No. 448, April 2024 (Replaces No. 133, January 2018)

Guideline No. 448: Prevention of Rh D Alloimmunization

The English document is the original version; translation may introduce small differences in the French version.

This clinical practice guideline was prepared by the authors and overseen by the SOGC MFM Committee (now part of the Clinical Obstetrics Committee). It was reviewed by the SOGC Clinical Practice Obstetrics Committee (now the Clinical Obstetrics Committee) and approved by the SOGC Guideline Management and Oversight Committee.
This clinical practice guideline supersedes No. 133 published in January 2018.

Authors

Karen Fung-Kee-Fung, MD, Ottawa, ON
Karen Wong, MD, Ottawa, ON
Jennifer Walsh, MD, Calgary, AB
Candyce Hamel, PhD, Ottawa, ON
Gwen Clarke, MD, Victoria, BC

J Obstet Gynaecol Can 2024;46(4):102449

https://doi.org/10.1016/j.jogc.2024.102449

SOGC MFM Committee (2022): James Andrews, Sheryl Choo, Elisabeth Codsi, Jillian Coolen, Amélie Guay, Janine Hutson, Venu Jain, Noor Niyar R. Ladhani, Heather Martin, Kirsten Niles, Christy Pylypjuk, Genevieve Quesnel, Karen Wong.

SOGC Clinical Practice Obstetrics Committee (2023): Cynthia Chan, Elissa Cohen, Christine Dallaie, Kirsten Duckitt, Sebastian Hobson, Isabelle Létourneau, Amy Metcalfe, J. Larry Reynolds, debbie Reynolds, Marie-Ève Roy-Lacroix, Katherine Tyndall, Kathryn Versteeg.

Disclosures: Statements were received from all authors. No relationships or activities that could involve a conflict of interest were declared by Dr. Karen Fung-Kee-Fung, Dr. Karen Wong, Dr. Jennifer Walsh, and Dr. Candyce Hamel. Dr. Gwen Clarke declares that she received payment from Prenatal Screening Ontario for medical consultation, travel support from the International Society for Blood Transfusion (ISBT), payment for reviewing the case findings in a medical lawsuit, travel support to attend the ISBT conference, and is the Secretary General of the ISBT. All authors have indicated that they meet the journal's requirements for authorship.

Subject Categories: maternal fetal medicine, obstetrics, abortion

Keywords : Rh isoimmunization; Rho(D) immune globulin; erythroblastosis; fetal

Corresponding Author: Karen Fung-Kee-Fung, kfung@toh.ca

This document reflects emerging clinical and scientific advances as of the publication date and is subject to change. The information is not meant to dictate an exclusive course of treatment or procedure. Institutions are free to amend the recommendations. The SOGC suggests, however, that they adequately document any such amendments.

Informed consent: Patients have the right and responsibility to make informed decisions about their care, in partnership with their health care provider. In order to facilitate informed choice, patients should be provided with information and support that is evidence-based, culturally appropriate, and personalized. The values, beliefs, and individual needs of each patient in the context of their personal circumstances should be considered and the final decision about care and treatment options chosen by the patient should be respected.

Language and inclusivity: While the SOGC as a rule uses gendered language, in respect for our mission to advance women's health, there are contexts in which it is important to use gender neutral language, and to be fully inclusive. This guideline speaks to the needs of people are often marginalized or "unseen." For this reason, except where citing the words of others, the SOGC has chosen to use gender neutral language in this guideline. The SOGC recognizes and respects the rights of all people for whom the information in this document may apply, including but not limited to transgender, non-binary, and intersex people. The SOGC encourages health care providers to engage in respectful conversation with their patients about their gender identity and preferred gender pronouns and to apply these guidelines in a way that is sensitive to each person's needs.

Weeks Gestation Notation: The authors follow the World Health Organization's notation on gestational age: the first day of the last menstrual period is day 0 (of week 0); therefore, days 0 to 6 correspond to completed week 0, days 7 to 13 correspond to completed week 1, etc.

RECOMMENDED CHANGES IN PRACTICE

1. Routine blood typing and antibody screening is not required for pregnant individuals before 8 weeks gestation.
2. For Rh D-negative pregnant individuals undergoing potential sensitizing events such as threatened or spontaneous abortion, or induced abortion, Rho(D) immune globulin is not required before 8 weeks gestation.
3. The identification of D variants such as "weak D" or "partial D" on routine blood typing should be further investigated with *RHD* genotyping to determine the risk of alloimmunization.
4. Weak D types 1, 2, or 3 identified on *RHD* genotyping do not pose a risk of alloimmunization, and therefore, Rho(D) immune globulin prophylaxis is not required.

KEY MESSAGES

1. Blood typing and antibody screening are not required before 8 weeks gestation.
2. Prophylactic administration of Rho(D) immune globulin for early complications of pregnancy is not required before 8 weeks gestation since there is no compelling evidence of benefit from this practice.
3. *RHD* genotyping is required when routine blood typing identifies D variants (weak or partial D), as Rho(D) immune globulin prophylaxis is not required for weak D types 1, 2, or 3.
4. Rho(D) immune globulin is effective in the prevention of rhesus alloimmunization. When routine Rho(D) immune globulin prophylaxis is correctly administered, both antepartum and postpartum, to Rh D-negative pregnant individuals, the rate of Rh D alloimmunization can be reduced to less than 1%.
5. In Rh D-negative pregnant individuals, fetal *RHD* genotyping via cell-free fetal DNA testing can identify an Rh D-negative fetus and eliminate the unnecessary administration of Rho(D) immune globulin prophylaxis to patients not at risk. It is recommended in jurisdictions where this test is found to be cost-effective for routine screening.

DEFINITIONS

Anti-D: Antibodies to the Rh D antigen found in serum; these antigens may signal active alloimmunization or passive immunization with Rho(D) immune globulin.
Rho(D) immune globulin: A blood product containing a high titre of antibody to Rh D antigens of red blood cells; this product is available in Canada under the brand name WinRho SDF.

ABSTRACT

Objective: This guideline provides recommendations for the prevention of Rh D alloimmunization (isoimmunization) in pregnancy, including parental testing, routine postpartum and antepartum prophylaxis, and other clinical indications for prophylaxis. Prevention of red cell alloimmunization in pregnancy with atypical antigens (other than the D antigen), for which immunoprophylaxis is not currently available, is not addressed in this guideline.

Target Population: All Rh D-negative pregnant individuals at risk for Rh D alloimmunization due to potential exposure to a paternally derived fetal Rh D antigen.

Outcomes: Routine postpartum and antepartum Rh D immunoprophylaxis reduces the risk of Rh D alloimmunization at 6 months postpartum and in a subsequent pregnancy.

Benefits, Harms, and Costs: This guideline details the population of pregnant individuals who may benefit from Rho(D) immune globulin (RhIG) immunoprophylaxis. Thus, those for whom the intervention is not required may avoid adverse effects, while those who are at risk of alloimmunization may mitigate this risk for themselves and/or their fetus.

Evidence: For recommendations regarding use of RhIG, Medline and Medline in Process via Ovid and Embase Classic + Embase via Ovid were searched using both the trials and observational studies search strategies with study design filters. For trials, the Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and Database of Abstracts of Reviews of Effects via Ovid were also searched. All databases were searched from January 2000 to November 26, 2019. Studies published before 2000 were captured from the grey literature of national obstetrics and gynaecology specialty societies, luminary specialty journals, and bibliographic searching. A formal process for the systematic review was undertaken for this update, as described in the systematic review manuscript published separately.

Validation Methods: The authors rated the quality of evidence and strength of recommendations using the SOGC's modified GRADE approach. See Appendix A (Tables A1 for definitions and A2 for interpretations of strong and conditional [weak] recommendations).

Intended Audience: The intended users of this guideline include prenatal care providers such as obstetricians, midwives, family physicians, emergency room physicians, and residents, as well as registered nurses and nurse practitioners.

Tweetable Abstract: An updated Canadian guideline for prevention of Rh D alloimmunization addresses D variants, cffDNA for fetal Rh type, and updates recommendations on timing of RhIG administration.

SUMMARY STATEMENTS:

1. The earliest that the Rh D antigen has been identified in a fetus is 7^{3} weeks gestation (*low*).
2. There are limited data regarding the incidence of Rh D sensitization in pregnancies earlier than 8 weeks gestation, as studies on the use of Rho(D) immune globulin have not included pregnancies below this gestational age (*very low*).
3. Certain variations in expression of the Rh D antigen, such as weak D types 1, 2, and 3, have no risk of alloimmunization. Other D variants (including other types of weak D or partial D) do confer a risk of alloimmunization (*moderate*).
4. In Rh D-negative pregnant individuals at risk for alloimmunization, routine antepartum and postpartum RhIG prophylaxis reduces the risk of alloimmunization in subsequent pregnancies (*moderate*).
5. Universal genotyping of fetal blood type in Rh D-negative pregnant individuals can reduce unnecessary administration of RhIG to individuals carrying an Rh D-negative fetus and who are therefore not at risk (*high*).
6. As little as 0.1 mL of Rh D-positive red blood cells is considered potentially sensitizing for Rh D-negative individuals (*low*).
7. In Rh D-negative pregnant individuals receiving routine antenatal prophylaxis at 28 weeks, there is insufficient evidence to recommend additional dosing at 40 weeks gestation if they have not delivered by that date. (*very low*).

RECOMMENDATIONS:

1. Routine blood group typing and antibody screening in pregnant individuals before 8 weeks gestation is not recommended (*conditional, low*).
2. For all pregnant individuals, we recommend blood group typing and antibody screening at the first prenatal visit after 8 weeks gestation (*good practice point*).
3. For prenatal patients with discrepant, weak, or inconclusive Rh D blood typing, further investigation with *RHD* genotyping is recommended to determine candidacy for Rho(D) immune globulin. Weak D types 1, 2, and 3 carry no risk of alloimmunization, and Rho(D) immune globulin prophylaxis is not recommended for these individuals. The presence of other weak D variants, or partial D carries a potential risk of alloimmunization, and Rho(D) immune globulin prophylaxis is recommended (*strong, moderate*).
4. For non-sensitized Rh D-negative pregnant individuals, administration of 300 μg of Rho(D) immune globulin at 28 weeks is recommended when the fetal blood type is unknown or known to be Rh D-positive (*strong, moderate*). Alternatively, 2 doses of 120 μg may be given (120 μg being the lowest dose currently available in Canada) at 28 and 34 weeks gestation (*strong, moderate*).
5. For non-sensitized postpartum Rh D-negative individuals with a Rh D-positive newborn, we recommend administration of 120 or 300 μg within 72 hours of delivery, with testing and additional Rho(D) immune globulin given for fetomaternal hemorrhage over 6 or 15 mL of fetal red blood cells, respectively (or 12 mL or 30 mL fetal blood, respectively) (*strong, moderate*). If Rho(D) immune globulin is not given within 72 hours of delivery, we recommend that 300 μg of Rho(D) immune globulin be given as soon as the need is recognized, for up to 28 days after delivery (*strong, moderate*).
6. For pregnant individuals with a suspected fetomaternal hemorrhage, we recommend routine quantitative testing with the Kleihauer-Betke test or flow cytometry after 20^0 weeks gestation in Rh D-negative individuals to tailor the appropriate dosage of Rho(D) immune globulin (*good practice point*).
7. For confirmed fetomaternal hemorrhage, the dose of Rho(D) immune globulin should be titrated to the volume of fetal blood (or fetal red blood cells) in the maternal circulation; for hemorrhage less than 12 mL (6 mL fetal red blood cells), 120 μg of Rho(D) immune globulin should be given; for hemorrhage between 12 and 30 mL (6−15 mL fetal red blood cells), 300 μg should be given; and for hemorrhage over 30 mL, an additional 10 μg should be given for every additional 1mL of fetal blood (0.5 mL fetal red blood cells) (*conditional, very low*). The dose given may be titrated to the next available vial size. The appropriate dosage should be given within 72 hours of the sensitizing event (*strong, moderate*).
8. For non-sensitized Rh D-negative individuals who have experienced threatened, spontaneous or induced abortion, ectopic pregnancy, or molar pregnancy before 8 weeks gestation, we recommend not administering Rho(D) immune globulin (*conditional, low*).
9. For non-sensitized Rh D-negative individuals who have experienced threatened, spontaneous, or induced abortion, ectopic pregnancy or molar pregnancy between 8 and 12 weeks gestation, we suggest not administering Rho(D) immune globulin. In individuals who are more risk averse, Rho(D) immune globulin may be considered (*conditional, low*).
10. For non-sensitized Rh D-negative individuals who have experienced threatened, spontaneous or induced abortion, ectopic pregnancy or molar pregnancy after 12 weeks gestation, we suggest administration of 300 μg of Rho(D) immune globulin (*conditional, low*). If the diagnosis of complete mole is certain, Rho(D) immune globulin is not required (*conditional, low*).
11. Following chorionic villous sampling in non-sensitized Rh D-negative individuals, we recommend Rho(D) immune globulin at a minimum dose of 120 μg during the first 12 weeks of gestation and 300 μg thereafter (*conditional, very low*).
12. For non-sensitized Rh D-negative individuals who have undergone second- or third-trimester invasive fetal procedures (e.g., amniocentesis, cordocentesis) or external cephalic version, we suggest 300 μg of Rho(D) immune globulin (*conditional, very low*).
13. For additional, ongoing, or recurrent potentially sensitizing events (e.g., recurrent antepartum hemorrhage, multiple fetal procedures), serial testing for fetomaternal hemorrhage and passive Rho(D) immune globulin is suggested to determine appropriate additional dosing (*good practice point*).

INTRODUCTION

Antenatal and postpartum administration of Rho(D) immune globulin (RhIG) to Rh D-negative mothers incompatible with their fetuses for paternally derived Rh D antigen has been a major public health success story over the last 50 years. In Canada, prior to the implementation of Rho(D) immunoprophylaxis, the incidence of Rh D sensitization within 6 months of delivery was approximately 7.2% for an Rh D-negative susceptible patient delivering an Rh D-positive infant. With Rho(D) immune globulin (RhIG), the incidence decreases to 1.8% after postpartum treatment alone, and 0.07% following the introduction of routine antenatal treatment at 28 weeks gestation.[1]

For low- and middle-income countries, however, where access to immunoprophylaxis is lacking, the burden of RhD alloimmunization remains high with neonatal mortality and morbidity rates estimated to be about 11 times higher than those of high-income countries.[2] Even with structured prophylaxis programs like those in Canada, treatment failures can and do occur. In addition, recent advances in maternal and fetal genotyping enhance opportunities to identify at-risk individuals who would benefit from immunoprophylaxis.

This update of the previously published guideline,[3] considers these recent developments and provides direction for obstetrical care providers. Two systematic reviews were conducted to identify studies published since 2003 and to evaluate the certainty of the evidence related to the administration of RhIG for immunoprophylaxis in pregnancy and in the peripartum period.[4] For these reviews, a formal GRADE approach was used to evaluate the evidence. This approach considers the risk of bias, indirectness, imprecision, inconsistency, and publication bias at the outcome level.

The recommendations in this guideline are labelled with the strength of the recommendation (i.e., strong, conditional) and the quality of the evidence (i.e., very low, low, moderate, high) informing the recommendation, following the SOGC's "modified GRADE" approach. This guideline does not address alloimmunization to atypical (i.e., non-Rh D) antigens, for which prophylaxis is not available.

ABBREVIATIONS

FMH	fetomaternal hemorrhage
HbF	fetal hemoglobin
RBC	red blood cell
RhIG	Rho(D) immune globulin

RHO(D) IMMUNE GLOBULIN

RhIG is a blood product containing a high titre of antibody to Rh D antigens of red blood cells (RBCs). It is obtained from pooled human plasma and is effective in the prevention of active rhesus alloimmunization.[5] Although several preparations are manufactured in North America, in Canada, availability is virtually limited to a single product, WinRho SDF (Emergent BioSolutions, formerly Cangene Corporation of Winnipeg). The product is available in vials of 600 IU (120 μg), 1500 IU (300 μg), and 5000 IU (1000 μg). This product can be administered by intravenous (IV) or intramuscular (IM) routes, with a half-life of 24 and 30 days, respectively.[6] An additional product, Hyper RHO S/D (syringe) is a product not licensed by Health Canada, but available under the Special Access Programme only.

The nature of polyclonal anti-D as a pooled blood product with potential infectious risks coupled with the relative shortage of the product in some jurisdictions, has prompted research into the development of a synthetic, recombinant monoclonal product. Completed phase two trials on the monoclonal product rozrolimupab have revealed some limitations, such as the need for administration of costly high doses and decreased efficacy compared with the polyclonal products.[7] This product is not currently available for routine use in Canada.

Route of Administration

RhIG may be administered either intravenously or intramuscularly; the chosen route may depend on institutional, physician, or patient preference. A systematic review identified two randomized controlled trials that compared IV and IM routes of administration of anti-D prophylaxis at 28 weeks gestation and found no difference between routes.[4]

ASSESSMENT OF RH D ALLOIMMUNIZATION RISK

Blood Grouping and Antibody Screening in Pregnancy

The prevalence of the Rh D-negative blood type is population-specific and related to relative gene frequencies in that population cohort. Incidence varies widely from less than 1% in the Han Chinese population[8] to 27% in the Spanish Basque population.[9] White populations have an approximate 15% incidence of Rh D-negative blood.[10]

Determination of maternal blood type as an element of prenatal screening at the first antenatal visit aims to identify Rh D-negative people at risk of rhesus alloimmunization as potential candidates for Rh D immunoprophylaxis.

The earliest gestational age at which the D antigen has been detected on embryonic RBCs was 52 days (7^3 weeks gestational age), or 38 days after conception.[11] There are limited data regarding the incidence of Rh D sensitization in pregnancies earlier than 8 weeks gestation, as studies on RhIG use have not included pregnancies below this gestational age.[12] Thus, we suggest that maternal blood group typing and antibody screening be done after 8 weeks gestation, as there is no requirement for prophylaxis before this gestational age. In uncomplicated pregnancies, this bloodwork may be scheduled to coincide with first-trimester screening (11−14 weeks), where available.

Pregnant individuals with Rh D-negative blood should be advised of this result, the possible repercussions of alloimmunization should sensitizing events occur during pregnancy, and the existence of established prophylaxis programs. Important points that may impact counselling should be elicited from the patient, including past pregnancy and neonatal outcomes, transfusion history, social risk factors for sensitization (e.g., needle-sharing), and the paternal Rh D type, if it is known.

Recommendations 1 and 2

D Variants (Weak D and Partial D)

At the time of antenatal serological testing, some individuals may have weakly reactive Rh D or results that are discrepant from prior testing. Historically this was termed *weak D or D^u*. Today, the genetic basis of these D variants is better understood.[13] The most common variants are called *weak D* and are characterized by a quantitative decrease in D antigens on the red cell surface. Another type of Rh D variant is the *partial D* phenotype, for which the Rh D antigen is present but altered, potentially allowing an individual to form alloantibodies to the epitopes on Rh D-positive RBCs that are different from their own.

Previously, a follow-up serologic weak D test was routine in Canada for individuals with a weakly reactive result on initial Rh D typing (i.e., agglutination graded ≤2+) to establish a serologic weak D phenotype. In line with the National Advisory Committee on Blood and Blood Products (NAC), it is now recommended that individuals with a weakly reactive Rh D result have *RHD* genotyping performed.[14] Similarly, individuals with discrepant or inconclusive Rh D test result should have *RHD* genotyping. *RHD* genotyping is available to all pregnant individuals in Canada through reference laboratories at Canadian Blood Services (Edmonton, AB) and Héma-Québec (Montréal, QC). Most commonly, genotyping identifies weak D types 1, 2, or 3.[14] These weak D types confer no risk for alloimmunization to the D antigen, and the patient can be safely considered Rh D positive, with no requirement for RhIG prophylaxis.[13,14] Conversely, if genotyping reveals any other weak D type or a partial D variant, the individual should be considered at risk for developing allo-anti-D if exposed to the D antigen. These patients should be offered RhIG prophylaxis at the usual times during pregnancy.[13,14] There is debate among experts regarding the management of weak D type 4; the conservative approach is to provide RhIG prophylaxis.[14]

Recommendation 3

Identification of Pregnant Individuals at Risk for Rh D Alloimmunization

RhIG is offered to all Rh D-negative pregnant individuals at risk for alloimmunization due to incompatibility with an Rh D-positive fetus. The Rh D antigen in a Rh D-positive fetus is paternally derived. This implies that in situations where paternity is certain and this individual is documented to be also Rh D-negative such an incompatibility will not exist and RhIG may be omitted. Caution must be exercised, however, as the reported incidence of misattributed paternity in Canada is estimated to be 4%.[15]

In some countries, universal genotyping of the fetal blood group using cell-free fetal DNA in maternal blood is integrated into routine antenatal care.[16,17] Such an approach provides the advantage of targeted administration of RhIG immunoprophylaxis to Rh D-negative pregnant people who carry Rh D-positive fetuses, without the need for paternal testing. Such an approach has the potential to prevent unnecessary administration of a pooled blood product to approximately 40% of Rh D-negative women whose fetuses are also negative for the Rh D antigen,[18] while preserving stocks of RhIG, which are limited in certain jurisdictions.

Several analyses of this universal approach to screening have been conducted with varying results.[19−21] The false-negative rate of fetal *RHD* genotyping is up to 0.2%, depending on the assay used.[18] While acknowledging the accuracy of the test and the benefits described above, most of these reports point to the cost of fetal genotyping as the rate-limiting step to cost-effectiveness. In Canada, fetal

genotyping is currently outsourced to international reference labs with added costs. Geographical distribution of patients within Canada may also limit timely access to testing.

Therefore, universal noninvasive fetal genotyping of Rh D-negative pregnant individuals who are not already alloimmunized is currently not the standard of care. Further research is required to revisit this question as the cost of the technology declines with high throughput testing and as patient preferences and values dictate.

Passive Versus Active Immunization

When anti-Rh D is identified on antibody screening in an Rh D-negative person, it may represent passive transfer of administered RhIG or active alloimmunization. Differentiation requires knowledge of whether the patient has received RhIG and the timing of administration.

One study demonstrated that passive RhIG was no longer detectable in patient serum 96 days (approximately 13[5] weeks) after receiving RhIG[22]; however, the ability to detect residual RhIG 10−13 weeks after administration depends on the sensitivity of the assay used.[23] Where there is uncertainty regarding whether the identified antibody is passive or active, we suggest serial antibody screening and titre. Passive antibody is expected to decrease over time. Individuals with anti-Rh D due to active alloimmunization *do not* require RhIG immunoprophylaxis, while those with confirmed passive transfer *do* require prophylaxis. If it is uncertain whether the antibody is passive or active, we suggest continued administration of RhIG immunoprophylaxis.

PROPHYLACTIC ADMINISTRATION OF RHIG

Prior to Administration of Prophylaxis

RhIG is indicated for prevention of Rh D alloimmunization in individuals not previously sensitized; however, there is debate among clinicians regarding routine screening for the presence of antibodies prior to administration. In Rh D-negative individuals, the rationale for repeat blood group typing and screening at 28 weeks prior to administration of RhIG is to identify those who have since become alloimmunized and therefore would no longer be candidates for this prophylaxis. A Canadian study at two hospitals providing low-risk obstetrical care found that the prevalence of new alloimmunization between the first-trimester antibody screen and 28 weeks gestation was 42 out of 17 568 (0.24%); none of the detected antibodies were anti-D.[24] Acknowledging that the incidence of developing anti-D by 28 weeks gestation is low, we continue to suggest pretreatment antibody screening to identify those who may need other, more intensive monitoring and/or treatment due to the presence of alloantibodies. However, RhIG administration should not be deferred pending the antibody screening result.

Informed consent must be obtained prior to administration of any blood product. If the individual refuses RhIG, they should be advised of the potential consequences of not receiving the prophylaxis. A patient information brochure is available from the manufacturer of RhIG.

Administration of Routine Prophylaxis

Postpartum Prophylaxis

Postpartum administration of RhIG to Rh D-negative mothers at risk has been part of routine prophylaxis programs in Canada since the 1970s. Data from both randomized controlled trials and observational studies have informed this practice, albeit with significant heterogeneity among the studies in terms of dosage and the number and timing of doses (i.e., 2 antenatal plus postpartum, 1 antenatal plus postpartum, postpartum only).[4]

The baseline risk of Rh D alloimmunization without routine prophylaxis has been reported as approximately 7% at 6 months postpartum[25] and 17% in a subsequent pregnancy.[26] A systematic review determined that postpartum administration of RhIG 24−72 hours after delivery reduced the number of individuals sensitized to Rh D at 6 months postpartum (70 per 1000 fewer, 95% CI 67−71 per 1000) and in a subsequent pregnancy (130 per 1000 fewer; 95% CI 117−139 per 1000) compared with not receiving RhIG, regardless of dosage used.[4] However, another systematic review found that a very low dose of 50 μg increased the risk of sensitization in a following pregnancy compared with higher doses.[5]

Newborn blood typing using cord blood is recommended to ascertain the need for postpartum prophylaxis. Since RhIG is available in Canada as vials of 120 or 300 μg, a single dose of 120 or 300 μg can be given, with quantification of fetomaternal hemorrhage (FMH) to determine whether additional RhIG is required. If the 120-μg dose was given, FMH over 12m L requires additional RhIG; if the 300-μg dose was used, FMH over 30 mL requires additional RhIG. If RhIG is not given within 72 hours of delivery, 300 μg of RhIG should be given as soon as the need is recognized, for up to 28 days after delivery.

Antepartum Prophylaxis

Following the success of the RhIG postpartum trials, attention turned to investigating the potential benefits of

additional antenatal administration of immunoprophylaxis to protect against immunizing events during pregnancy and delivery. It is estimated that without antenatal RhIG prophylaxis, 1%–2% of Rh D-negative women at risk become sensitized.[27] In a systematic review, use of antepartum prophylaxis together with routine postpartum prophylaxis resulted in fewer individuals (9 per 1000; 95% CI 2–11 per 1000) sensitized to Rh D at delivery and up to 12 months postpartum.[4]

There is debate regarding use of a single dose (300 μg) at 28 weeks gestation compared with a 2-dose regimen (120 μg/dose) at 28 and 34 weeks gestation. The Royal Australian and New Zealand College of Obstetricians and Gynaecologists[28] and the British Society for Haematology (BSH)[29] support a 2-dose regimen. The 2-dose approach may achieve a higher circulating concentration of RhIG as term approaches than the single larger dose. Turner et al. conducted a bias-adjusted meta-analysis of studies assessing various doses of antepartum RhIG and concluded that a 1250 IU (250 μg) dose at 28 and 34 weeks was the most effective at preventing alloimmunization with a decreased odds of sensitization (OR 0.19; 95% CI 0.03–0.53), while a single dose of 1500 IU (300 μg) at 28–30 weeks had an OR of 0.42 (95% CI 0.17–0.73).[30] However, in a U.K.-based cohort study, White et al. reported lower compliance in administration of a 2-dose regimen compared with a 1-dose regimen.[31] Thus, given the added cost and risk of lower compliance with 2 doses and evidence for acceptable efficacy with 1 dose, we continue to recommend a single dose of 300 μg RhIG at 28 weeks, although the 2-dose regimen may also be used.

Figure 1 presents a flow chart for routine antepartum and postpartum Rh D prophylaxis in Rh D-negative pregnant individuals.

Recommendations 4 and 5

Repeat dosing at 40 Weeks Gestation

Twelve weeks after injection of 300 μg (1500 IU) of RhIG, 56%–75% of individuals have no residual anti-D.[32] Bowman and Pollock noted that 3 of 9 failures of antenatal prophylaxis occurred in women delivering at least 13[5] weeks after the antenatal dose of RhIG.[1] A policy of induction of labour before 42 weeks gestation may therefore influence the rate of Rh D sensitization and the decision to administer an additional dose of RhIG after 40 weeks gestation. There is currently insufficient evidence for or against administering another dose of RhIG to an unsensitized Rh D-negative individual who has not delivered by 40 weeks.

Additional Clinical Indications for RhIG Prophylaxis

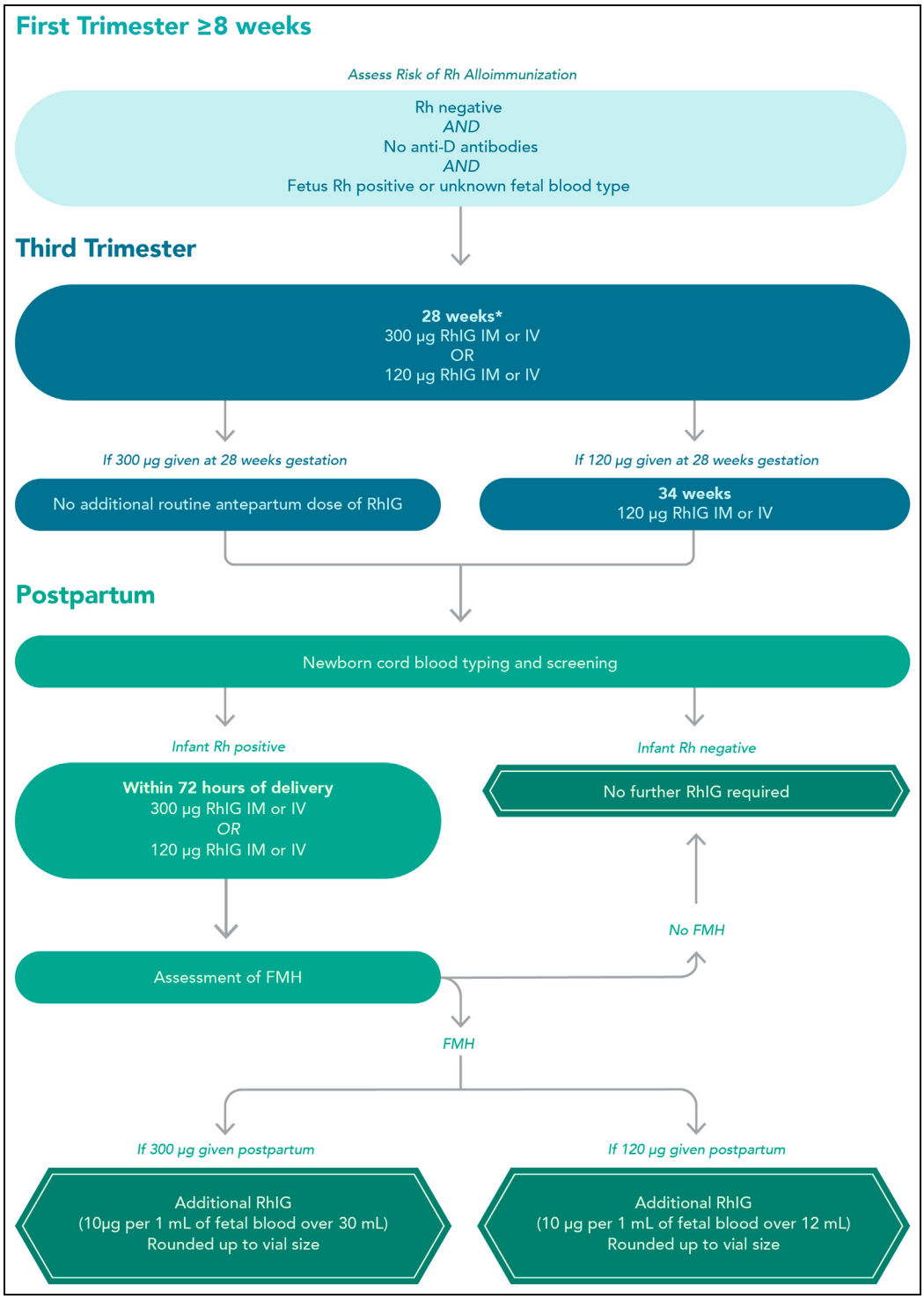
Fetomaternal Hemorrhage

Experiments in D-negative men injected with D-positive cells and RhIG established that 20 μg of RhIG protects against 1 mL of D-positive RBCs (about 2 mL of fetal blood).[33] A dose of 300 μg of RhIG protects against 30 mL of fetal blood (15 mL of fetal RBCs), and 120 μg protects against 12 mL of fetal blood (6 mL of fetal RBCs). It has been demonstrated that even 0.1 mL of D-positive RBCs can sensitize some Rh D-negative women,[34] thus this volume of fetal RBCs is considered potentially sensitizing.

Fetoplacental blood volume varies by gestational age and fetal weight. At 20 weeks gestation, the fetoplacental blood volume is estimated to be 30 mL.[35] Thus, in the case of a sensitizing event before 20 weeks gestation, the entirety of the fetal blood volume would be covered by a single 300 μg dose of RhIG, and quantification of FMH is not required (Figure 2). After 20 weeks gestation, additional RhIG may be required depending on the quantity of fetal blood within maternal circulation, and quantification of FMH is recommended (Figure 3).

Tests for FMH may be qualitative or quantitative.[36] The rosette test is a qualitative test for Rh D-negative individuals carrying an Rh D-positive fetus. In this test, maternal blood is mixed with exogenous Rh D antibodies, which adhere to any Rh D-positive fetal cells. *Indicator* RBCs are then added, which cluster around the antibody-coated fetal cells, forming rosettes that can be identified under the microscope. Quantitative tests include the Kleihauer-Betke test and flow cytometry. The former is an acid elution test, in which a smear of maternal venous blood is obtained, subjected to an acid solution, and stained. Adult hemoglobin is easily eluted by acid, in contrast to fetal hemoglobin (HbF). As a result, when viewed under a microscope, adult RBCs that do not contain HbF will appear as clear ghost cells, whereas fetal RBCs containing HbF will maintain their colour. Fetal cells are counted and expressed as a percentage of adult cells. This percentage is then used to calculate the volume of FMH. There may be interobserver variability in identification of fetal ghost cells, and the calculation of FMH volume assumes a constant maternal blood volume of 5 L. Because of the reliance on the presence of HbF, this test may also be inaccurate for those with hemoglobinopathies

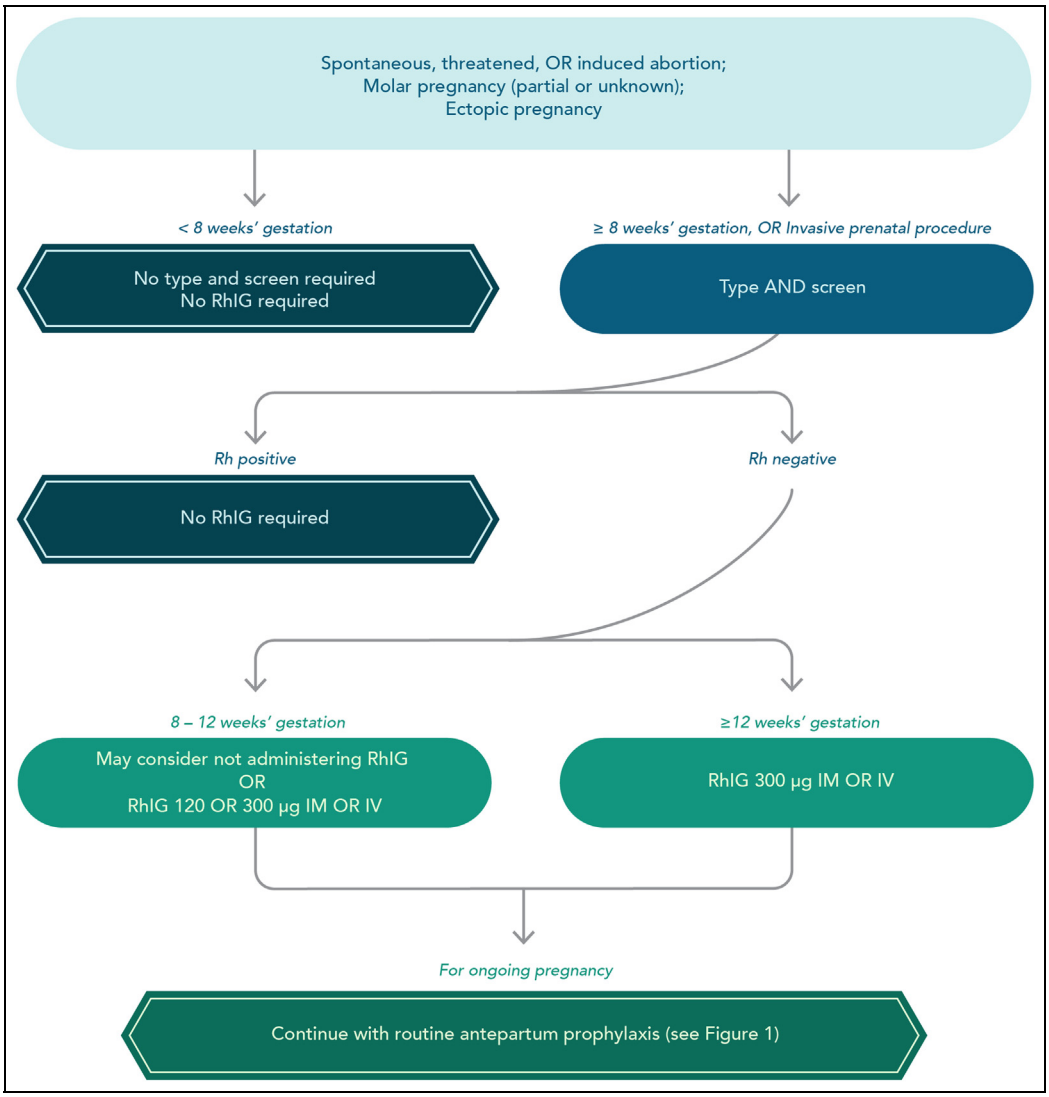
Figure 1. Routine prophylaxis against Rh D alloimmunization for pregnant individuals (*non-sensitized).



FMH: fetomaternal hemorrhage; IM: intramuscular; IV: intravenous; RhIG: Rho(D) immune globulin.

or near term, when the fetus begins to produce adult hemoglobin. Flow cytometry is another quantitative test for FMH that identifies the presence of HbF in fetal RBCs. This test is less operator-dependent than Kleihauer-Betke testing and can differentiate false-positive test results in those with hemoglobinopathies. Fluorescent monoclonal antibodies to HbF are mixed with the blood sample, and the fluorescence intensity is measured. This technique differentiates between adult and fetal HbF-containing cells using differential fluorescence intensity between HbF-containing adult and fetal cells, mean cell volume, and/or the presence of other RBC antigens. This

Figure 2. Potentially sensitizing events in pregnancy at less than 20 weeks gestation and RhIG prophylaxis.



IM: intramuscular; IV: intravenous; RhIG: Rho(D) immune globulin.

test is typically performed with multiple samples at once (i.e., batched); hence, is not available for single urgent cases. Laboratories in Canada have varying capacities to provide one or both of these tests for detecting FMH.

Clinical conditions and events associated with potential placental trauma or disruption of the fetomaternal interface (e.g., placental abruption, external cephalic version, blunt trauma to the abdomen, placenta previa with bleeding) can lead to sensitizing FMH. Measurement of FMH volume in these scenarios is prudent. No studies were identified in the systematic review specific to placental trauma.[4]

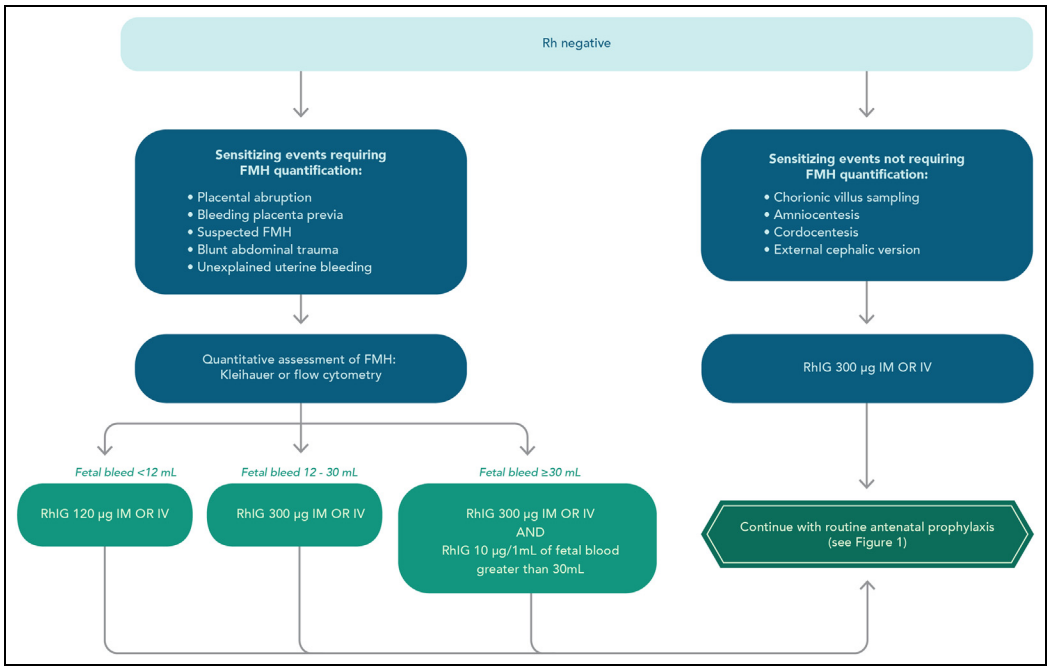
Spontaneous massive FMH may rarely occur and presents as decreased fetal movement with abnormal fetal heart rate pattern, fetal anemia, hydrops, or stillbirth. Quantitative FMH testing may be used in such cases to make the diagnosis, and in the Rh D-negative patient, determine the dosage of RhIG required.

Recommendations 6 and 7

Bleeding and Induced Abortion Before 20 Weeks Gestation

Prior to 8 weeks gestation, there is no evidence or pathophysiologic rationale for the use of RhIG. Using flow cytometry, Horvath et al. demonstrated that, in those undergoing uterine aspiration in the first trimester, fetal RBCs do enter the maternal circulation but in quantities below the threshold for sensitization (0.1 mL per 4 L of maternal blood).[37] This group also found that following

Figure 3. Potentially sensitizing events in pregnancy at greater than 20 weeks gestation and RhIG prophylaxis.



FMH: fetomaternal hemorrhage; IM: intramuscular; IV: intravenous; RhIG: Rho(D) immune globulin.

induced medical or surgical abortion before 12 weeks' gestation, circulating fetal RBCs in maternal circulation did not exceed the threshold for sensitization.[38]

However, there are reports of Rh D immunization occurring following spontaneous or induced abortion after 8 weeks,[39,40] and therefore there may be a role for RhIG prophylaxis. A systematic review[4] identified two randomized controlled trials that evaluated RhIG in women after spontaneous or induced abortion: one compared 300 μg with placebo[41] and the other compared a higher dosage (300 μg) with a lower dosage (50 μg) of RhIG.[42] Among the 803 participants in these trials, none became immunized at 6 months of follow-up. Another systematic review identified two other studies that assessed the use of RhIG after miscarriage or abortion up to 12 weeks gestation, with a total of 214 Rh D-negative patients. Sensitization at 6 months of follow-up only occurred in individuals not receiving RhIG; however, meta-analysis was not conducted because of significant study heterogeneity, and the authors concluded there was limited evidence on the effectiveness of anti-D in early miscarriage or abortion.[43] Thus, we do not recommend blood group typing, antibody screening, or provision of RhIG prior to 8 weeks gestation. For spontaneous or induced abortion between 8-12 weeks gestation, there is some evidence that the fetal RBCs entering maternal circulation are not sufficient to lead to sensitization, clinicians may consider not administering RhIG.[38] However, there are rare reports of sensitization following procedures at this gestational age, and clinicians may administer RhIG in this group following a discussion of risks and benefits. We continue to recommend RhIG for pregnancy loss after 12 weeks gestation, as there is a possible risk of sensitization.

No conclusive evidence was identified concerning the use of RhIG in ectopic pregnancy. However, 25% of individuals with a ruptured tubal pregnancy have a significant number of fetal RBCs in their circulation, suggesting that RhIG is indicated.[44]

Complete hydatidiform mole is composed entirely of trophoblastic tissue. Since trophoblastic tissue does not express Rh D antigen, expert consensus maintains that RhIG may be omitted when complete mole is diagnosed in non-sensitized Rh D-negative individuals. However, by the same reasoning, RhIG should not be omitted for partial mole or uncertain diagnosis of molar pregnancy because fetal RBCs are present. No studies were identified in the systematic review specific to molar pregnancy.[4]

There is little evidence to support what dosage of RhIG should be provided in the first trimester.

Recommendation 8

Invasive Fetal Diagnostic Procedures and External Cephalic Version

Even with sonographic placental localization, a potentially immunizing volume of FMH (>0.1 mL) occurs in at least 2% of pregnancies undergoing amniocentesis.[45] Fourteen percent of first-trimester chorionic villi sampling procedures results in FMH.[46] FMH can occur following cordocentesis, particularly if a transplacental route is chosen, and the prevalence of FMH following cordocentesis exceeds that following amniocentesis.[47]

There are few studies evaluating the administration of RhIG after invasive fetal diagnostic procedures. A small comparative cohort study from the U.K. Medical Research Council (Working Party on Amniocentesis) in 1978 evaluated 117 women who had received amniocentesis before 20 weeks gestation.[48] Half of the women (n = 59) were given RhIG after the procedure and 58 women were not. The report did not specify dosage. Among the 117 women, 3 who did not receive RhIG became sensitized. In a systematic review, no studies involving chorionic villi sampling and cordocentesis were identified.[4]

FMH has been identified in 1%–6% of attempted or successful external cephalic version attempts.[49]

Recommendations 9 and 10

Ongoing Hemorrhage

There is no evidence to inform Rh D immunoprophylaxis strategies in the context of ongoing antenatal hemorrhage. Considering international guidance, the BSH suggests serially testing patients with ongoing hemorrhage after 20 weeks gestation by quantitative methods (i.e., the Kleihauer-Betke test).[29] Additional RhIG is administered at a dose sufficient for the quantity of FMH thus identified. However, guidelines from the American College of Obstetricians and Gynecologists (ACOG) recommend only performing the Kleihauer-Betke test and providing an additional RhIG dose for those with a negative indirect Coombs test (i.e., no remaining passive RhIG).[50] The BSH recommends a conservative testing interval of every 2 weeks in those with ongoing intermittent hemorrhage, while ACOG suggests assessment can occur every 3 weeks.

Similar to the above international guidelines, serial testing for FMH and the presence of passive RhIG every 2–3 weeks is suggested for ongoing antepartum hemorrhage, in line with ACOG and BSH recommendations (see Figure 4). Where this testing is positive, RhIG should be given at a dose sufficient for the quantity of hemorrhage identified. If antibody screening results are negative (i.e., no remaining passive RhIG), RhIG should be administered. If antibody screening results are positive and quantitative testing did not identify FMH, additional RhIG is not required.

Routine antepartum and postpartum RhIG prophylaxis should continue on schedule, regardless of additional RhIG provided for potentially sensitizing events.

Recommendation 11

PATIENT SAFETY ISSUES RELATED TO ADMINISTRATION OF RHIG

To understand the true safety profile of a drug, monitoring and reporting of adverse drug events and medication errors is necessary. There is a dearth of information regarding the event rate of adverse drug effects and medication errors surrounding the use of RhIG because of the absence of mandatory regulatory reporting requirements for such events in most jurisdictions.

Medication Errors

In the U.K., where mandatory reporting exists for RhIG products, a 2016 report described RhIG-related errors, including late administration or omission for eligible patients and laboratory error. These errors resulted in 3 patients becoming sensitized in their subsequent pregnancies.[51]

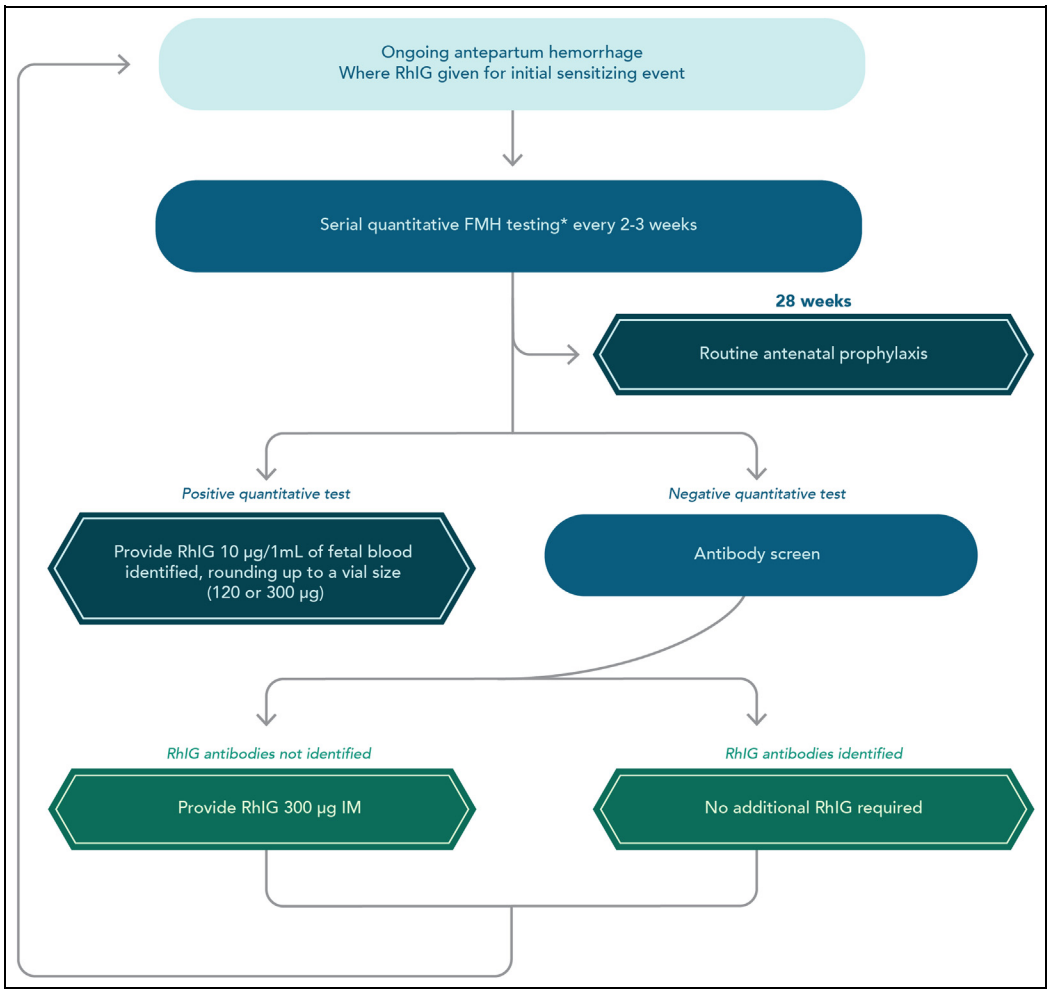
Regarding the Canadian experience, one study reported an 85% antenatal treatment rate in eligible Rh D-negative women compared with a 98% postpartum treatment rate.[52] Factors influencing this observation included lack of prenatal care prior to the third trimester, transfer from an outside facility, and licensing of attending physicians prior to 1980.

These reports underscore the need for broad education strategies, along with the use of checklists to ensure adherence to local protocols and robust record keeping and traceability of product use to facilitate communication between the various obstetrical care providers and lab personnel in a progressively complex clinical environment.

Adverse Events

Early RhIG preparation involved Cohn fractionation techniques, which resulted in residual impurities and

Figure 4. Ongoing antepartum hemorrhage after 20 weeks gestation and management of RhIG prophylaxis.



*Kleihauer-Betke test or flow cytometry.
FMH: fetomaternal hemorrhage; IM: intramuscular; IV: intravenous; RhIG: Rho(D) immune globulin.

higher risks of anaphylactic events. In addition, an outbreak of hepatitis C in Ireland in the late 1970s was attributed to contaminated RhIG, though this was prior to the identification of the hepatitis C virus. The Cangene Corporation of Winnipeg used ion exchange chromatography to create a purer product, WinRho, that could be administered intramuscularly or intravenously. No cases of transmission of infectious pathogens have been reported in North America since implementation of the newer preparations of RhIG.[53]

High doses of RhIG in conditions such as immune thrombocytopenic purpura have been associated with disseminated intravascular coagulation, renal failure, and intravascular hemolysis. These events have not been reported in pregnant individuals given usual doses for Rh D immunoprophylaxis. Minor symptoms of pain at the injection site, headache, flushing, and general malaise have been reported.[6] Case reports and small case series acknowledge the possibility of rare adverse effects in certain identifiable at-risk populations. Anaphylaxis following RhIG administration has been reported in patients with immunoglobulin A (IgA) deficiency or antibodies to IgA, and use of RhIG preparations that are IgA-depleted is advised. Caution is also advised in individuals with insulin-dependent diabetes when RhIG products that have been stabilized in maltose (e.g., WinRho SDF) are given, as this product can interfere with certain glucose monitoring systems (i.e., glucose testing strips using GDH-PQQ methodology), giving falsely elevated glucose readings and potentially leading to over-administration of insulin and subsequent hypoglycemia.[54]

Management of Hypersensitivity Reactions

Both anaphylaxis and delayed transfusion reactions have been reported with RhIG. Once hypersensitivity to RhIG is suspected, it presents a management challenge for the subsequent pregnancy. Referral to an allergist is suggested, with possible cautious drug challenge testing in a hospital setting in collaboration with an obstetrician and anesthesiologist. Fetal *RHD* genotyping may play a role in determining whether Rh D immunoprophylaxis is indeed necessary. Strategies to mitigate risks in susceptible women with Rh D-positive fetuses include restriction of RhIG to the postpartum period and progressive desensitization to RhIG by successive, small interval dosing (e.g., 10%, 30%, and 60% of the desired total dosage administered at 30-minute intervals).[55]

Neonatal Effects

The transplacental passage of RhIG has raised concerns over potential adverse effects to the fetus, especially if born prematurely and shortly after the routine antenatal administration of RhIG. Limited retrospective and observational data suggest the risk is minimal. In a cohort of 94 preterm infants born at 28–34 weeks gestation whose mothers received RhIG at 28 weeks gestation, slightly higher bilirubin levels were encountered in the first 3 days of life without evidence of significant hemolysis requiring phototherapy or transfusion. Hematocrit levels also remained stable.[56]

CONCLUSION

Routine use of RhIG in Rh D-negative individuals has significantly reduced the incidence of Rh D alloimmunization in the past several decades. This guideline outlines the judicious use of RhIG in the Canadian context, including refined recommendations for pregnancies earlier than 8 weeks gestation and updates on testing for and management of D variants. Multidisciplinary collaboration and communication between care providers and transfusion medicine laboratories will maximize effectiveness while minimizing medication error and treatment failures related to RhIG administration.

Acknowledgements: The authors would like to acknowledge and thank special contributor Mina Majd of Ottawa, ON.

REFERENCES

1. Bowman JM, Pollock JM. Failures of intravenous Rh immune globulin prophylaxis: an analysis of the reasons for such failures. Transfus Med Rev 1987 Aug;1(2):101–12.
2. Bhutani VK, Zipursky A, Blencowe H, Khanna R, Sgro M, Ebbesen F, et al. Neonatal hyperbilirubinemia and Rhesus disease of the newborn: incidence and impairment estimates for 2010 at regional and global levels. Pediatr Res 2013 Dec;74(Suppl 1):86–100.
3. Fung Kee Fung K, Eason E. Prevention of Rh Alloimmunization. SOGC Clinical Practice Guidelines. No. 133. J Obstet Gynaecol Can 2018;40(1):e1–10.
4. Hamel C, Esmaeilisaraji L, Thuku M, Michaud A, Sikora L, Fung-Kee-Fung K. Antenatal and postpartum prevention of Rh alloimmunization: A systematic review and GRADE analysis. PLoS One 2020;15(9):e0238844.
5. Crowther C, Middleton P. Anti-D administration after childbirth for preventing Rhesus alloimmunisation. Cochrane Database Syst Rev 2000;(2):CD000021.
6. KI BioPharma LLC. WinRHO SDF Product Monograph. Health Canada Drug Product Database June 2022. 44p. Available from: https://pdf.hres.ca/dpd_pm/00066449.PDF
7. Kumpel BM. Lessons learnt from many years of experience using anti-D in humans for prevention of RhD immunization and haemolytic disease of the fetus and newborn. Clin Exp Immunol 2008 Oct;154(1):1–5.
8. Liu J, Zhang S, Wang Q, Shen H, Zhang Y, Liu M. Frequences and ethnic distribution of ABO and RhD blood groups in China: a population-based cross-sectional study. BMJ Open 2017;7(12):e018476.
9. Van der Heide HM, Magnee W, Van Loghem JJ. Blood group distribution in basques. Am J Hum Genet 1951;3(4):356–61.
10. Huchcroft S, Gunton P, Bowen T. Compliance with postpartum Rh isoimmunization prophylaxis in Alberta. CMAJ 1985;133(9):871–5.
11. Bergstrom H, Nilsson LA, Nilsson L, Ryttinger L. Demonstration of Rh antigens in a 38-day-old fetus - ScienceDirect. American Journal of Obstetrics & Gynecology 1967;99(1):130–3.
12. Karanth L, Jaafar SH, Kanagasabai S, Nair NS, Barua A. Anti-D administration after spontaneous miscarriage for preventing Rhesus alloimmunisation. Cochrane Database Syst Rev 2013;(3):CD009617.
13. Sandler SG, Flegel WA, Westhoff CM, Denomme GA, Delaney M, Keller MA, et al. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. College of American Pathologists Transfusion Medicine Resource Committee Work Group. Transfusion 2015 Mar;55(3):680–9.
14. RHD Genotyping for Prenatal Patients [Internet]. National Advisory Committee on Blood and Blood Products; 2017 Mar [cited 2022 May 16]. Available from. https://nacblood.ca/en/resource/rhd-genotyping-prenatal-patients
15. Bellis MA, Hughes K, Hughes S, Ashton JR. Measuring paternal discrepancy and its public health consequences. J Epidemiol Community Health 2005 Sep;59(9):749–54.
16. Neovius M, Tiblad E, Westgren M, et al. Cost-effectiveness of first trimester noninvasive fetal RhD screening for targeted antenatal anti-D prophylaxis in RhD-negative pregnant women: a model-based analysis. BJOG 2016;123(8):1337–46.
17. Darlington M, Carbonne B, Mailloux A, et al. Effectiveness and costs of non-invasive foetal RHD genotyping in rhesus-D negative mothers: a French multicentric two-arm study of 850 women. BMC Pregnancy Childbirth 2018;18(1):496.
18. Finning K, Martin P, Summers J, Massey E, Poole G, Daniels G. Effect of high throughput RHD typing of fetal DNA in maternal plasma on use of anti-RhD immunoglobulin in RhD negative pregnant women: prospective feasibility study. BMJ 2008;336:816–8.
19. Moise KJ, Hashmi SS, Markham K, et al. Cell free fetal DNA to triage antenatal rhesus immune globulin: is it really cost-effective in the United States? Prenatal Diagnosis 2019;39(3):238–47.
20. Duplantie J, Gonzales OM, Bois A, et al. Cost-effectiveness of the management of Rh negative pregnant women. J Obstet Gynaecol Can 2013;35(8):730–40.
21. Teitelbaum L, Metcalfe A, Clarke G, et al. Costs and benefits of non-invasive fetal RhD determination. Ultrasound Obstet Gynecol 2015;45(1):84–8.

22. Kennedy MS, McNanie J, Waheed A. Detection of anti-D following antepartum injections of Rh immune globulin. Immunohematology 1998;14(4):138–40.
23. Szkotak AJ, Lunty B, Nahirniak S, Clarke G. Interpretation of pretransfusion testing in obstetrical patients who have received antepartum Rh immunoglobulin prophylaxis. Vox Sang 2016;110(1):51–9.
24. Heddle NM, Klama L, Frassetto R, O'Hoski P, Leaman B. A retrospective study to determine the risk of red cell alloimmunization and transfusion during pregnancy. Transfusion 1993;33(3):217–20.
25. Pollack W, Gorman JG, Freda VJ, Ascari Q, Allen AE, Baker WJ. Results of clinical trials of RhoGAM in women. Transfusion 1968;8(3):151–3.
26. Ascari WQ, Levine P, Pollack W. Incidence of maternal Rh immunization by ABO compatible and incompatible pregnancies. Br Med J 1969;1(5641):399–401.
27. Pilgrim H, Lloyd-Jones M, Rees A. Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. Health Technol Assess 2009;13(10).
28. RANZCOG. Guidelines for the use of Rh(D) Immunoglobulin (Anti-D) in obstetrics in Australia [Internet]. 2015 [cited 2019 Apr 9]. Available from: http://www.ranzcog.edu.au/RANZCOG_SITE/media/RANZCOG-MEDIA/Women's%20Health/Statement%20and%20guidelines/Clinical-Obstetrics/Guidelines-for-the-use-of-Rh(D)-Immunoglobulin-(Anti-D)-(C-Obs-6)-Review-November-2015.pdf?ext=.pdf
29. Qureshi H, Massey E, Kirwan D, Davies T, Robson S, White J, et al. BCSH guideline for the use of anti-D immunoglobulin for the prevention of haemolytic disease of the fetus and newborn. Transfus Med 2014 Feb;24(1):8–20.
30. Turner RM, Lloyd-Jones M, Anumba DOC, Smith GCS, Spiegelhalter DJ, Squires H, et al. Routine antenatal anti-D prophylaxis in women who are Rh(D) negative: meta-analyses adjusted for differences in study design and quality. PLoS One 2012;7(2):e30711.
31. White SW, Cheng JC, Penova-Veselinovic B, Wang C, White M, Ingleby B, et al. Single dose v two-dose antenatal anti-D prophylaxis: a randomised controlled trial. The Medical Journal of Australia 2019 Aug 26;211(6):261–5.
32. Sorensen K, Stjern HE, Karlsen BAG, Tomter G, Ystad I, et al. Following targeted routine antenatal anti-D prophylaxis, almost half of the pregnant women had undetectable anti-D prophylaxis at delivery. Acta Obstet Gynecol Scand 2022;101(4):431–40.
33. Stern K, Goodman HS, Berger M. Experimental Isoimmunization to Hemoantigens in Man. The Journal of Immunology 1961 Aug 1;87(2):189–98.
34. Zipursky A, Israels LG. The pathogenesis and prevention of Rh immunization. Can Med Assoc J 1967 Nov 18;97(21):1245–57.
35. Nicolaides KH, Clewell WH, Rodeck CH. Measurement of human fetoplacental blood volume in erythroblastosis fetalis. Am J Obstet Gynecol 1987;157(1):50–3.
36. Wylie B, D'Alton ME. Fetomaternal hemorrhage. Obstet Gynecol 2010;115(5):1039–51.
37. Horvath S, Tsao P, Huang ZY, Zhao L, Du Y, et al. The concentration of fetal red blood cells in first-trimester pregnant women undergoing uterine aspiration is below the calculated threshold for Rh sensitization. Contraception 2020;102(1):1–6.
38. Horvath S, Huang ZY, Koelper NC, Martinez C, Tsao PY, et al. Induced abortion and the risk of Rh Sensitization. JAMA 2023;330(12):1167–74.
39. Queenan JT, Kubarych SF, Shah S, Holland B. Role of induced abortion in rhesus immunization. Lancet 1971;1(7704):815–7.
40. Gavin PS. Rhesus sensitization in abortion. Obstet Gynecol 1972;39(1):37–40.
41. Visscher RD, Visscher HC. Do RhD-negative women with an early spontaneous abortion need Rh immune prophylaxis? Am J Obstet Gynecol 1972 May 15;113(2):158–65.
42. Stewart FH, Burnhill MS, Bozorgi N. Reduced dose of Rh immunoglobulin following first trimester pregnancy termination. Obstet Gynecol 1978 Mar;51(3):318–22.
43. Chan MC, Gill RK, Kim CR. Rhesus isoimmunization in unsensitized RhD-negative individuals seeking abortion at less than 12 weeks' gestation: a systematic review. BMJ Sex Reprod Health 2022;48:163–8.
44. Hartwell EA. Use of Rh immune globulin: ASCP practice parameter. American Society of Clinical Pathologists. Am J Clin Pathol 1998 Sep;110(3):281–92.
45. Bowman JM, Pollock JM. Transplacental fetal hemorrhage after amniocentesis. Obstet Gynecol 1985 Dec;66(6):749–54.
46. Brambati B, Guercilena S, Bonacchi I, Oldrini A, Lanzani A, Piceni L. Feto-maternal transfusion after chorionic villus sampling: clinical implications. Hum Reprod 1986 Jan;1(1):37–40.
47. Bowman JM, Pollock JM, Peterson LE, Harman CR, Manning FA, Menticoglou SM. Fetomaternal hemorrhage following funipuncture: increase in severity of maternal red-cell alloimmunization. Obstet Gynecol 1994 Nov;84(5):839–43.
48. An assessment of the hazards of amniocentesis. Report to the Medical Research Council by their Working Party on Amniocentesis. Br J Obstet Gynaecol 1978;85(Suppl 2):1–41.
49. Lau TK, Stock A, Rogers M. Fetomaternal haemorrhage after external cephalic version at term. Aust N Z J Obstet Gynaecol 1995 May;35(2):173–4.
50. ACOG. Practice Bulletin No. 181: Prevention of Rh D Alloimmunization. Obstetrics & Gynecology 2017 Aug;130(2):e57.
51. Bolton-Maggs PHB. SHOT conference report 2016: serious hazards of transfusion - human factors continue to cause most transfusion-related incidents. Transfus Med 2016 Dec;26(6):401–5.
52. Koby L, Grunbaum A, Benjamin A, Koby R, Abenhaim HA. Anti-D in Rh(D)-negative pregnant women: are at-risk pregnancies and deliveries receiving appropriate prophylaxis? J Obstet Gynaecol Can 2012 May;34(5):429–35.
53. Hirose TG, Mays DA. The safety of RhIG in the prevention of haemolytic disease of the newborn. J Obstet Gynaecol 2007 Aug;27(6):545–57.
54. Frias JP, Lim CG, Ellison JM, Montandon CM. Review of adverse events associated with false glucose readings measured by GDH-PQQ-based glucose test strips in the presence of interfering sugars. Diabetes Care 2010 Apr;33(4):728–9.
55. Rutkowski K, Nasser SM. Management of hypersensitivity reactions to anti-D immunoglobulin preparations. Allergy 2014 Nov;69(11):1560–3.
56. Maayan-Metzger A, Leibovitch L, Schushan-Eisen I, Morag I, Strauss T. Maternal anti-D prophylaxis during pregnancy and risk of hemolysis among preterm infants. J Perinatol 2014 Dec;34(12):906–8.

APPENDIX A

Table A1. Key to Grading of Recommendations, Assessment, Development and Evaluation Quality of Evidence

Grade	Definition
Strength of recommendation	
Strong	High level of confidence that the desirable effects outweigh the undesirable effects (strong recommendation for) or the undesirable effects outweigh the desirable effects (strong recommendation against)
Conditional (weak)[a]	Desirable effects probably outweigh the undesirable effects (weak recommendation for) or the undesirable effects probably outweigh the desirable effects (weak recommendation against)
Quality of evidence	
High	High level of confidence that the true effect lies close to that of the estimate of the effect
Moderate	Moderate confidence in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low	Limited confidence in the effect estimate: The true effect may be substantially different from the estimate of the effect
Very low	Very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Adapted from GRADE Handbook (2013), Table 5.1.
[a]Do not interpret conditional (weak) recommendations to mean weak evidence or uncertainty of the recommendation.

Table A2. Implications of Strong and Conditional (Weak) recommendations, by guideline user

Perspective	Strong Recommendation • "We recommend that…" • "We recommend to not…"	Conditional (Weak) Recommendation • "We suggest…" • "We suggest to not…"
Authors	The net desirable effects of a course of action outweigh the effects of the alternative course of action.	It is less clear whether the net desirable consequences of a strategy outweigh the alternative strategy.
Patients	Most individuals in the situation would want the recommended course of action, while only a small proportion would not.	The majority of individuals in the situation would want the suggested course of action, but many would not.
Clinicians	Most individuals should receive the course of action. Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator.	Recognize that patient choices will vary by individual and that clinicians must help patients arrive at a care decision consistent with the patient's values and preferences.
Policy makers	The recommendation can be adapted as policy in most settings.	The recommendation can serve as a starting point for debate with the involvement of many stakeholders.

Adapted from GRADE Handbook (2013), Table 6.1.