of antenatal and postnatal RhIg. The prevalence of both newly detected and of all RhD-immunizations in RhD-negative pregnant women has nowadays reached unprecedented low percentages of 0.31% and 0.63% respectively. This is in line with previously reported figures of large studies. 31-33 With a frequency of 15% of RhD-negative women, RhD immunization now concerns only 0.09% of all pregnant women in the Netherlands. Half of the RhD immunizations were detected in the first trimester of pregnancy.

Caesarean section was the main risk factor for RhD immunization in women with a previous pregnancy of an RhD-positive child, despite RhIg prophylaxis was given, confirming our earlier study.20 Other risk factors pointing to a possible large FMH were: surgical removal of placenta, post-maturity (≥ 42 weeks), postnatal bleeding (>1000mL) and perinatal death, but these often occurred in combination.

The miscarriage rate in our study was significantly higher than that in the Dutch population (21% vs 10-12.5% p<0.001). This finding can be fully attributed to the excessively high miscarriage rate (35%) in the group of women lacking a previous pregnancy with an RhD positive child. In the so-called ‘exposed’ group the miscarriage rate was not significantly different from the population rate.

### Strengths and limitations

This is the largest study on risk factors for RhD immunization in a pregnant population, participating in a high-coverage RhD immunization prevention program. A strength of our study is that we were able to collect national data on all RhD-immunized women and their previous non-immunized and immunized pregnancies, covering a 2-year period. This created the opportunity to evaluate all potential obstetrical and non-obstetrical incidents that may induce RhD immunization.

A limitation in the design of this study is that we could not include a control group. Our design therefore only allowed univariate analysis, comparing our data with published data in other populations or Dutch national data, available via open access. However, the current data set substantiates the outcome of our previous prospective study on risk factors in a smaller but more defined group of primigravidae, in which a control group was included.20 In our view, the present data can therefore be used to fine-tune the advice for an additional RhIg dose, in case risk factors for FMH in pregnancy occur.20

### Interpretation

In our study, we found caesarean section to be a significant univariate risk factor for RhD immunization, having almost no interrelations with other events potentially increasing FMH. Current Dutch guidelines recommend to estimate the volume of FMH by performing a KBT after caesarean section and, depending on the results, to increase the RhIg dose.1-3 In only a few countries, a KBT is routinely performed around delivery or upon risk factors related to increased FMH.34 Our study shows that a caesarean section has a strong association with RhD immunization, which is in line with other studies 15-17, 19, 20, 29 Therefore, we advocate to make FMH testing mandatory for every RhD-negative woman having a caesarean section, to enable timely adjustment of RhIg dosing, or alternatively to double the dose of RhIg (2x1,000 IU), in settings were FMH testing is not available.

A history of post-maturity (gestational age ≥42 weeks), was only present in four cases and in all but one an additional risk factor for FMH was present. These findings therefore may not point to a failure of antenatal RhIg prophylaxis, but more to events related to a complicated delivery. In current obstetrical practice in developed countries, post-maturity past 42 weeks has become rare, as most pregnancies are nowadays induced before or around 41 weeks. 35 In this context, adjustment of RhD-prophylaxis in post term pregnancies seems not to be indicated.

Surgical removal of the placenta (SRP) and postnatal bleeding (> 1000 mL) in the preceding pregnancy with an RhD-positive child were significant risk factors for RhD immunization. As may be expected, both events were strongly related (bleeding >1000 mL occurred in 5/7 cases of SRP). Postnatal excessive bleeding will always be a sign of a more complex delivery with an additional risk of a larger FMH, increasing the risk of alloimmunization in RhD-negative women. Therefore, in these cases and in accordance with the recommendation after a caesarean section, estimation of FMH volume and adjustment of RhIg dosing may best be routinely performed.

Perinatal death appeared in our study to be associated with a higher risk of RhD immunization. This finding supports the current policy to routinely perform a KBT in the work-up of perinatal death cases.

Surprisingly, the miscarriage rate in our study was high, 35% in the combined non-exposed and possibly exposed group. In our study the average maternal age at the foregoing pregnancy without RhD-immunization was 27 years. The expected miscarriage rate in the age group between 25-30 years is around 12%, but in the non-exposed and possibly exposed groups, we found almost tripled miscarriage rates.28 In this group, there were no other known risk factors which may explain the RhD immunization. Our results suggest that miscarriage may be a potential sensitizing event in RhD-incompatible pregnancies, independent of RhIg administration and gestational age. Several authors suggested that, although there is only a small amount of fetal blood cells in the maternal circulation, it may be sufficient to induce primary immunization, which may at first be undetectable by insensitive laboratory tests.13, 36, 37 This may well explain the fact that half of the RhD immunizations after miscarriage were first detected in the third trimester of the next pregnancy. Hypothetically, FMH in the current pregnancy may serve as a boosting event after immunization due to a previous miscarriage, or alternatively be the cause of primary immunization. Further studies are needed to explore the role of miscarriage as a primary sensitizing event in RhD immunization, including the effectiveness of RhIg in preventing immunization after all spontaneous or induced (including instrumental) abortions. This policy is in accordance with current international guidelines. 2, 3 Our observations underscore the poor adherence to current guidelines regarding RhD prophylaxis in cases of miscarriage or abortion.

Overall, we did not find evidence that potential antenatal risk factors for FMH in the current pregnancy were associated with RhD immunization. These events (e.g. invasive diagnostic procedures, twin pregnancies, antenatal bleeding and abdominal trauma) are relatively rare and probably, the compliance with the national prevention program and with the guidelines has contributed to this.1-3 In case of antenatal bleeding in pregnancies before 16 weeks, extra RhIg is currently not recommended, and based on our findings, we would not advise to change this policy.

## Conclusion

We advocate to be strict in the policy of recognizing risk factors, determination of FMH volume and adjustment of RhIg dosing, especially in pregnancies with complicated deliveries, including cases of major bleeding and surgical interventions, such as caesarean section and surgical removal of the placenta. Finally, our data suggest that miscarriage may be an additional risk factor for RhD immunization, requiring further studies, in order to reconsider the current RhIg policy. For future research, we recommend to critically and prospectively evaluate any adjustments to the RhD immunization prevention program made.

## Declaration

### Ethical considerations

The Medical Ethics Committee of the Leiden University Medical Center approved the protocol (P15.101/NV/nv) at April 28th 2015. Written informed consent was obtained from all women included in this study.

### Disclosure of interests

None declared. Completed disclosure of interests form available to view online as supporting information.

### Contribution to authorship

All authors were involved in designing the study. YMS and CZ carried out the collection and extraction of the data. YMS carried out the analysis and interpretation of the data, drafted the article and is responsible for the integrity of the work as a whole. JMK, ILvK and MdH advised on the interpretation of the data, revised the article critically for intellectual content and approved the final draft for publication. CZ, DO and EvdS assisted with the interpretation of the data, revised the article critically for intellectual content and approved the final draft for publication.

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Table 1 Baseline characteristics of 193 RhD-immunized pregnant women.

|  |  |  |
| --- | --- | --- |
|  | Cases | Population prevalence |
|  | N(%) | N(%) |
| Maternal age at delivery before the immunized pregnancy (y)# (N=113) | 27.4 (4.0) | 29.5 (4.5) |
| Pre-pregnancy weight (kg) (n=155)1# | 71.2 (13.5) | 70.4 (12.6) |
| Blood transfusion in history | 32 (16.5) | - |
| Nulliparous | 65 (34) | 44.7 |
| Multiparous | 128 (66) | 55.3 |
| Miscarriage2@ | 40 (20.7) | 12.5 |
| Moment of detection RhD-antibodies |  |  |
| Early first trimester screening$ | 102 |  |
| First screening 20th- 27th week | 3 |  |
| Routine third trimester (27th week) screening | 84 |  |
| Around delivery | 4 |  |
| Before current pregnancy | 2 |  |
| Variables with other comparable evidence than the Dutch perinatal registration: 1Pre-pregnancy weight, Bakker et al 2011, Miscarriage, 2Dutch general practitioner’s guideline “Miscarriage”, for comparison mean of 10-15% miscarriage rate used.28, 38  #Mean (SD) and mean difference (95% CI)  @Nulliparous or multiparous with one or more miscarriages before immunized pregnancy  Number of women delivered in the Netherlands in 2015 is 166.733 and 73,121 nulliparous. | | |

Table 2 Potential risk factors for RhD immunization in multiparous women exposed to the RhD-antigen in previous pregnancy >16 weeks.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Prevalence** | | | | | | | |
| Risk factors | | | Cases (N=113) | Population prevalence | Odds ratio 95%CI | P-value | |
|  |  | | N (%) | % |  | | |
| Risk factors around previous delivery, ongoing pregnancies above 16 weeks | | | | | | | |
| Caesarean section | | 32 (28.3) | | 18.7 | 1.7 (1.1-2.6) | | 0.009 |
| Assisted birth | | 18 (16) | | 16.4 | 1.0 (0.6-1.6) | | 0.89 |
| Surgical removal placenta | | 7 (6.1) | | 1.5 | 4.3 (2.0-9.3) | | <0.001 |
| Twins | | 3 (2.7) | | 1.1 | 2.4 (0.8-7.7) | | 0.13 |
| Gestational age >=41 weeks | | 21 (19) | | 14.5 | 1.3 (0.8-2.2) | | 0.22 |
| Gestational age >=42 weeks | | 4 (3.5) | | 0.6 | 6.1 (2.2-16.6) | | <0.001 |
| Perinatal death | | 3 (2.7) | | 0.78 | 3.5 (1.1-10.9) | | 0.03 |
| Postnatal bleeding >1000 ml1 | | 12 (10.6) | | 5.9 | 2.0 (1.1-3.6) | | 0.02 |
| Blood transfusion2 | | 8 (7.1) | | 3.9 | 1.9 (0.95-4.0) | | 0.07 |
| Male gender (N=103) | | 62 (60) | | 51 | 1.4 (0.98-2.2) | | 0.07 |
| External cephalic version6# | | 5 (4.4) | | 2.4 | 1.9 (0.76-4.61) | | NS |
| Risk factors during current pregnancy, before detection of RhD immunization | | | | | | | |
| Invasive prenatal testing3 | | 1 (0.9) | | 1.68 | 0.52 (0.07-3.75) | | NS |
| Antenatal bleeding <16 weeks4 | | 7 (6) | | 21.5 | 0.27 (0.13-0.59) | | 0.001 |
| Antenatal bleeding >16 weeks | | 2 (1.8) | | 1.3 | 1.4 (0.3-5.6) | | NS |
| Abdominal trauma5# | | 6 (5.3) | | 5-7 | 0.87 (0.39-2.0) | | NS |
|  | |  | |  |  | |  |
| Variables with other comparable evidence than the Dutch perinatal registration:  1,2Postnatal bleeding >1000 mL and blood transfusion pregnancy related - van Stralen et al 2016, 3 Prenatal diagnosis - WPDT and Liefers 2015, 4Antenatal bleeding prior 16 weeks - Hossain et al 2007, 5Abdominal trauma - Cheng et al 2012, 6 External cephalic version - Vlemmix et al 2010.29, 39-43  #Abdominal trauma without anti-D N=3. External cephalic version without anti-D N=1 and unknown N=1.  Number of delivered women in the Netherlands in 2015 is 166.733, number of nulliparous was 73,121. | | | | | | | |

Table 3 Potential risk factors for RhD immunization before or during pregnancy in women previously non-exposed or possibly exposed to the RhD-antigen.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primigravid women, nulliparous women with a miscarriage in history and multiparous women with an RhD-negative child and with or without miscarriage in history (n=80) | | | | |
|  | Cases (n=80)  N (%) | Population prevalence (%) | Odds ratio 95%CI | P-value |
| Miscarriage\* | 28 (35) | 10-15 | 4.3 (2.7-6.8) | <0.001 |
| Blood transfusion non pregnancy related | 16 (20) | - | - | - |
| Blood transfusion pregnancy related | 4 (5) | 3.9 | 1.7 (0.69-4.22) | NS |
| Invasive Prenatal testing~ | 2 (2.5) | 1.68 | 1.52 (0.37-6.19) | NS |
| Antenatal bleeding < 16 weeks# | 4 (5) | 21.5 | 0.19 (0.07-0.52) | 0.001 |
| Abdominal trauma& | 3 (3.8) | 5-7 | 0.61 (0.19-1.93) | NS |
| \*Miscarriage after 10 weeks gestation without or unknown anti-D N=10, curettage without anti-D N=1, ~Invasive prenatal testing without anti-D N=2, #Antenatal bleeding without anti-D N=4, &Abdominal trauma without anti-D N=2 | | | | |