Disease-severity in subsequent pregnancies with RhD immunization: a nationwide cohort

Carolien Zwiers[1], Yolentha Slootweg[2], Joke Koelewijn[3], Peter Ligthart[4], Johanna van der Bom[5], Inge Kamp[1], Enrico Lopriore[2], Ellen van der Schoot[3], Dick Oepkes[6], and Masja de Haas[4]

[1]Leiden Universitair Medisch Centrum
[2]Leiden University Medical Center
[3]Sanquin Research
[4]Sanquin Blood Supply Foundation
[5]Sanquin Bloedvoorziening
[6]Leiden University Medical Centre

December 29, 2021

Abstract

OBJECTIVE(S): to evaluate the severity of HDFN in subsequent pregnancies with RhD immunization and to identify predictive factors for severe disease. DESIGN: prospective cohort. SETTING: the Netherlands. POPULATION: nationwide selection of all pregnant women with RhD antibodies. METHODS: women with two subsequent RhD immunized pregnancies with RhD-positive children after antibodies were detected were included. MAIN OUTCOME MEASURE: the severity of HDFN in the first and subsequent pregnancy at risk. RESULTS: 62 RhD immunized women with a total of 150 RhD-positive children were included. The severity of HDFN increased significantly in the subsequent pregnancy (P<.001), although it remained equal or even decreased in 44% of women. When antibodies were already detected at first trimester screening in the first immunized pregnancy, severe HDFN in the next pregnancy was uncommon (22%), especially when no therapy or only non-intensive phototherapy was indicated during the first pregnancy (6%), or if the ADCC result remained <10%. Contrarily, women with antibodies detected during the first pregnancy of a RhD positive child (>= 27th week), most often before they had ever received RhIg prophylaxis, were most prone for severe disease in a subsequent pregnancy (48%). CONCLUSION(S): RhD-mediated HDFN in a subsequent pregnancy is generally more severe than in the first pregnancy at risk and can be estimated using moment of antibody detection and severity in the first immunized pregnancy. Women developing antibodies in their first pregnancy of a RhD-positive child are at highest risk of severe disease in the next pregnancy.

Disease-severity in subsequent pregnancies with RhD immunization: a nationwide cohort

Carolien Zwiers, MD PhD, Department of Obstetrics, Leiden University Medical Center, Leiden, the Netherlands; Department of Experimental Immunohematology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

Yolentha M. Slootweg, MD, Department of Obstetrics, Leiden University Medical Center, Leiden, the Netherlands;

Joke M. Koelewijn, MD PhD, Department of Experimental Immunohematology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; Department of Immunohematology Diagnostic Services, Sanquin, Amsterdam, the Netherlands.

Peter C. Ligthart, MSc, Department of Immunohematology Diagnostic Services, Sanquin, Amsterdam, the Netherlands.

Johanna G. van der Bom, MD PhD, Center for Clinical Transfusion Research, Sanquin Research, Leiden, the Netherlands; Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands.

Inge L. van Kamp, MD PhD, Department of Obstetrics, Leiden University Medical Center, Leiden, the Netherlands.

Enrico Lopriore, MD PhD, Department of Pediatrics, Leiden University Medical Center, Leiden, the Netherlands.

C. Ellen van der Schoot, MD PhD, Department of Experimental Immunohematology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

Dick Oepkes, MD PhD, Department of Obstetrics, Leiden University Medical Center, Leiden, the Netherlands.

Masja de Haas, MD PhD, Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, the Netherlands; Immunohematology Diagnostic Services, Sanquin Blood Supply, Amsterdam, the Netherlands.

Corresponding author: Carolien Zwiers, Department of Obstetrics, Room K6-034, Leiden University Medical Center, PO box 9600, 2300 RC Leiden, the Netherlands, c.zwiers@lumc.nl, +31612766799, fax number +31715266741.

Running title: HDFN severity in subsequent pregnancies.

ABSTRACT

OBJECTIVE(S): to evaluate the severity of HDFN in subsequent pregnancies with RhD immunization and to identify predictive factors for severe disease.

DESIGN: prospective cohort.

SETTING: the Netherlands.

POPULATION: nationwide selection of all pregnant women with RhD antibodies.

METHODS: women with two subsequent RhD immunized pregnancies with RhD-positive children after antibodies were detected were included.

MAIN OUTCOME MEASURE: the severity of HDFN in the first and subsequent pregnancy at risk.

RESULTS: 62 RhD immunized women with a total of 150 RhD-positive children were included. The severity of HDFN increased significantly in the subsequent pregnancy (P <.001), although it remained equal or even decreased in 44% of women. When antibodies were already detected at first trimester screening in the first immunized pregnancy, severe HDFN in the next pregnancy was uncommon (22%), especially when no therapy or only non-intensive phototherapy was indicated during the first pregnancy (6%), or if the ADCC result remained <10%. Contrarily, women with antibodies detected during the first pregnancy of a RhD positive child (>= 27^{th} week), most often before they had ever received RhIg prophylaxis, were most prone for severe disease in a subsequent pregnancy (48%).

CONCLUSION(S): RhD-mediated HDFN in a subsequent pregnancy is generally more severe than in the first pregnancy at risk and can be estimated using moment of antibody detection and severity in the first immunized pregnancy. Women developing antibodies in their first pregnancy of a RhD-positive child are at highest risk of severe disease in the next pregnancy.

FUNDING: grant from Sanquin Blood Supply (L2181).

KEYWORDS: Alloimmunization in pregnancy, Foetal anaemia, Foetal hydrops, Haemolytic disease of the foetus and newborn, Intra-uterine transfusion, Natural course of disease, Pregnancy complications, Red cell immunization in pregnancy.

TWEETABLE ABSTRACT

The moment of RhD antibody detection and previous HDFN severity help to predict HDFN severity in a next pregnancy.

INTRODUCTION

Haemolytic disease of the foetus and newborn (HDFN) is a serious, and nowadays rare condition, caused by maternal alloantibodies against foetal red cells. The subsequent haemolysis may result in neonatal anaemia and hyperbilirubinemia, evoking the need for phototherapy, red cell transfusions or exchange transfusions. In severe cases, anaemia occurs prenatally and intervention with intrauterine transfusion(s) (IUT) is needed. Although the introduction of RhIg-prophylaxis has greatly reduced the RhD immunization-rate, it still has remained the major cause of severe HDFN cases.[1]

As blood transfusions are nowadays always ABO- and RhD-matched, RhD alloimmunization is mostly the result of maternal exposure to foetal red cell antigens, inherited from the father.[2] The risk of alloimmunization depends on the duration and amount of foetomaternal haemorrhage, characteristics of the maternal immune system and of the red blood cell antigens.[3]

A generally accepted idea is that the severity of HDFN increases in every subsequent pregnancy, as a rise in the amount of stillbirths in every following pregnancy affected with HDFN was already reported in 1957, before the introduction of RhIg.[4, 5] As the administration of RhIg is thought to have a long lasting suppressive effect on the strength of the immune response,[6, 7]this generally accepted idea cannot simply be applied to the current setting.

The aim of this study is to assess the severity of HDFN in consecutive pregnancies with RhD immunization and RhD-positive foetuses, in the presence of routine antenatal and postnatal RhIg prophylaxis, in order to properly counsel and manage women after a first RhD immunized pregnancy. Furthermore, we evaluated which factors from the first immunized pregnancy are associated with severe disease in a subsequent pregnancy at risk.

METHODS

Setting

To prevent RhD immunization induced by pregnancy, RhD-negative mothers carrying RhD-positive foetuses receive both antenatal (around 30 weeks gestation) and postnatal anti-D prophylaxis (RhIg) in the Netherlands.

All pregnant women are screened for the presence of allo-antibodies in the first trimester of pregnancy. Furthermore, RhD-negative and c-negative women are additionally screened in week 27. The coverage of this screening program is almost 100%.[8] 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 referral laboratories (Sanquin Diagnostic services and Special Institute for Blood group Investigations (BIBO)). Here, the clinical relevance of the antibody is evaluated by, amongst others, assessing whether the foetus is antigen-positive, with serological typing of the father as the first step in most of the pregnancies in this study. If the foetus is RhD-positive, the risk on foetal haemolysis is assessed by serially determining the antibody titer and antibody-dependent cell-mediated cytotoxicity (ADCC, performed only at Sanquin Diagnostic Services), a monocyte based assessment of the destructive capacity of the antibodies.[9, 10]

Study design and population

This study was part of the OPZI 2.0 study, a nationwide cohort study on RhD immunization in pregnancy. All pregnant women with a positive screening for RhD antibodies at any moment in pregnancy, identified

at Sanquin Diagnostic Services during our study period, were eligible for inclusion. Positive screenings as a result of a RhIg administration were not included. Women were identified from two time periods (for practical reasons): from July 1, 2014 to March 31, 2015 and from August 1, 2015 to February 28, 2017. Women were excluded if the mother additionally had another antibody with a titer higher than that of RhD (and an antigen-positive child).

The local care provider of eligible pregnant women was contacted in order to obtain patient's informed consent. Subsequently, clinical data were collected from the care provider in a detailed questionnaire. If outcome data were incomplete, the researchers made at least three attempts to contact care providers or study participants directly to complete the questionnaire. Missing data on receiving RhIg in a previous pregnancy was obtained from the Department for Vaccine Supply and Prevention Programs (RIVM-DVP).

To test the hypothesis that HDFN is more severe in the subsequent pregnancy with RhD immunization than in the first immunized pregnancy, we selected all women with at least two pregnancies with RhD antibodies and RhD-positive foetuses from the OPZI 2.0 cohort. In order to assess the risk of selection and non-response bias, characteristics of included and non-included cases were compared (supplemental text).

Sample size calculation

Based on the literature[6, 11] and an interim analysis of our data, we expected approximately 20% of cases to be treated with IUT, exchange transfusion or ending in foetal or neonatal death in the first immunized pregnancy, and 45% in the second pregnancy. With a significance of 0.05 and a power of 0.8, a total of 56 women with two immunized pregnancies of RhD-positive foetuses would be required.

Data collection and outcome definitions

Relevant clinical data from all previous non-immunized and immunized pregnancies were collected in the OPZI 2.0 database. Furthermore, we obtained treatment details to assess the severity of HDFN of all pregnancies **with RhD antibodies and RhD-positive foetuses** . From Sanquin Diagnostic Services, laboratory data were retrieved (including antibody titers, ADCC results and the presence of additional antibodies).

In the current study, '**first immunized pregnancy** ' is defined as the first pregnancy with RhD antibodies and a RhD-positive child. '**Subsequent pregnancy'** is defined as the second pregnancy with RhD antibodies and a RhD-positive child.

Our main outcome was disease severity, which was categorized as follows:

1. No HDFN: no antenatal or postnatal treatment
2. Mild HDFN: non-intensive phototherapy ([?]2 lamps), or only one day intensive phototherapy (>2 lamps), with or without a red blood cell transfusion during the first month after birth
3. Moderate HDFN: intensive phototherapy (>2 lamps) for more than one day or neonatal exchange transfusion (in the Netherlands neonatal exchange transfusion has been gradually replaced by intensive phototherapy)
4. Severe HDFN: intrauterine transfusion or HDFN-related death.

In case of missing data on disease severity, patients were assigned to a disease category based on the other, non-missing disease parameters (laboratory results, phototherapy duration and intensity, etc.). In twin pregnancy, disease severity was categorized according to the most severely affected child.

Ethical considerations

The medical ethics committee of the Leiden University Medical Center approved the protocol (P15.101/NV/nv). Written informed consent was obtained from all mothers included in this study.

Funding

This research was supported by a grant from Sanquin Blood Supply (L2181). The design, conduct or publication of the study was not influenced by this financial support.

Statistical analysis

All outcomes were analysed according to a predefined analysis strategy that was conducted in collaboration with our clinical epidemiologist (JGB).

For our main outcome, sensitivity and subgroup analyses on the difference in severity of HDFN between two subsequent pregnancies, a Wilcoxon Signed-Rank test was used. With this test, the number of positive differences in severity (+1 to +3 disease categories), negative differences (-1 to -3) and ties are ranked.

Differences in severity of HDFN between two non-paired groups were analysed with a multinomial logistic regression. In other, non-paired analyses, the Pearson's Chi-square test or logistic regression (or Fisher's exact test if appropriate) was used for the comparison of proportions. Comparisons of non-parametric outcomes were analysed with the Mann-Whitney U test. A sensitivity analysis was performed among patients in whom all the information on disease outcome was available and disease severity was thus not imputed. As the mechanism and thus severity of HDFN might be different if RhD antibodies are developed after giving birth to a RhD-positive child and thus after receiving anti-D at least twice (group A), or in the first pregnancy at risk for immunization (group B), a subgroup analysis was performed in these groups.

In order to identify factors possibly predicting severe HDFN (IUT or death) in a subsequent pregnancy for counselling purposes, a prediction model was constructed including variables known or thought to be associated with HDFN severity from the literature, the potential predictors. All potential predictors with a P -value<.25 in univariate analysis were included in a multivariate logistic regression model. The prediction model was further improved by applying manual backward selection, excluding the variable with the highest P -value at every step. Eventually, all variables with a P -value<.1 remained in the final prediction model.

RESULTS

Selection and characteristics of study population

311 pregnant women with RhD immunization were found eligible for inclusion in the OPZI 2.0 study. Figure 1 shows how the study population for the present analysis on HDFN severity in subsequent pregnancies was selected. In total, 62 women were included, with 155 pregnancies complicated by RhD antibodies and with a RhD-positive child (38 women with two, 19 women with three, four women with four and one woman with six pregnancies of RhD-positive children after her RhD antibodies were detected). Including two twins makes a total of 157 RhD-positive children. Table 1 shows the characteristics of included women and their children. To assess the risk of selection bias by selecting women with two or more subsequent pregnancies only, disease severity in the first immunized pregnancy of patients with and without a subsequent pregnancy was compared and showed a similar distribution (Table S1).

Severity of HDFN in the first immunized and the subsequent pregnancy

In this cohort of 157 RhD-positive children out of pregnancies complicated by RhD antibodies, no children died as a result of HDFN. One foetal death occurred due to a cause other than HDFN (severe growth restriction and placental infarction by pathological examination). As the severity of HDFN of this deceased child cannot be categorized nor compared to the subsequent children (4 RhD-positive children since detection of antibodies), it is not reported in outcome tables and figures.

In two twins, both in the first immunized pregnancy and all RhD-positive, all children showed mild disease.

Table 2 demonstrates that the severity of HDFN was significantly higher in the subsequent pregnancies, compared to the first immunized pregnancy (P <.001). HDFN was more severe in the subsequent pregnancy in 34/61 women (56%, maximum of three categories more), equally severe in 19/61 (31%) and less severe in 8 women compared to the first immunized pregnancy (13%, maximum of one HDFN category less). For two patients HDFN severity was missing an thus imputed, the sensitivity analysis without these patients

showed a similar result (P <.001). Figure 2A demonstrates the severity of HDFN in subsequent pregnancies in relation to the severity in the first immunized pregnancy. HDFN severity in third and later pregnancies is available in supplemental table S2 and the accompanying text.

Table S3 presents the raw data on indicators of HDFN and treatment details in first immunized pregnancies and in subsequent pregnancy with a RhD-positive child. Most of these disease parameters indicated more severe disease in the second immunized compared to the first immunized pregnancy (upon eyeballing).

Severity of HDFN according to the time of antibody detection

Figure 2B and C illustrate severity of HDFN in subsequent pregnancies in relation to the severity in the first immunized pregnancy for the subgroups with RhD antibodies detected after giving birth to a RhD-positive child (A), or during the first pregnancy at risk for immunization (B). The median HDFN severity was mild in the first immunized pregnancy in both subgroups and did not differ significantly between group A and B (P =.794). In both subgroups, disease severity increased significantly in the subsequent pregnancy as compared to the first pregnancy (more severe in 14/27 (52%) women in group A, equal in 10 (37%) equal and less severe in 3 (11%), P =.007, and 18/25 (72%) more severe, 4 (16%) equal and 3 (12%) less severe in group B, P =.001). The change in HDFN severity in subsequent pregnancies did not differ between these subgroups (both median +1 HDFN categories change, range -1 to +3), P =.053).

In group A, 6/27 (22%) of women experienced severe HDFN in the second pregnancy, as opposed to 12/25 (48%) of women that developed antibodies in the first pregnancy at risk (group B, OR 3.22, 95% CI 1.0-10.8, P =.051).

Predicting severe disease in the second pregnancy with RhD antibodies

The association between severity in the first and the second pregnancy with RhD antibodies was assessed in both subgroups. In group A, 5/11 (45%) women with moderate to severe disease in the first pregnancy with RhD immunization developed severe disease in the second pregnancy as compared to 1/16 women with previous no or mild disease (6%, OR 12.5, 95% CI 1.2-130.6, P =.027). In group B, this was 5/8 (63%) as compared to 7/17 women with previous no or mild disease (41%, OR 2.4, 95% CI 0.4-13.4, P =.411).

Factors from the first immunized pregnancy possibly predicting severe disease in the subsequent pregnancy with a RhD-positive foetus were assessed in a multivariate prediction model per subgroup (supplemental Table S4). In group A, the highest ADCC result in the first immunized pregnancy remained as the only factor associated with severe disease in the subsequent pregnancy. The predictive value of this test is summarized in Table S5. The negative predictive value of an ADCC test result >10% appeared most useful: if the ADCC test did not exceed 10% in the first pregnancy, 89% (95% CI 55-98%) of subsequent RhD-positive children will not be treated with intrauterine transfusion(s).

In group B, no predictive factors were found in this multivariate analysis.

DISCUSSION

Main findings

In this unselected national cohort of 157 RhD-positive children of 62 women with RhD antibodies, HDFN severity in the first pregnancy with anti-RhD antibodies with a RhD-positive child and subsequent pregnancies at risk was evaluated. The severity of HDFN increased significantly in 56% of women. Women who developed RhD antibodies in the first pregnancy at risk for immunization seemed more prone for severe disease in the subsequent pregnancy.

Interpretation

In this study, severe HDFN occurred more often in subsequent (31%) compared to first immunized (3%) pregnancies, in line with findings of others. For example, Tiblad et al. found 1.7% (5/288) severe HDFN in first immunized pregnancies, according to our definitions, and 19% in the second pregnancy at risk.[11] Similar to our findings, mothers that were already immunized during their first ongoing pregnancy (before giving

birth to a RhD-positive child) received more treatment for HDFN, although not significantly. Other authors observed 0% severe disease in first immunized pregnancies and 19% in 'reactivation' of RhD immunization.[12] Our study is however the first study directly comparing the first and subsequent immunized pregnancy of the same woman, which demonstrated that the severity of HDFN did not increase in 44% of the cohort. This challenges the general accepted concept that every next child at risk for HDFN will be more severely affected.

We found that the proportion of severe disease in a subsequent pregnancy of women that developed antibodies during their first pregnancy of a RhD-positive child, before RhIg could even be administered (group A), was as high as 48%. This is twice as much as compared to the subgroup that developed antibodies after giving birth to a RhD-positive child, despite receiving full prophylaxis (group B, 22%), a finding that approached statistical significance (figure 2B and C). Several mechanisms might contribute to this difference in course of disease.

First: women developing antibodies as a result of a large immunizing event (e.g. birth, group A) are 'low-responders', as compared to women with an antibody response to a small foetomaternal haemorrhage during pregnancy (group B, potential 'high-responders). Recent publications revealed associations between the intensity of an antibody response and a combination of genetic risk factors such as carrying HLA-DRB1*1501 and FCRIIC-ORF alleles.[13-15] If in the future 'high responders' could be identified early, additional anti-D prophylaxis before the conventional antenatal administration might prevent immunization during the first pregnancy at risk. In this study, no association was found in this subgroup between clinical or biochemical (ADCC/titer) disease severity and severe disease in the subsequent pregnancy. Therefore, all women who develop RhD antibodies in their first pregnancy at risk for immunization are to be monitored closely.

Second: the immune response to RhD-antigens is not prevented by anti-D prophylaxis but is merely suppressed, causing a stronger antibody response in women that have never received anti-D (group B), as opposed to women that received prophylaxis at least twice (group A), which has earlier been suggested by others.[6, 7, 11, 16]

A *third* hypothesis is that women in group A and B have different IgG-Fc-glycosylation profile of their anti-D antibodies, which correlates with clinical and biochemical (ADCC) HDFN severity.[17, 18] Interestingly, we have previously shown that there exists immunological memory for this Fc-glycosylation profile, meaning that this profile is sustained in subsequent pregnancies.[18] Already before RhIg was available, disease severity in subsequent pregnancies seemed to be interrelated.[4] This correlates with our finding of a persistent tendency to milder disease in the subsequent pregnancy in group A: only one of 16 women with no or mild disease in her first immunized pregnancy developed severe disease, and a low ADCC result in the first immunized pregnancy was the best predicting factor for no severe HDFN in the next pregnancy. These associations were not found in group B, possible reflecting a different IgG-Fc-glycosylation profile.

Lastly, an additional factor influencing the relation between severity in the first and subsequent immunized pregnancies might be the inherited foetal Fc-receptor profiles, as we have previously shown that this profile influences the risk of severe HDFN.[15]

Strengths and limitations

The major strength of our study is the unselected study population: as coverage of the national screening program is near 100% in the Netherlands[8] and serological assessment (titers and ADCC tests) for the risk on HDFN is performed at Sanquin Diagnostic services only, all women with D antibodies in the Netherlands that were pregnant during our study period were identified.

Another strong point of this study was our response rate of 73%. Furthermore, no selection bias seems to be induced by selecting women with two or more subsequent pregnancies only (supplemental text and Table S1).

A limitation of this study is however that cut-offs for the disease categories are somewhat arbitrary, as the clinical rationale for treatment decisions is not always clear in retrospect and might vary over time. Our

main finding that disease severity increases in the majority of subsequent pregnancies at risk is however supported by the increase in almost all raw disease characteristics in Table S3.

CONCLUSION

The severity of anti-RhD mediated HDFN increases in the majority of subsequent pregnancies with RhD-positive foetuses. The risk of severe HDFN in a subsequent pregnancy can be estimated using the moment of antibody detection, antibody characteristics as reflected by ADCC test results and the severity of HDFN in the first immunized pregnancy. Mothers with antibodies occurring during their first pregnancy of a RhD-positive child, who never received RhIg, detected at 27^{th} week screening, are more at risk for developing severe disease in a subsequent pregnancy. Further research should focus on identifying this group of 'high-responders' to establish whether an additional, early administration of RhIg could be beneficial. Furthermore, the development of more effective non-invasive treatment options for foetuses affected by HDFN could possibly ameliorate outcome.

ACKNOWLEDGEMENTS

Thanks to Fleur Zwanenburg and Cindy de Jong, department of Obstetrics, LUMC, Leiden, the Netherlands, for their efforts in collecting and transforming study data without financial compensation.

DISCLOSURE OF INTEREST

Authors report no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

CZ and YMS: conception, planning, carrying out, analyzing and writing. JK, IvK, EL, DO, MdH: conception, planning, reviewing. PL: planning and analysing. JGvdB: conception, analysing, reviewing. EvdS: conception, reviewing. All authors have read and approved the final manuscript.

DETAILS OF ETHICS APPROVAL

The medical ethics committee of the Leiden University Medical Center approved the protocol (P15.101/NV/nv). Written informed consent was obtained from all mothers included in this study.

FUNDING

This research was supported by a grant from Sanquin Blood Supply (L2181). The design, conduct or publication of the study was not influenced by this financial support.

REFERENCES

1. Zwiers C, Oepkes D, Lopriore E, Klumper FJ, de Haas M, van Kamp IL. The near disappearance of foetal hydrops in relation to current state-of-the-art management of red cell alloimmunization. Prenatal diagnosis. 2018;38(12):943-50.

2. CentraalBegeleidingsOrgaan. Richtlijn Bloedtransfusie. 2011.

3. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Haemolytic disease of the foetus and newborn. Vox sanguinis. 2015;109(2):99-113.

4. Walker W, Murray S, Russell JK. Stillbirth due to haemolytic disease of the newborn. J Obstet Gynaecol Br Emp. 1957;64(4):573-81.

5. Mollison P, Engelfriet, CP, Contreras M. Blood Transfusion in Clinical Medicine. Oxford: Blackwell Science; 1993.

6. Koelewijn JM, de Haas M, Vrijkotte TG, Bonsel GJ, van der Schoot CE. One single dose of 200 microg of antenatal RhIG halves the risk of anti-D immunization and haemolytic disease of the foetus and newborn in the next pregnancy. Transfusion. 2008;48(8):1721-9.

7. Derrick Tovey LA, Robinson AE. Reduced severity of Rh-haemolytic disease after anti-D immunoglobulin. British medical journal. 1975;4(5992):320-2.

8. van der Ploeg CPB, Schönbeck Y, Oomen P, Vos K. Prenatale Screening Infectieziekten en Erytrocytenimmunisatie (PSIE). Procesmonitor 2016.: RIVM and TNO; 2018 23-7-2018.

9. Urbaniak SJ, Greiss MA, Crawford RJ, Fergusson MJ. Prediction of the outcome of rhesus haemolytic disease of the newborn: additional information using an ADCC assay. Vox sanguinis. 1984;46(5):323-9.

10. Oepkes D, van Kamp IL, Simon MJ, Mesman J, Overbeeke MA, Kanhai HH. Clinical value of an antibody-dependent cell-mediated cytotoxicity assay in the management of Rh D alloimmunization. Am J Obstet Gynecol. 2001;184(5):1015-20.

11. Tiblad E, Westgren M, Pasupathy D, Karlsson A, Wikman AT. Consequences of being Rhesus D immunized during pregnancy and how to optimize new prevention strategies. Acta obstetricia et gynecologica Scandinavica. 2013;92(9):1079-85.

12. Dajak S, Roje D, Haspl ZH, Maglic PE. The importance of antenatal prevention of RhD immunisation in the first pregnancy. Blood transfusion = Trasfusione del sangue. 2014;12(3):410-5.

13. Schonewille H, Doxiadis, II, Levering WH, Roelen DL, Claas FH, Brand A. HLA-DRB1 associations in individuals with single and multiple clinically relevant red blood cell antibodies. Transfusion. 2014;54(8):1971-80.

14. Hall AM, Cairns LS, Altmann DM, Barker RN, Urbaniak SJ. Immune responses and tolerance to the RhD blood group protein in HLA-transgenic mice. Blood. 2005;105(5):2175-9.

15. Stegmann TC, Veldhuisen B, Nagelkerke SQ, Winkelhorst D, Schonewille H, Verduin EP, et al. RhIg-prophylaxis is not influenced by FCGR2/3 polymorphisms involved in red blood cell clearance. Blood. 2017;129(8):1045-8.

16. MacKenzie IZ, Bowell P, Gregory H, Pratt G, Guest C, Entwistle CC. Routine antenatal Rhesus D immunoglobulin prophylaxis: the results of a prospective 10 year study. British journal of obstetrics and gynaecology. 1999;106(5):492-7.

17. Sonneveld ME, Koelewijn J, de Haas M, Admiraal J, Plomp R, Koeleman CA, et al. Antigen specificity determines anti-red blood cell IgG-Fc alloantibody glycosylation and thereby severity of haemolytic disease of the foetus and newborn. British journal of haematology. 2017;176(4):651-60.

18. Kapur R, Della Valle L, Sonneveld M, Hipgrave Ederveen A, Visser R, Ligthart P, et al. Low anti-RhD IgG-Fc-fucosylation in pregnancy: a new variable predicting severity in haemolytic disease of the foetus and newborn. British journal of haematology. 2014;166(6):936-45.