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RESEARCH ARTICLE

Fetal Medicine

Risk factors for RhD immunisation in a high coverage prevention programme 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 2-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 >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% versus 12.5%, $P < 0.001$).

Conclusion: Complicated deliveries, including cases of major bleeding and surgical interventions (CS, MRP), must be recognised as a risk factor, requiring estimation of fetomaternal haemorrhage volume and adjustment of RhIg dosing. The higher miscarriage rate suggests that existing RhIg protocols need adjustment or better compliance.

KEY WORDS

alloimmunisation, foetal medicine, immunology, screening, serum

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Tweetable abstract: Complicated delivery (caesarean section, manual removal placenta, major bleeding) is the most valid risk factor for RhD immunization despite antenatal and postnatal RhIg.

1 | 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 fetomaternal haemorrhage (FMH).¹⁻³ This has led to a major reduction in the number of fetuses and newborns suffering from haemolytic disease.^{4,5} However, RhD immunisation still occurs in RhD-negative women pregnant with an RhD-positive child, with an estimated incidence of 0.3 to 1.3%.⁶⁻⁹ RhD immunisation has a 30% risk of severe disease of the fetus or newborn.^{10,11}

As blood transfusions have been routinely RhD-matched for decades, the main cause of RhD immunisation is exposure to RhD-positive red blood cells (RBC) from the fetus, due to FMH during pregnancy or around delivery.¹² Even small amounts of FMH can lead to alloimmunisation.¹³ Minor FMH occurs frequently during pregnancy (44% during the third trimester and 64% at delivery).¹⁴ A major FMH (>5 ml of fetal 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 alloimmunisation.²⁰

In this study, we evaluated in a prospectively collected cohort for 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.¹ Since 2011, routine RhIg administration has been based on fetal *RHD* typing.

2 | METHODS

2.1 | 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.²¹ 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.¹² Accepted screening tests are those with a sensitivity similar to 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.¹¹ The coverage of this screening programme, monitored annually, is almost 100%.²² Following Dutch guidelines, RhIg (1000 IU) is given at 30 weeks of gestation and again within 48 h after birth in the case of an RhD-positive fetus, 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 the case of abdominal trauma or antenatal bleeding after 16 weeks. After a delivery, quantitation (KBT) is recommended only when a large FMH is suspected, 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) or, for the north-eastern part of the Netherlands, the laboratory of the University Medical Centre Groningen (UMCG).^{23,24} Fetal 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.²⁵

2.2 | 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 OCPs. If needed, the OCP or study participants were contacted by telephone up to three times to complete the dataset. If it was unclear whether women received RhIg in a previous pregnancy, this information was obtained from the Department for Vaccine Supply and Prevention Programmes (RIVM-DVP).

2.3 | 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 were collected 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). Miscarriages preceding the current ongoing pregnancy were considered 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 fetal 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 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 had a previous pregnancy of an RhD-positive child or a miscarriage. Birth-related risk factors were analysed in the group of multiparous women (the RhD-exposed group), and 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 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 2016.²⁶

2.4 | Statistical analysis

The associations between potential risk factors and the occurrence of RhD-alloimmunisation were described as odds ratios (OR) and 95% confidence intervals (CI; categorical variables) or as mean difference with 95% CI (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) (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.

3 | RESULTS

3.1 | 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 had likely been immunised before immigration to the Netherlands were excluded ($n = 15$). Anti-D was newly detected at the screening early in pregnancy in 0.18% of RhD-negative women, and during routine screening in week 27 of pregnancy in 0.13% of RhD-negative women. 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.

3.2 | 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 women (27.5%); the most common antibodies were anti-RhC (19.7%) and anti-RhE (3.1%) ([Table S1](#)).

3.3 | General risk factors for RhD immunisation

When compared with the Dutch pregnant population, multiparous women were significantly overrepresented in our study group (66% versus 55.3%, $P = 0.002$), but there was still a large number of women in their first ongoing pregnancy ([Table 1](#), details of population rates in [Table S2](#)). We found a

higher miscarriage rate in RhD-immunised women than in the general Dutch population (21% versus 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 of 16 women (69%) 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 occurred in 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'.

3.4 | Risk factors for RhD immunisation in previously RhD-exposed women

As shown in Table 2, caesarean section, manual removal of the placenta, postpartum bleeding >1000 ml, delivery at

gestational age ≥ 42 weeks and history of a perinatal death were significant risk factors for RhD immunisation in the 'exposed' group 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 27-week screening, fetal RHD typing was positive in all cases. In the 'exposed group', all of whom had had a previous pregnancy with an RhD-positive fetus, 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%.²⁸

The incidence of vaginal blood loss before 16 weeks could only be compared with one prospective cohort study, performed in two US general hospitals, as our national Perined database does not collect these data.²⁹ This study reported a 21.5% incidence, whereas we found an incidence of 5.3% in our group.

For antenatal bleeding after 16 weeks, we could use the Dutch perinatal registration data.³⁰ None of the risk factors



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

FIGURE 1 Composition of the study population

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) (<i>n</i> = 113)	27.4 (4.0)		29.5 (4.5)	
Pre-pregnancy weight (kg) (<i>n</i> = 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,a}		40 (20.7)		12.5
Moment of detection of RhD-antibodies				
Before current pregnancy ^b		2 (1)		
Early first trimester screening [§]		102 (53)		-
First screening 20 th –27th week		3 (2)		-
Routine third trimester (27th week) screening ^c		84 (43)		-
Around delivery		2 (1)		-

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 a mean miscarriage rate of 10–15% was used.^{28,40}

In 2015, the number of women delivered in the Netherlands was 166 733, of whom 73 121 were nulliparous.

^aFetal RHD typing result was positive in all cases.

^bNulliparous or multiparous with one or more miscarriages before immunised pregnancy.

^cPre-transfusion screening.

[§]Routine first trimester screening at the booking visit around 11th week of gestation.

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

Risk factors	Cases (<i>n</i> = 113)		Population prevalence	Odds ratio (95% CI)	<i>P</i> -value
	<i>N</i> (%)	%			
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 of 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 by 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 (<i>n</i> = 103)	62 (60.2)	51	1.4 (0.98–2.2)	0.07	
External cephalic version ^{6,a}	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,b}	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). ³Prenatal diagnosis—WPDT and Liefers (2015). ⁴Antenatal bleeding prior to 16 weeks—Hossain et al. (2007). ⁵Abdominal trauma—Cheng et al. (2012). ⁶ External cephalic version—Vlemmix et al. (2010).^{29,41–45}

There were 166 733 of women delivered in the Netherlands in 2015; 73 121 were 73 121.

^aAbdominal trauma without RhIg (*n* = 3).

^bExternal cephalic version without RhIg (*n* = 1) and unknown (*n* = 1).

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' than in the general population.

3.5 | Combined parturition-related risk factors

Figure 2 shows that some parturition-related risk factors occurred in combination, hence some of these could be considered confounders. Postpartum bleeding >1000 ml occurred in eight of 12 pregnancies (67%) in combination with other risk factors, most often with manual removal of the placenta. A further case of excessive postpartum 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 of 32 (94%) pregnancies.

3.6 | 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 that occurred 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). Only one woman with a history of miscarriage had an additional incident (antenatal bleeding <16 weeks) during the current pregnancy, before anti-D was detected in the third trimester. Twenty per cent 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.

4 | DISCUSSION

4.1 | 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 1000 IU as per our national guideline: caesarean section, manual removal of the placenta, excessive postpartum 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 rates 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 immunisation now occurs in 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.²⁰ The second risk factor, postpartum haemorrhage >1000 ml, was in the majority of the cases

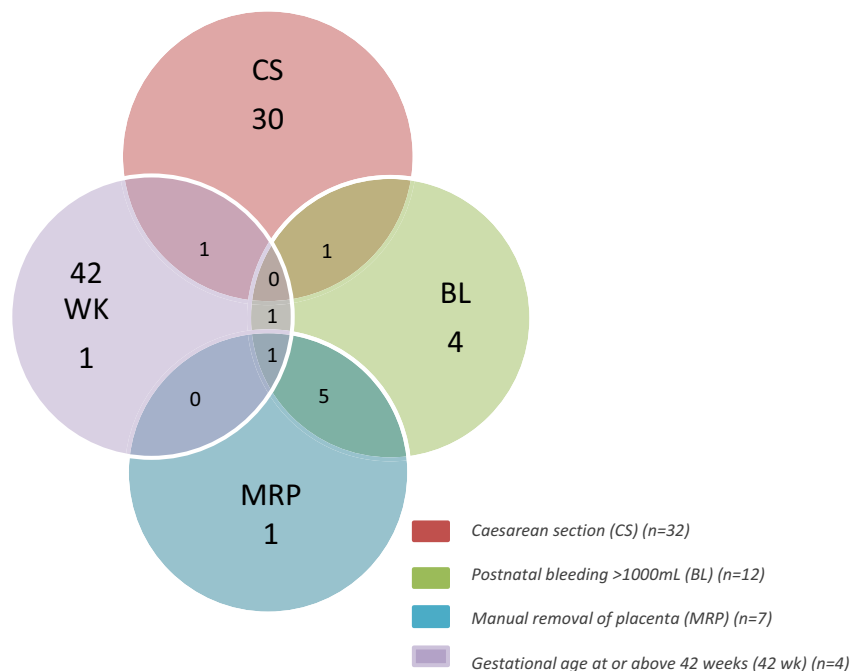


FIGURE 2 Association of significant parturition-related risk factors for RhD immunisation

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 history of miscarriage and multiparous women with a history of an RhD-negative child and with or without miscarriage (n = 80)				
	Cases (n = 80) n (%)	Population prevalence (%)	Odds ratio (95% CI)	P-value
Miscarriage ^a	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 ^b	2 (2.5)	1.68	1.52 (0.37–6.19)	NS
Antenatal bleeding <16 weeks ^c	4 (5.0)	21.5	0.19 (0.07–0.52)	0.001
Abdominal trauma ^d	3 (3.8)	6	0.61 (0.19–1.93)	NS

^aMiscarriage after 10 weeks' gestation without or unknown RhIg (n = 10), curettage without RhIg (n = 1).

^bInvasive prenatal testing without RhIg (n = 2).

^cAntenatal bleeding without RhIg (n = 4).

^dAbdominal trauma without RhIg (n = 2).

(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 ≥ 42 weeks) was a less common risk factor, associated with excessive postpartum bleeding and caesarean section in three of four cases.

The overall miscarriage rate in our study was significantly higher than that in the Dutch population (21% versus 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 the majority of 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.^{6,34}

4.2 | 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 programme. 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.²⁰

4.3 | 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.^{15–17,19,20,29}

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} This is, however, not mandatory. In some countries, a KBT is routinely performed after delivery or in the case of risk factors related to increased FMH.^{2,35} In some prophylaxis programmes, a higher dose of RhIg of 1500 IU is routinely used 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 where 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.²⁰ 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.³⁶ 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.³⁷

An alternative explanation could be that some women respond more strongly to a relatively small amount of fetal 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.²⁸ 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 fetal RhD-positive red cells, will the antibody levels increase and then may become first detectable at the 27-week screening.^{13,38,39} 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.^{2,3}

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.¹⁻³ In the case of antenatal bleeding in pregnancies before 16 weeks, extra RhIg is currently not recommended and, based on our findings, we would not advise changing this policy.

5 | CONCLUSION

We advocate strictly following 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 possibly reconsidering the current RhIg policy. For future research, we recommend that critical and prospective evaluation of any adjustments to the RhD immunisation prevention programme be done.

DETAILS OF ETHICS APPROVAL

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

CONFLICT OF INTERESTS

None declared. Completed disclosure of interest forms are 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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DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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