Improving care for red blood cell alloimmunized pregnant women

Slootweg, Y.M.

Chapter 2

Risk factors for RhD immunisation in a high coverage prevention program of antenatal and postnatal RhIg: a nationwide cohort study

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Abstract

Objective: To evaluate which risk factors for RhD immunisation remain, despite adequate routine antenatal and postnatal RhIg prophylaxis (1000 IU RhIg) and additional administration of RhIg. The second objective was assessment of the current prevalence of RhD immunisations.

Design: Prospective cohort study.

Setting: The Netherlands.

Population: Two-year nationwide cohort of alloimmunised RhD-negative women.

Methods: RhD-negative women in their first RhD immunised pregnancy were included for risk factor analysis. We compared risk factors for RhD immunisation, occurring either in the previous non-immunised pregnancy or in the index pregnancy, with national population data derived from the Dutch perinatal registration (Perined).

Results: In the two-year cohort, data from 193 women were eligible for analysis. Significant risk factors in women previously experiencing a pregnancy of an RhD positive child (N=113) 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 (>1000 mL) (OR 2.0, 95% CI 1.1-3.6), manual removal of the placenta (MRP) (OR 4.3, 95% CI 2.0-9.3); these factors often occurred in combination. The miscarriage rate 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, MRP) need to be recognised as risk factor, requiring estimation of foetomaternal haemorrhage volume and adjustment of RhIg dosing. The higher miscarriage rate suggests that existing RhIg protocols either need adjustment or better compliance.

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Introduction

In high-income countries, the incidence of RhD immunisation has decreased after implementing routine antenatal and postnatal Rh immunoglobulin prophylaxis (RhIg), combined with administration of RhIg after events likely causing foetomaternal haemorrhage (FMH).(26, 64, 65) This has led to a major reduction of foetuses and newborns suffering from haemolytic disease.(14, 25) However, RhD immunisation still occurs in RhD-negative women pregnant of an RhD-positive child, with an estimated incidence of 0.3 to 1.3%. (10, 66-68) RhD immunisation has a 30% risk of severe disease of the foetus or newborn.(3, 7)

Since blood transfusions are routinely RhD matched for decades, the main cause of RhD immunisation is exposure to RhD-positive red blood cells (RBC) from the foetus, due to FMH during pregnancy or around delivery.(32) Small amounts of FMH can already lead to alloimmunisation.(69) Minor FMH occurs frequently during pregnancy (44% during the third trimester and 64% at delivery).(70) A major FMH (> 5 ml of foetal cells) occurs less frequently, with an estimated range of 0.1-6% of pregnancies. (5, 70-73) If there is a risk for a major FMH, administration of extra RhIg is often indicated in guidelines.(26, 64, 65) 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.(53, 71, 72, 74) 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 alloimmunisation.(53)

In this study, we evaluated in a prospectively collected cohort which risk factors for RhD immunisation 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 organisation of obstetricians.(26) 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 screened for the presence of alloantibodies against RBCs in the first trimester of pregnancy, preferably before the 13th week of gestation.(75) RhD- and Rhc-negative women are screened again in week 27. Certified Dutch laboratories (n = 90) process the screening test according to existing national guidelines.(32) Accepted screening tests are those with a sensitivity similar or better than the bovine albumin indirect antiglobulin test (IAT) to detect clinically relevant antibodies. In daily practice, column testing is used. Sensitive techniques with addition of enzymes are not used in the screening.(3) The coverage of this screening program, monitored annually, is almost 100%.(76) Following Dutch guidelines, RhIg (1,000 IU) is given at 30 weeks of gestation and again within 48 hours after birth in case of an RhD-positive foetus, after spontaneous abortion when the pregnancy was at least 10 weeks, and following instrumental evacuation of the uterus irrespective of gestational age. An extra dose of RhIg is advised to be given, after invasive prenatal testing or external cephalic version and, after estimating FMH with a microscopic Kleihauer Betke test (KBT) or a flow cytometry-based quantitation of HbF containing red blood cells (both referred to as KBT) in case of abdominal trauma or antenatal bleeding after 16 weeks. After a delivery, only when a large FMH is suspected, quantitation (KBT) is recommended, followed, if needed, by adjustment of the RhIg dose. Guidelines to calculate the adjusted dosing are available.

When at routine screening or at any other moment in pregnancy red cell alloantibodies are detected, a maternal (and if possible paternal) blood sample is sent to one of the two national reference laboratories: Sanquin Diagnostic Services (90% of all tests) and, for the north-eastern part of the Netherlands, the laboratory of the University Medical Center Groningen (UMCG).(22, 77) Foetal RHD genotyping is routinely performed in all RhD-immunised pregnancies. This typing as well as the antibody-dependent cell-mediated cytotoxicity (ADCC) test, to determine the biological activity of RBC antibodies, is centralised at Sanquin Diagnostic Services in Amsterdam.(19)

Study design and population

This study was part of the OPZI 2.0 study (unpublished data), a nationwide cohort study on RhD immunisation in pregnancy. All pregnant women with a positive screening test for anti-D antibodies, identified at Sanquin Diagnostic Services during our study period, were eligible for inclusion. In some cases, a positive screening test

was found shortly after RhIg administration, these were excluded. The study period ranged (for practical reasons) from July 1, 2014, to March 31, 2015, and from August 1, 2015, to February 28, 2017, a total of 28 months.

Written informed consent was obtained by the obstetric care provider (OCP). Clinical data were collected using a questionnaire, sent to the OCP's. If needed, the OCP or study participants were contacted by telephone up to three times, in order to complete the data set. 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, gestational age at antibody detection, pre-pregnancy blood transfusions) and relevant clinical data from all previous non-immunised and immunised pregnancies were collected in the OPZI 2.0 database. Data on all RhIg administrations and possible sensitising 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, manual removal of placenta, assisted birth and pregnancy-related RBC transfusion), were collected. Miscarriages preceding the current ongoing pregnancy were considered as possible sensitising events.

To identify risk factors for RhD immunisation, occurring despite antenatal and postnatal RhIg administration, we selected all women in their first RhD-immunised 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. When the RHD type of the child was not registered, but the complete RhIg prophylaxis was given, the foetal RHD type was considered positive. We evaluated potential risk factors in the following three groups: the first group 'exposed to the RhD antigen' consisted of women with a previous pregnancy (> 16 weeks) of an RhD-positive child; the second group 'possibly exposed to the RhD antigen' had a previous miscarriage (< 16 weeks) without a prior pregnancy of an RhD-positive child; the third group 'non-exposed to the RhD-antigen' had neither a previous pregnancy of an RhD-positive child nor a miscarriage. Birth-related risk factors were analysed in the group of multiparous women (the RhD exposed group), whereas risk factors in the current pregnancy were analysed in the other two groups. The prevalence of potential risk factors for RhD immunisation was 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 prevalence of both newly detected and already existing RhD immunisations, we used data from the year 2016, collected in the OPZI 2.0 cohort. The denominators to assess the prevalence of RhD immunisation were derived from the monitor of the National Institute of Public Health and Environment of 2016.(78)

Statistical analysis

The associations between potential risk factors and the occurrence of RhD-alloimmunisation were described as Odds Ratios and 95% confidence intervals (categorical variables) or as mean difference with 95% confidence intervals (normally distributed continuous variables) according to Altman, 1991.(79) 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). Risk factors were tested univariately. The mutual interrelation of univariate significant risk factors was depicted in a vector diagram.

Results

Prevalence of RhD immunisation

The prevalence of newly detected RhD immunisations in 2016 was 0.31% (79/25,170) of RhD-negative pregnant women in the Netherlands. Pregnancies from women who were likely immunised before immigration to the Netherlands were excluded (N=15). In 0.18% of RhD-negative women anti-D was newly detected at the screening early in pregnancy and in 0.13% during routine screening in week 27 of pregnancy. The prevalence of all RhD immunisations (including immigrants) in 2016 was 0.09% of all pregnant women (158/171,727) and 0.63% of all RhD-negative pregnant women.

Selection of the study population

During the study period, 304 RhD-immunised 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-immunisation despite RhIg prophylaxis. After exclusion, 193 women remained, 65 of whom were nulliparous (33.7%) and 128 multiparous (66.3%). Of this group 113 women were

exposed to the RhD antigen, 28 were possibly exposed and 52 were non-exposed, respectively. Only one woman carried an RhD variant (in the 'possibly exposed group'). 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 immunisation

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). We found a higher miscarriage rate in RhD-immunised women compared to the general Dutch population (21% vs 12.5% p<0.001). A total

of 40 women had a miscarriage preceding the RhD-immunised pregnancy (25 nulliparous and 15 multiparous women). Eleven out of sixteen (69%) women who had a miscarriage past 10 weeks' gestation or a curettage did not receive the advised RhIg (Table S3).

First detection of anti-D after a negative first trimester screening concerned 44% (86/193) of all cases (Table 1). Mostly these antibodies were found at the routine third trimester screening: 36% (41/113) of the women from the 'exposed group', 43% (12/28) of the women from the 'possibly exposed' group and 60% (31/52) of those from the 'non exposed group'.

Figure 1 *Composition of the study population*

The dark blue box reflects the number of eligible women before exclusion. Red boxes reflect exclusions and the green box the number of included women, eligible for the risk factor analysis. We stratified into three groups: exposed to RhD, most likely non-exposed to RhD and possibly exposed to RhD in history.

* RhD-antigen previous child unknown (n=21), Antenatal and postnatal RhIg prophylaxis was given, therefore the child was considered to be RhD positive

Table 1 *Baseline characteristics of 193 RhD-immunised pregnant women.*

	Cases		General pregnant prevalence	
	Mean (SD)	N (%)	Mean (SD)	(%)
Maternal age at delivery before the immunised 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 (33.7)		44.7
Multiparous		128 (66.3)		55.3
Miscarriage[2@]		40 (20.7)		12.5
Moment of detection of RhD-antibodies				
Before current pregnancy*		2 (1)		
Early first trimester screening[$]		102 (53)		-
First screening 20th- 27th week		3 (2)		-
Routine third trimester (27th week) screening[#]		84 (43)		-
Around delivery		2 (1)		-

Variables with other comparable evidence than the Dutch perinatal registration: [1]Pre-pregnancy weight, Bakker et al 2011, Miscarriage, [2]Dutch general practitioner's guideline "Miscarriage", for comparison a mean miscarriage rate of 10-15% was used.(80, 81)
In 2015, the number of women delivered in the Netherlands was 166.733, of which 73,121 were nulliparous
[@]Nulliparous or multiparous with one or more miscarriages before immunised pregnancy
**Pre transfusion screening*
[#]Foetal RHD typing result was positive in all cases

Risk factors for RhD immunisation in previously RhD-exposed women

As shown in table 2, caesarean section, manual removal of the placenta, post-partum bleeding >1000 mL, delivery at gestational age ≥42 weeks and perinatal death in history were significant risk factors for RhD immunisation 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 analysed risk factors in the previous pregnancy. In 61% of these cases, anti-D was detected during the first trimester. Of the women whose RhD immunisation was first detected at the 27th week screening, foetal RHD typing was positive in all cases. In the 'exposed group', who all had

a previous pregnancy with an RhD-positive foetus, 10.6% (12/113) women had a miscarriage in between the previous and the current pregnancy. This miscarriage rate was not different from the population rate of 12.5%.(81)

The incidence of vaginal blood loss before 16 weeks could only be compared with one prospective cohort study, performed in two US general hospitals, since our national Perined database does not collect these data.(82) This study reported a 21.5% incidence, while we found an incidence of 5.3% in our group.

For antenatal bleeding after 16 weeks, we could use the Dutch perinatal registration data.(83) None of the risk factors currently regarded as indication to administer (extra) RhIg prophylaxis (abdominal trauma, antenatal bleeding after 16 weeks and cephalic version), occurred more frequently in women of the 'exposed group' compared to the general population.

Combined parturition-related risk factors

Figure 2 shows that some parturition-related risk factors occurred in combination, hence some of these could be considered as confounders. Post-partum bleeding > 1000 mL occurred in 8 out of 12 (67%) pregnancies in combination with other risk factors, most often with manual removal of the placenta. One more case of excessive post-partum bleeding occurred in combination with a perinatal death (not depicted in Figure 2). Delivery from 42 weeks onwards was an isolated risk factor only once. Caesarean section was an isolated risk factor in 30 out of 32 (94%) pregnancies.

Risk factors for RhD immunisation in 'non-exposed' or 'possibly RhD-exposed' women

In the combined group of 'non-exposed' and 'possibly exposed' women (n=80), we analysed possible sensitising moments, occurring either before or during the current pregnancy (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 (table S3). 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. Twenty percent of women (16/80) reported a blood transfusion in their history, unrelated to pregnancy. There are no comparable population data on incidence of non-pregnancy related blood transfusions in the history of women of fertile age.

Figure 2 *Association of significant parturition-related risk factors for RhD immunization.*

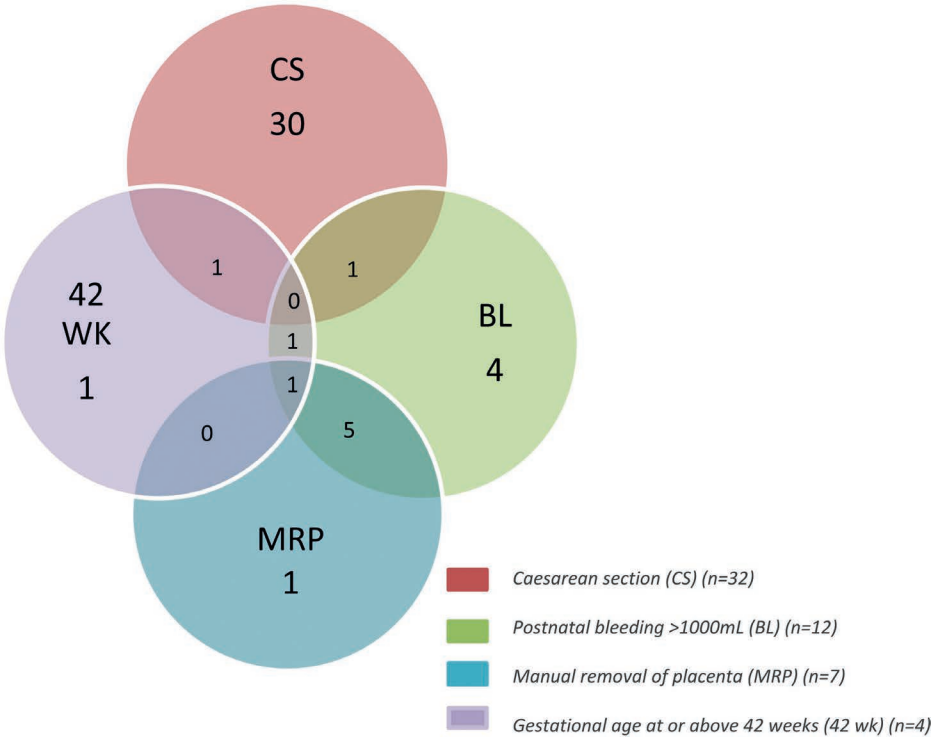

Table 2 *Potential risk factors for RhD immunisation in multiparous women exposed to the RhD-antigen in previous pregnancy >16 weeks.*

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 (15.9)	16.4	1.0 (0.6-1.6)	0.89
Manual 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 delivery >=41 weeks	21 (18.6)	14.5	1.3 (0.8-2.2)	0.22
Gestational age delivery >=42 weeks	4 (3.5)	0.6	6.1 (2.2-16.6)	<0.001
Perinatal death	3 (2.7)	0.8	3.5 (1.1-10.9)	0.03
Postnatal bleeding >1000 ml[1]	12 (10.6)	5.9	2.0 (1.1-3.6)	0.02
Blood transfusion[2]	8 (7.1)	3.9	1.9 (0.95-4.0)	0.07
Male gender (N=103)	62 (60.2)	51	1.4 (0.98-2.2)	0.07
External cephalic version[6#]	5 (4.4)	2.4	1.9 (0.76-4.61)	NS
Risk factors during current pregnancy, before detection of RhD immunisation in week 27				
Invasive prenatal testing[3]	1 (0.9)	1.7	0.52 (0.07-3.75)	NS
Antenatal bleeding <16 weeks[4]	7 (5.3)	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 trauma[5*]	6 (5.3)	6	0.87 (0.39-2.0)	NS

Variables with other comparable evidence than the Dutch perinatal registration:
[1,2]Postnatal bleeding >1000 mL and blood transfusion pregnancy related - van Stralen et al 2016, [3]Prenatal diagnosis - WPDT and Liefers 2015, [4]Antenatal bleeding prior 16 weeks - Hossain et al 2007, [5]Abdominal trauma - Cheng et al 2012, [6] External cephalic version - Vlemmix et al 2010. (82, 84-88)
[#]Abdominal trauma without RhIg N=3.
**External cephalic version without RhIg 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 immunisation 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 (35.0)	10-15	4.3 (2.7-6.8)	<0.001
Blood transfusion non pregnancy related	16 (20.0)	-	-	-
Blood transfusion pregnancy related	4 (5.0)	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 (5.0)	21.5	0.19 (0.07-0.52)	0.001
Abdominal trauma&	3 (3.8)	6	0.61 (0.19-1.93)	NS

**Miscarriage after 10 weeks gestation without or unknown RhIg N=10, curettage without RhIg N=1, ~Invasive prenatal testing without RhIg N=2, #Antenatal bleeding without RhIg N=4, &Abdominal trauma without RhIg N=2*

Discussion

Main findings

In this study, we found the following risk factors for RhD immunisation to remain, despite adequate routine antenatal and postnatal RhIg prophylaxis of 1,000 IU as per our national guideline: caesarean section, manual removal of the placenta, excessive post-partum haemorrhage (1000 ml), delivery at or past 42 weeks and perinatal death. These risk factors occurred often in combination.

The prevalence of both newly detected and of all RhD-immunisations 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.(44, 89, 90) With a frequency of 15% of RhD-negative women, RhD immunisation now concerns only 0.09% of all pregnant women in the Netherlands. Half of the RhD immunisations were detected in the first trimester of pregnancy.

Caesarean section was the main and most often single risk factor for RhD immunisation in our cohort, confirming findings from our earlier study.(53) The second risk factor, post-partum haemorrhage >1000ml, was in the majority of the cases (9/12) associated with one (or more) of the other risk factors we observed, including manual placental removal (6/7 cases), and perinatal death (1/3), suggesting a cascade of possibly immunising events. Post-maturity (delivery $\geq$42 weeks) was a less common risk factor, associated with excessive post-partum bleeding and caesarean section in three out of four cases.

The overall 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 high miscarriage rate (35%) in the group of women in their first ongoing pregnancy with an RhD positive baby. In most cases, these women did not have a positive RhD antibody screen during the first trimester, but only at the 27-week test, as has been described before.(10, 91)

Strengths and limitations

This is the largest study to date on risk factors for RhD immunisation in pregnant women participating in a high-coverage RhD immunisation prevention program. A strength of our study is that we were able to collect national data on all RhD-immunised women and their previous non-immunised and immunised pregnancies.

This created the opportunity to evaluate all potential obstetrical and non-obstetrical incidents that may induce RhD immunisation.

A limitation of this study design is that we could not include a control group. We had to compare our findings with published data in other populations or Dutch national registry data. 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.(53)

Interpretation

In our study, we found caesarean section to be a significant risk factor for RhD immunisation, having almost no interrelations with other events potentially increasing FMH. These findings confirm data reported by other smaller studies.(53, 71-74, 82)

Current Dutch guidelines recommend estimating the volume of FMH by performing a KBT after caesarean section and, depending on the results, to increase the RhIg dose.(26, 64, 65) This is however no obligation. In some countries, a KBT is routinely performed after delivery or in case of risk factors related to increased FMH.(64, 92) In some prophylaxis programs, a higher dose of RhIg of 1,500 IU is used routinely, in order to reduce the risk of RhD immunisation. Our data support the concept that a caesarean section should be regarded as a risk for RhD immunisation. We hypothesise that making FMH testing mandatory might further reduce the number of RhD immunisations. Alternatively, a double dose of RhIg could be given after caesarean section, especially in settings were FMH testing is not easily available.

Previously, we hypothesised that post-maturity may lead to a failure of antenatal RhIg prophylaxis, due to the long interval between the administration of prophylaxis and delivery.(53) The current study however suggests that immunisation in post-maturity is mostly related to complications during 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.(93) In this context, adjustment of RhD-prophylaxis in post-term pregnancies is no priority.

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 alloimmunisation in RhD-negative women. In addition, perinatal death appeared to be associated with a higher risk of RhD immunisation. Therefore, if these risk factors occur, estimation of FMH volume and adjustment of RhIg dosing is advised. Surprisingly, in one third of women who previously had given birth to an RhD positive baby, none of the high-risk features that we found to be related to RhD immunisation were reported. Possibly, a

larger but subclinical FMH than could be covered by the RhIg prophylaxis occurred, as has been reported earlier.(94) An alternative explanation would be that some women respond more strongly to a relatively low amount of foetal blood entering their circulation around delivery.

The miscarriage rate in the combined non-exposed and possibly exposed group was almost three times higher than in a comparable age group.(81) Half of the RhD immunisations in ongoing pregnancies after a miscarriage were first detected in the third trimester. This finding confirms the theory that the miscarriage may be a primary sensitising event, however with such a low level of RhD antibodies that these are still undetectable in the first trimester of the subsequent pregnancy. Only after renewed contact with foetal RhD-positive red cells, the antibody levels increase and may become first detectable at the 27-week screening.(69, 95, 96) Our observations regarding current guidelines to administer RhIg prophylaxis in cases of miscarriage or abortion suggest insufficient adherence. Further studies are needed to explore the effectiveness of RhIg in preventing immunisation after all spontaneous or induced (including instrumental) abortions.(64, 65)

Overall, we did not find evidence that potential antenatal risk factors for FMH in the current pregnancy were associated with RhD immunisation. These events (invasive diagnostic procedures, twin pregnancy, antenatal bleeding and abdominal trauma) are relatively rare and there is likely sufficient awareness of the prophylactic measures that need to be taken.(26, 64, 65) 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 recognising 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 manual (surgical) removal of the placenta. Our data suggest that miscarriage may be an additional risk factor for RhD immunisation, requiring further studies, and possible to reconsider the current RhIg policy. For future research, we recommend to critically and prospectively evaluate any adjustments to the RhD immunisation prevention program made.

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Table S1. *Additional antibody specificities in women with RhD- immunization.*

Additional antibody specificities			N	(%)
C			38	19.7
E			6	3.1
K			1	0.5
G			2	1.0
Jk(a)			1	0.5
Fy(a)			1	0.5
C	E		3	1.6
C	E	K	1	0.5
	Total		53	27.5

Table S2. *Content of evidence, other than perinatal registration Netherlands.*

Variable	Reference	Year	Population (N)*
Prenatal diagnosis	WPDT/ Liefers	2015	2796:56,685
Blood transfusion pregnancy related	Van Stralen	2016	93,864:2,406,784
Postnatal bleeding >1000 ml	Van Stralen	2016	142,000:2,406,784
Miscarriage	NHG Guideline	2015	20,842:166,733
Maternal weight	Bakker	2011	8,623

**numerator: denominator*

Table S3. *Details of previous miscarriages in the groups of Possibly RhD exposed and RhD Exposed women*

	Possibly exposed (N=28) N (%)	Exposed (N=12) N (%)
Median duration pregnancy (days)	53 (35-76)	63 (51-72)
Gestational age <10 weeks	13 (46)	7 (58)
No anti-D or unknown	11 (85)	6 (86)
Gestational age >10 weeks and/curettage	11 (39)	5 (42)
No anti-D or unknown	8 (73)	3 (60)
Gestational age unknown	4 (14)	0
No anti-D	4 (100)	
Screen positive 1st trimester	14 (50)	4 (33)
Screen positive 3rd trimester	12 (43)	8 (67)
Screen positive delivery/operation/blood transfusion	2 (7)	0

Possibly exposed and exposed women to the RhD antigen with a miscarriage before the RhD immunization was detected (N=40)