Use of Rh Immune Globulin

ASCP Practice Parameter

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Abstract

The use of Rh immune globulin (RhIG) has dramatically decreased the incidence of hemolytic disease of the fetus and newborn resulting from the production of anti-D by an Rh-negative woman. However, despite the widespread use of RhIG, instances of Rh immunization continue to occur, most likely through failure to administer RhIG when indicated or in the appropriate dose. This utilization gap can be closed only through continued active surveillance by health care providers. The following report summarizes recommendations for the administration of RhIG, the dose required in various circumstances, prenatal and postnatal serologic testing of the obstetric patient, and the methods used to determine the degree of fetomaternal hemorrhage or the amount of Rh-positive RBCs in the circulation.

Hemolytic disease of the fetus and newborn (HDN) is a condition resulting from the destruction of the infant's antigen-positive RBCs by a corresponding IgG alloantibody produced by the mother. Although numerous alloantibodies are known to cause HDN, the most notorious is anti-D directed against the D antigen of the Rh blood group system. This antibody can be produced by Rh-negative (D-negative) women who are exposed to Rh-positive (D-positive) fetal RBCs during pregnancy. The D antigen has been demonstrated on fetal RBCs as early as the sixth gestational week.[1] The binding of maternal anti-D to the fetal RBCs often leads to extravascular hemolysis that ranges from minimal to extreme and this, in part, determines the severity of HDN. At its worst, the anemia leads to fetal hepatosplenomegaly, hydrops fetalis, heart failure, and death in utero or soon after birth.[2] Infants who survive are at risk for the development of kernicterus and resultant neuronal damage.

There is a dose-dependent relationship between the volume of Rh-positive RBCs to which the Rh-negative person is exposed and the incidence of Rh immunization, with volumes as small as 0.1 mL resulting in antibody formation.[3] Consequently, the degree of fetomaternal hemorrhage (FMH) is an important consideration in the maternal development of anti-D. During a normal first-trimester pregnancy, approximately 3% of women have detectable fetal RBCs in their circulation, although, typically, this is less than 0.1 mL.[4] As pregnancy progresses, the frequency and volume of fetal RBCs in the maternal circulation increases, with 12% of women in the second trimester and 45% of women in the third trimester having detectable fetal RBCs in their circulation.[4] At the time of delivery, up to 50% of women delivering an ABO-compatible infant will have demonstrable circulating fetal RBCs.[5] Therefore, the major immunizing event for Rh-negative women occurs late in pregnancy and primarily at the time of delivery when

placental separation occurs. As the volume of FMH increases, so does the incidence of Rh immunization.

In 1941, Levine and colleagues[6] made the association between hemolytic disease of the newborn and the presence of anti-D in the mother. This discovery was followed by several landmark studies that offered new insights into the development of Rh immunization during pregnancy.[3,5,7,8] In 1963, the concept of passive immunization using a preparation of anti-D as a means of protecting Rh-negative persons against sensitization to Rh-positive RBCs was tested by Freda et al[7] and Clarke et al.[8] Following the success of initial studies in Rh-negative male volunteers, clinical trials in Rh-negative pregnant women were undertaken. These were enormously successful, with a reduction in the incidence of Rh immunization occurring after pregnancy from 12% to 13% to 1% to 2%.[9] In 1968, Rh immune globulin (RhIG) was licensed for postpartum use in the United States.

The incidence of Rh immunization decreased dramatically after this time; however, evidence began to accumulate that some Rh-negative women continued to form anti-D even after appropriate RhIG postpartum prophylaxis.[10] Approximately 1.8% of Rh-negative women were apparently sensitized during pregnancy as a result of small transplacental hemorrhages. Subsequent studies in primigravidas verified that small amounts of fetal RBCs entering the maternal circulation were the usual cause of Rh immunization occurring during pregnancy.[11] In 1968, a clinical trial of antenatal RhIG prophylaxis began in Winnipeg, Manitoba, Canada.[12] With the administration of an antepartum dose of RhIG, in addition to the recommended postpartum dose, the incidence of Rh immunization fell to 0.1%.[9] After the success of these trials, the use of antepartum RhIG prophylaxis for Rh-negative women at 28 to 30 weeks' gestation was endorsed by the American College of Obstetricians and Gynecologists.[13–15]

Preparation and Administration of Rh Immune Globulin

RhIG is a sterile concentrated protein solution containing IgG anti-D that is derived from human plasma. The preparation process results in a product that typically is not associated with the transmission of infectious diseases, such as HIV.[16] Isolated instances of transmission of hepatitis C virus via contaminated intravenous preparations of RhIG have been reported in Europe; however, this was before the routine screening of plasma donors for anti–hepatitis C virus.[17] The majority of products available for use in the United States are for intramuscular (IM) injection only; however, in 1995, an intravenous (IV) preparation was approved for use as well. Adverse reactions associated with administration of RhIG are rare. Since the final product contains small amounts of globulins, including IgA, caution should be exercised before administration to persons known to have had hypersensitivity reactions to any of these components.

The exact mechanism by which RhIG prevents sensitization to the D-antigen remains to be elucidated, although several hypotheses have been proposed.[18,19] One of the more favored mechanisms postulates that the passively administered anti-D attaches to the D antigen sites on the Rh-positive RBCs in the circulation and interferes with the host primary immune response to the foreign antigen.

Early studies determined that 20 μg of purified RhIG would provide protection against 1 mL of Rh-positive RBCs.[20] This finding subsequently led to the standardization of RhIG preparation and packaging. One standard effective IM dose or vial (roughly 1 mL) of RhIG typically contains approximately 300 μg of anti-D and affords protection against 15 mL of Rh-positive RBCs or 30 mL of Rh-positive whole blood. A smaller 50-μg IM dose is available for limited use after first trimester pregnancy terminations only and will counteract the effects of approximately 2.5 mL of Rh-positive RBCs. A recently approved preparation of RhIG (WinRho SD, Cangene, Winnipeg, Manitoba, Canada) is available that can be administered by the IV or IM route. Potency of this product is expressed in IU; 5 IU is the equivalent of 1 μg. Therefore, a 300-μg dose of IV RhIG contains 1,500 IU of anti-D. This product, which requires reconstitution with 0.9% sodium chloride injection, is prepared by using a solvent detergent–treatment step that inactivates lipid-enveloped viruses, including HIV, hepatitis B, and hepatitis C. It is available in vials containing 600 IU (120 μg) and 1,500 IU (300 μg). The dose is the same for this preparation whether administered by the IM or IV route. The half-life of an injected (IV or IM) dose of RhIG varies from 21 to 30 days.[21,22]

If more than a single 300-μg dose is required to counteract the effects of a large volume of Rh-positive RBCs, up to 5 doses may be administered at one time.[21] In such instances, administration of IV RhIG may be preferable to decrease patient discomfort. If additional IM doses are needed, these can be administered at alternative sites every 12 hours until the total dose requirement is achieved.[2]

RhIG is classified as a drug and requires a prescription for use. The IV preparation of RhIG is substantially more expensive than the IM preparation and is more commonly used for treatment of immune thrombocytopenic purpura (ITP) than for suppression of Rh immunization. Since RhIG must be stored at 2°C to 8°C until ready for use, it is typically dispensed through the hospital blood bank or pharmacy, but also may be stocked in the obstetric office or clinic. Careful record keeping is advised for all dispensing facilities.

Indications for Use

An outline of the suggested indications for use of RhIG and the recommended doses to be given are presented in ▮Table 1▮ and ▮Table 2▮.

Routine Antepartum Prophylaxis

Antepartum administration of a standard 300-µg dose (IV or IM) of RhIG is indicated between 28 and 30 weeks of gestation in all pregnant Rh-negative women who have not already developed anti-D. This recommendation is based on studies that 92% of Rh-negative women who develop anti-D during pregnancy do so at 28 weeks or later.[23] If the father of the fetus is known and can be shown conclusively to be Rh-negative, RhIG is not indicated.

The antepartum injection of RhIG is designed to prevent the immunization of the 1.8% of Rh-negative women who become sensitized late in pregnancy and, therefore, are not protected by postpartum prophylaxis alone.[12] Although early concerns existed about the effect of passively administered anti-D on the fetus,[24,25] these have not been supported in numerous studies.[26] Small amounts of anti-D may cross the placenta and attach to fetal Rh-positive RBCs, causing a weakly positive direct antiglobulin test at birth, but the antibody has not been shown to have adverse effects on the infant.[23,27]

The half-life of a standard dose of RhIG varies from 21 to 30 days[21,22]; therefore, administration of RhIG at 28 to 30 weeks will confer protection for approximately 12 weeks. Since 10% or less of the original antenatal dose will be present at 40 weeks' gestation, Rh-negative women are still candidates for RhIG administration after delivery, provided the infant is Rh-positive. Most women who receive antepartum administration of RhIG will develop a positive antibody screen owing to the passively acquired anti-D. This anti-D may still be detectable at the time of delivery and should not be cause for withholding further administration of RhIG. Therefore, it is mandatory that complete records be kept of antenatal administration of RhIG to avoid mistakenly classifying the postpartum Rh-negative mother as being actively, rather than passively, immunized to the D antigen.

▮Table 1▮
Indications for Use of Rh Immune Globulin

Antepartum (routine at 28 weeks)
Postpartum (infant must be Rh-positive)
Termination of pregnancy
Ectopic pregnancy
Amniocentesis
Percutaneous umbilical blood sampling
Chorionic villus sampling
Obstetric complications
Abdominal trauma
Abruptio placentae
Placenta previa
Manual removal of placenta
Threatened abortion
Antepartum vaginal bleeding
Death in utero
External cephalic version
Trophoblastic disease or neoplasm
Tubal ligation
Immune thrombocytopenic purpura
Transfusion of Rh-positive blood

▮Table 2▮
Dosage of Rh Immune Globulin

Indication	Route of Administration	Dose
Pregnancy termination, <12 weeks' gestation	IM	50 µg
Abortion, miscarriage, ectopic pregnancy, or other pregnancy complication, >12 weeks' gestation	IM, IV	300 µg
Amniocentesis, chorionic villus sampling, <34 weeks' gestation	IM	300 µg
	IV	300 µg*
Amniocentesis, chorionic villus sampling, or other manipulation during pregnancy, >34 weeks' gestation	IM	300 µg†
	IV	120 µg†
Obstetrical complication (eg, abruptio placentae, placenta previa)	IM, IV	300 µg
Antepartum, 28 weeks' gestation	IM, IV	300 µg
Postpartum‡	IM	300 µg§
	IV	120 µg§
Transfusion of Rh-positive blood	IM	20 µg/mL RBCs
	IV	18 µg/mL RBCs
ITP	IV	50 µg/kg‖
		25–40 µg/kg¶

IM = intramuscular; IV = intravenous; ITP = immune thrombocytopenic purphra.
*To be repeated at 12-week intervals until delivery.
†Same dose should be administered if procedure is repeated >21 days after first dose.
‡Infant must be Rh-positive.
§Dose should be adjusted for fetomaternal hemorrhage of >15 mL.
‖If patient's hemoglobin level is ≥10 g/dL.
¶If patient's hemoglobin level is <10 g/dL.

It has been argued by some that antenatal RhIG prophylaxis is not cost-effective and will benefit few women compared with the potential risks.[25,28] However, the 1% to 2% incidence of Rh immunization and the resultant perinatal morbidity and mortality can be decreased substantially through this program, and the continued use of antenatal RhIG is strongly recommended.[29]

Postpartum Prophylaxis

RhIG should be administered to all Rh-negative women who deliver an Rh-positive infant, provided the woman has not formed anti-D.[30] Rh-negative women who have been immunized to the D antigen or who deliver Rh-negative infants and women who are Rh-positive are not candidates for postpartum RhIG prophylaxis. Although ABO incompatibility between mother and infant confers some protection against Rh immunization, RhIG should be given without regard to ABO blood groups. RhIG is intended for administration to Rh-negative mothers and not their infants.

Some difficulty may exist in determining whether anti-D detected at the time of delivery represents active or passive immunization. Typically, the standard antenatal dose of RhIG will not be responsible for a titer greater than 4. Titers higher than this are likely to represent active immunization. It is imperative that the distinction between active and passive immunization be made to prevent the inadvertent omission of postpartum RhIG prophylaxis. If serologic test results are questionable and cannot be resolved in a timely manner, it is advisable to administer RhIG rather than withhold it.

Typically, at least 1 full standard dose of 300 μg RhIG should be administered intramuscularly within 72 hours of delivery. For IV preparations, the recommended standard postpartum dose is 600 IU (120 μg). If a 300-μg dose of RhIG was administered during the 21-day period before delivery (eg, following amniocentesis), additional RhIG is not necessarily indicated after delivery, providing excessive FMH has been excluded.[21]

Since the initial studies and clinical trials used a 72-hour interval as the maximum period for administration of RhIG after the immunizing event, this policy recommendation continues to the present day. However, if RhIG is inadvertently not given within 72 hours of delivery, it should not be withheld. Administration of RhIG up to 13 days after exposure to Rh-positive RBCs has been shown to be somewhat protective in preventing primary Rh immunization[31] and is preferable to withholding RhIG altogether. The patient should be advised, however, that sensitization may still occur.

A small percentage of women will experience a significant FMH at the time of delivery. The standard 300-μg dose of RhIG protects against up to 15 mL of fetal RBCs; therefore, if there is an FMH in excess of 15 mL RBCs, additional doses of RhIG should be administered.

Pregnancy Termination (Abortion) and Ectopic Pregnancy

FMH may occur with spontaneous and elective terminations of pregnancy. Studies have shown that fetal RBCs can be detected in the circulation of up to 32% of women after spontaneous abortion; in 26% of these patients, the volume of FMH was 0.05 mL or more.[32] Since the D antigen has been detected on RBCs of a 38-day-old fetus,[1] the potential for early Rh sensitization of the Rh-negative mother exists. The overall risk of Rh immunization after induced or spontaneous abortion is estimated to be between 3% and 6%,[33,34] although some calculate a risk as high as 10%.[35]

Risk figures for Rh immunization after ectopic pregnancy are not as easily ascertained. A substantial number of fetal RBCs can be found in the circulation of up to 24% of women who experience rupture of a tubal pregnancy.[36,37] Therefore, it would seem prudent to consider these women as candidates for RhIG prophylaxis and administer at least the standard 300-μg IM dose.

It is recommended that RhIG be given to all nonimmunized Rh-negative women within 72 hours after pregnancy termination, whether the termination is a spontaneous miscarriage, induced abortion, or ectopic pregnancy. The assumption is made that the fetus is Rh-positive unless the biologic father is known with certainty to be Rh-negative and this information is appropriately documented. RhIG may be considered for women with a molar pregnancy. With a complete hydatidiform mole, vascularization of villi is typically absent or incomplete; therefore, the risk of immunization would be minimal to nonexistent. The D antigen has not been detected on the villous trophoblast of the hydatidiform mole.[38]

A 50-μg IM dose of RhIG may be used for pregnancy terminations occurring up to and including 12 weeks of gestation.[39] This smaller dose will protect against 2.5 mL of Rh-positive fetal RBCs or 5 mL of Rh-positive whole blood. It is unlikely that the total fetal blood volume during the first trimester would approach this amount, and complete exsanguination of the fetus at the time of termination would be extremely unusual.

Amniocentesis

Some degree of FMH is a known risk of amniocentesis even with careful placental localization by using ultrasonography.[40,41] Approximately 2% of women undergoing amniocentesis at various gestational ages will have an FMH of at least 0.1 mL.[40] Therefore, it is recommended that all Rh-negative nonimmunized women receive 300 μg IM of RhIG immediately following amniocentesis, regardless of gestational age or the indication for the procedure.

For IV preparations of RhIG, if amniocentesis is performed before 34 weeks' gestation, the 300-µg dose is recommended; when performed after 34 weeks' gestation, the 120-µg dose is recommended. These women should all receive a second 300 µg of RhIG if amniocentesis is repeated more than 21 days later. This practice has not been found to be harmful to the fetus.[42]

If delivery of an Rh-positive infant occurs within 21 days after RhIG administration following amniocentesis, a postpartum injection of RhIG may not be necessary unless testing reveals a large FMH.[21] If amniocentesis is performed within 48 hours of delivery for assessment of fetal lung maturity, RhIG can be withheld until after delivery. In such instances, if the infant is found to be Rh-positive, the standard 300-µg dose (IV or IM) of RhIG should be administered to the mother.

Other Obstetric Conditions

Recent studies indicate the prevalence of FMH after percutaneous umbilical blood sampling is several times greater than after amniocentesis.[43] Although chorionic villus sampling is usually performed at an earlier gestational age than percutaneous umbilical blood sampling, FMH also occurs.[2] It would seem prudent to recommend that Rh-negative nonimmunized women who undergo either of these procedures receive RhIG prophylaxis within 72 hours of the procedure unless the father or fetus is known and documented to be Rh-negative. The recommended dose after either procedure is 300 µg (IV or IM).

Obstetrical complications such as abruptio placentae, placenta previa with bleeding, manual removal of the placenta, vaginal hemorrhage (threatened abortion), external cephalic version, death in utero, and antepartum hemorrhage from any cause all are associated with an increased risk of FMH.[2,42] It is also well-documented that trauma that occurs during pregnancy is associated with a 4 to 5 times increased risk of FMH.[44–46] Anterior placement of the placenta, uterine tenderness after trauma, and the wearing of restraints during a motor vehicle crash all have a positive correlation with a larger volume of FMH.[44] All Rh-negative nonimmunized pregnant women should receive at least one 300-µg dose of RhIG as prophylaxis after an obstetrical complication or trauma. If it is determined that more than a 15-mL FMH has occurred, additional doses of RhIG should be given. Since RhIG confers protection for only approximately 12 weeks, if delivery has not occurred by this time, an additional dose of RhIG should be administered.

Tubal Ligation

The use of RhIG after postpartum or postabortal bilateral tubal ligation in Rh-negative women is controversial. It can be argued that such women, although currently at risk for Rh immunization, will not be at future risk for carrying an infant affected by HDN. However, it also can be argued that it is unacceptable to needlessly risk alloimmunization when appropriate prophylaxis is available.[47] Although tubal sterilization is generally considered a permanent procedure, there is a tendency for younger women to request reversal of the procedure, often owing to a change in a marital relationship.[48] In addition, approximately 0.3% of all tubal ligation procedures fail, and a subsequent pregnancy occurs.[49] For women who have or plan to undergo a sterilization procedure after pregnancy termination, it would seem prudent to offer RhIG prophylaxis.

Transfusion of Rh-Positive Blood

In certain situations, Rh-negative persons may be transfused with blood components containing Rh-positive RBCs and, consequently, be at risk for forming anti-D. This may occur inadvertently or because of a shortage of compatible Rh-negative blood. Blood components associated with a risk for Rh immunization include whole blood, RBCs, platelets (prepared from whole blood and by cytapheresis), and granulocytes (prepared by cytapheresis). Although the Rh blood group antigens are not present on platelets, platelet preparations may contain up to 0.5 mL of contaminating RBCs, which do possess the D antigen.[21] Granulocyte concentrates typically have significant contamination with RBCs owing to the method of preparation.

Depending on the volume of Rh-positive RBCs transfused, the childbearing potential of the recipient, and the clinical situation, RhIG prophylaxis should be considered for Rh-negative patients who receive Rh-positive blood components. RhIG prophylaxis is virtually 100% effective in such situations with timely administration of the appropriate RhIG dose.[50] Since the amount of RhIG required to suppress Rh immunization is 20 µg/mL of RBCs,[51] the amount of RhIG administered should be based on this calculation. A single 300-µg dose (IV or IM) of RhIG will confer protection against approximately 30 random-donor Rh-positive platelet concentrates. For single-donor plateletpheresis concentrates, the RhIG dose should be calculated based on the approximate number of random-donor platelet equivalents transfused.

RhIG should be administered within 72 hours of the first transfusion of Rh-positive blood products. To minimize pain, no more than 5 doses should be injected at 1 site. The injections also can be spaced over a 2- to 3-day period, particularly if larger doses are required. In such instances, the IV preparation of RhIG may be preferable. A dose of 600 µg (1,500 IU) can be infused every 8 hours until the total calculated dose is administered.[22]

Published data on the administration of massive doses of RhIG to a person with a large circulating volume of Rh-positive RBCs are minimal. One publication from the early 1970s reported that the IM administration of 17,700 µg (59 vials) of RhIG was well tolerated with transient elevation of bilirubin levels and a decrease in the hemoglobin and hematocrit levels.[50] In a more current case of massive FMH equivalent to 180 mL of fetal Rh-positive RBCs, 6,900 µg (23 vials) of RhIG was administered IM; effects on the mother were not addressed.[52] More recent literature addresses the IV administration of RhIG to Rh-negative persons inadvertently transfused with Rh-positive blood. In 4 persons receiving from 3,000 to 7,750 µg of IV RhIG, reactions ranged from minimal to fever and hemoglobinuria.[53] For administration of such large doses of RhIG by the IV route, the author suggested premedication with 100 mg of IV hydrocortisone followed by an initial dose of not more than 2,500 µg RhIG infused over 1 hour. If no reaction occurs following this dose, the remainder of the dose may be administered after 12 hours.[53]

Immune Thrombocytopenic Purpura (ITP)

Intravenous RhIG has documented efficacy in the treatment of certain subgroups of nonsplenectomized Rh-positive patients with ITP: children with acute or chronic ITP; adults with chronic ITP; and children and adults with HIV-related ITP.[22,54,55] A recent study showed administration of IV RhIG provided a hemostatic platelet increase in 72% of nonsplenectomized patients in the aforementioned groups.[55] This does not seem to be an effective form of treatment for splenectomized or Rh-negative patients with ITP.

An initial dose of 250 IU (50 µg) per kilogram of body weight is recommended unless the hemoglobin level is less than 10 g/dL (100 g/L), for which a smaller dose of 125 to 200 IU/kg is indicated.[22] The initial dose can be administered at one time or divided in 2 doses given on separate days. A decrease in hemoglobin levels will occur since the passively administered anti-D attaches to the D antigen on the patient's RBCs; the mean maximum decrease in hemoglobin is 1.70 g/dL (170 g/L).[55] Based on the clinical needs of the patient, additional RhIG may be administered in doses of 125 to 300 IU/kg.

Relative Contraindications for Use of RhIG

RhIG prophylaxis is not indicated in the following circumstances:

1. Rh-positive or weak D–positive women.
2. Rh-negative women who deliver an Rh-negative infant.
3. Rh-negative women who are carrying an Rh-negative fetus.
4. Rh-negative women who are immunized to the D antigen. It should be verified that the anti-D detected represents active rather than passive immunization and is not related to antenatal RhIG therapy.

Testing of the Obstetric Patient

Serologic testing of the obstetric patient is designed to identify Rh-negative women who are candidates for antepartum and postpartum RhIG prophylaxis.

Prenatal Assessment

At the first prenatal visit, for every pregnant woman, a blood sample should be tested for ABO and Rh type and the presence of clinically significant serum antibodies. In women who are determined to be Rh-negative by initial testing, a test for the weak-D phenotype must be performed. It is preferable to avoid reliance on old records of previous serologic testing if it was performed elsewhere.[56]

In some instances, Rh-positive (D-positive) RBCs with a weak expression of the D antigen are not directly agglutinated by all anti-D reagent typing sera; women with such weak expression can erroneously be classified as Rh-negative (D-negative) unless a more sensitive test for the presence of the D antigen is performed. The blood of such individuals is most correctly termed "weak D" and is often designated as "D+w." This designation is preferred over the term "D^u."[21] The frequency of the weak-D phenotype is approximately 0.2% in white persons.[57] Women who have this weakened expression of the D antigen are genetically classified as Rh-positive and are not considered to be at risk for Rh immunization.[58] RhIG prophylaxis is not necessary or recommended for these women. Nevertheless, the validity of this practice has been questioned by some persons who maintain that all women with weak D be considered candidates for RhIG administration.[59,60]

A much more unusual occurrence is the "partial D" or "D mosaic" phenotype. In persons with this phenotype, a portion or epitope of the D antigen is missing. Such persons occasionally form anti-D if exposed to normal D-positive RBCs via transfusion or pregnancy.[61–65] A pregnant woman with the partial D variant may, therefore, become immunized if FMH occurs from a fetus whose RBCs have all portions of the D antigen. Although there is a documented report of fatal HDN occurring as a result of the production of anti-D by a woman with a partial D antigen,[66] the experience of others indicates that the likelihood of this occurrence is low.[42,67,68] In addition, it has not been shown that administration of RhIG to these women after delivery of an Rh-positive infant would prevent Rh immunization. If it can be determined through genetic testing or family studies that the mother is a

true partial D phenotype, then antepartum RhIG prophylaxis may be considered.[69] This particular phenotype is extremely rare compared with the more common weak D.

Pregnant women who are found to be Rh-negative and have not formed anti-D should be scheduled for RhIG prophylaxis at 28 to 30 weeks' gestation. Before the administration of RhIG, it is recommended that Rh-negative women again be tested for unexpected antibodies to document the absence of alloimmunization to the D antigen at this time.

Although controversy exists, it is unnecessary to repeat Rh testing at 28 to 30 weeks if the results are known.[56] The current American Association of Blood Banks (AABB) Standards for Blood Banks and Transfusion Services mandate Rh testing only in women undergoing delivery, abortion, or an invasive obstetric procedure or if there is a request for RBC transfusion.[29] The AABB has agreed that 2 separate tests of Rh type with agreement of results is sufficient as long as the testing was performed at an accredited laboratory and the results are on file.[70] However, others maintain that repeated testing at 28 to 30 weeks will serve as confirmation that the patient is Rh-negative. Of interest, a study showed that prenatal Rh typing errors had occurred in approximately 2% to 3% of all patients tested at the time of delivery.[71]

Screening for FMH is unnecessary before routine antepartum RhIG administration. However, any situation associated with an increased likelihood of FMH occurring during pregnancy should be cause for consideration of additional administration of RhIG as determined by pertinent testing. The dose administered and the date given should be documented in the patient's medical record. A patient refusal of antepartum RhIG must also be documented in the medical record.

An alternative to antepartum RhIG prophylaxis is determination of the Rh status of the biologic father. If he is shown conclusively to be Rh-negative, the fetus will be Rh-negative and, therefore, unable to immunize the mother to the D antigen. The woman would not be considered a candidate for antepartum RhIG prophylaxis. However, paternity must be established with a high degree of certainty since failure to administer antepartum RhIG could result in severe consequences for future pregnancies.

Postpartum Assessment

Maternal Testing

At the time of delivery, many physicians request the same battery of serologic tests as performed antenatally: ABO, Rh, and unexpected antibody screen. However, in the era of utilization management, repeating the ABO group or antibody screen may not be justifiable if this information is on record from earlier in the pregnancy. Nevertheless, many obstetricians consider it prudent to perform the ABO, Rh, and antibody screen on all patients admitted for delivery in the event an emergency cesarean section is warranted.

Rh typing, on the other hand, must be determined at the time of delivery; a record of concordant results on 2 separate samples before delivery serves to meet this criterion.[70] If Rh typing is performed at delivery, it should include a test for weak D. Use of appropriate testing methods that will prevent the mistyping of an Rh-negative mother as Rh-positive, owing to the presence of a large FMH from an Rh-positive infant, is imperative.

The unexpected antibody detection test may demonstrate the presence of a weakly reactive anti-D if the patient received antenatal RhIG. If there is a history of antenatal RhIG administration in a woman who previously had a negative antibody screen, only limited identification studies are necessary to rule out the presence of other potentially clinically significant alloantibodies. Typically the antibody due to the administration of RhIG is weakly reactive (≤1+ at the antihuman globulin phase), of low titer (4 or less), and IgG in nature. A newly detected anti-D that fails to meet these characteristics is suggestive of primary Rh immunization and deserves further investigation.

Cord Blood Testing

It is standard practice in hospitals and health care agencies to collect a sample of umbilical cord blood at the time of delivery. The degree of testing performed on the sample varies from institution to institution. Some facilities prefer that all cord blood samples undergo ABO, Rh, and direct antiglobulin testing; in others, the samples are stored in the laboratory until specific testing is requested as determined by the clinical status of the infant or the Rh type of the mother.

Infants born to Rh-negative mothers should have their Rh type determined, including a test for weak D. Performance of a direct antiglobulin test (DAT) on the cord blood sample is usually unnecessary; however, it may be performed routinely as a matter of convenience. If the result for the direct agglutination test for the D antigen on the cord blood is negative, a test for weak D should be performed that uses the antiglobulin phase of testing. A DAT may be performed concurrently to serve as a control for interpretation of this test.

For infants of Rh-negative women who are potential RhIG candidates, a positive cord blood DAT result usually necessitates further serologic testing. Contamination of the cord blood sample with Wharton jelly during collection can result in a false-positive DAT result, and the DAT should be repeated on a sample of capillary or venous blood from the infant. If the DAT result remains positive, several circumstances should be considered:

1. Antepartum RhIG given to the mother may result in a weakly positive DAT result in the infant at birth. This can be confirmed by testing an eluate prepared from the infant's RBCs that will demonstrate the presence of anti-D. Since the infant is Rh-positive, the mother should receive postpartum RhIG prophylaxis.
2. Mother and infant may be ABO incompatible. An eluate will confirm the presence of anti-A, anti-B, or both on the infant's RBCs.
3. The mother may have a clinically significant IgG alloantibody that crossed the placenta and attached to the corresponding antigen on the infant's RBCs. The antibody screen on the mother will generally demonstrate the presence of the offending antibody; the same antibody will be detected in an eluate prepared from the infant's RBCs.

A postpartum blood sample from all Rh-negative nonimmunized women who deliver an Rh-positive infant must be tested for FMH in excess of 30 mL whole blood.[29] Approximately 0.3% to 1.0% of all deliveries result in a transplacental hemorrhage of more than 30 mL,[3,72,73] and one 300-μg dose of RhIG would be insufficient to confer protection. If excessive FMH is determined to be present, the degree of hemorrhage should be quantitated and the appropriate dose of RhIG administered.

Testing for Fetomaternal Hemorrhage

The implications of a large FMH occurring in an Rh-negative woman are obvious. Failure to administer sufficient RhIG prophylaxis in a timely manner can result in maternal Rh immunization that might otherwise have been prevented.

The incidence or degree of FMH often cannot be predicted. In several series, the majority of FMHs of more than 30 mL occurred in women without an evident predisposing condition.[72] It is imperative that Rh-negative women who are candidates for RhIG prophylaxis be considered at risk for excessive FMH, regardless of whether they fall into a high-risk category. The AABB requires that for all Rh-negative nonimmunized women who deliver an Rh-positive infant, a postpartum blood sample be screened for FMH to determine whether more than a single dose of RhIG is required.[30] During the antenatal period, a screen for FMH may be performed if clinical circumstances are such that excessive transplacental hemorrhage is suspected after 20 weeks' gestation. Earlier than 20 weeks' gestation, the total fetal blood volume does not exceed 30 mL[74]; therefore, screening for FMH before this time is not indicated since 1 dose of RhIG will be sufficient, if needed.

Several methods for the detection or quantitation of FMH are available. These include the erythrocyte rosette test, enzyme-linked antiglobulin test (ELAT), flow cytometry, and variations of the acid elution test of Kleihauer-Betke. Some of these tests are designed to detect Rh-positive RBCs in the circulation of an Rh-negative person; others detect fetal RBCs regardless of Rh type. Although used extensively in the past, methods such as the microscopic D^u test no longer are acceptable for detecting excessive FMH since the sensitivity of other methods is superior. If a screening test for FMH is performed and found to be positive, the degree of FMH must be quantitated and the dose of RhIG calculated accordingly.

Erythrocyte Rosette Test

The rosette test is a qualitative screening method for the identification of FMH.[75,76] It will detect approximately 5 mL of Rh-positive fetal RBCs (or 10 mL of Rh-positive whole blood). Samples containing less than 2.5 mL of Rh-positive fetal RBCs should give negative test results. The rosette test provides a qualitative result only; all positive results must be evaluated by using a quantitative test. It has been argued that this method is overly sensitive because unnecessary follow-up testing will be performed. However, since positive results will be obtained on only approximately 1% to 3% of women who are candidates for RhIG,[75] very few samples will require more extensive testing.

In the rosette test, reagent anti-D is added to a suspension of maternal RBCs. During incubation, the reagent antibody binds to any fetal Rh-positive RBCs that are present. Indicator Rh-positive RBCs are then added to the test system. These indicator cells will bind with the anti-D present on the fetal Rh-positive RBCs, forming rosettes around each antibody-coated fetal cell.

If the fetal RBCs are the weak-D phenotype, weak to negative reactions may be seen with the rosette test. In such instances, it is recommended that a test based on detection of fetal hemoglobin, such as the Kleihauer-Betke test, be performed. If the maternal RBCs are the weak-D phenotype, strongly positive results will be obtained with the rosette test. This may be difficult to distinguish from a massive FMH; specific testing for fetal RBCs is then indicated.

Enzyme-Linked Antiglobulin Test

Like the erythrocyte rosette test, the ELAT detects Rh-positive RBCs whether or not they are of fetal origin. The ELAT provides qualitative and quantitative results.[77] This method involves 3 phases during which a suspension of RBCs is incubated successively with anti-D, conjugated anti-IgG, and an enzyme substrate. Reactions are read by using a spectrophotometer; the optical density is proportional to the number of Rh-positive RBCs present in the

original suspension. The sensitivity of the test is such that all women who may require more than one 300-μg dose of RhIG will be identified. The ELAT is capable of detecting at least 12.5 mL of Rh-positive whole blood; approximately 50% of samples with as little as 3 mL of Rh-positive blood will give a positive result.[78]

Flow Cytometry

Anti-D and fluorescent-conjugated anti-D are used in a 2-stage flow cytometric technique to detect Rh-positive RBCs.[79] A more recent variation combines biotinylated anti-D with fluorescent-conjugated streptavidin in an attempt to provide better distinction between weak D maternal RBCs and fetal Rh-positive RBCs.[80] Like the rosette test and the ELAT, flow cytometry distinguishes Rh-positive cells from Rh-negative cells without regard to origin (fetal or maternal). Flow cytometry is a very sensitive method and reproducibly detects 0.1% Rh-positive RBCs,[81,82] which corresponds to an FMH of approximately 15 mL whole blood. Although a large number of cells can be analyzed, many clinical laboratories lack the facilities, expertise, or finances to operate a flow cytometer for purposes of determining excessive FMH.

Kleihauer-Betke Test

The Kleihauer-Betke test is based on the principle that fetal hemoglobin, but not adult hemoglobin, is resistant to acid elution.[83] When a blood smear of the maternal sample is exposed to an acid buffer, the adult hemoglobin will be dissolved and, when counterstained, the maternal RBCs will appear light pink to white ("ghosts") when examined with a microscope. The fetal RBCs will stain dark red to pink owing to the presence of hemoglobin F. At least 1,000 (preferably 2,000–5,000) adult cells are counted and the ratio of fetal to adult cells determined. From the percentage of fetal RBCs present, the volume of FMH can be calculated and the appropriate dose of RhIG administered. Several commercial kits for performing the Kleihauer-Betke test are available.

The Kleihauer-Betke test traditionally is one of the most commonly used tests for the quantitation of FMH.[84,85] Unfortunately, it is subject to various pitfalls. Technique is very important, and blood smear interpretation relies heavily on subjective observer evaluation. The Kleihauer-Betke test tends to overestimate the amount of RhIG to be administered[84] and assumes that less than 1% of cells in the healthy individual contain hemoglobin F and any level above this represents the presence of fetal RBCs. Unfortunately, persons with hereditary persistence of fetal hemoglobin will have false-positive results, as will women with other hemoglobinopathies such as sickle cell disease and sickle cell trait. In addition, it is known that the levels of hemoglobin F often increase during pregnancy in up to 25% of women.[86]

Despite these pitfalls, when performed by properly trained personnel and with the proper controls, the Kleihauer-Betke test is a reliable method for quantitation of FMH. The sensitivity of the test is such that all samples containing at least 0.5% fetal cells will be detected reliably[57]; the specificity of the test is influenced by the aforementioned variables.

Various formulas exist for calculation of the volume of FMH based on the results of the Kleihauer-Betke test. Using one published formula, the percentage of fetal RBCs detected is multiplied by 50 (corresponding to a presumed maternal blood volume of 5,000 mL); this represents the volume of FMH in milliliters of whole blood.[21] Since one 300-μg dose of RhIG protects against 30 mL of Rh-positive whole blood, the volume of FMH is divided by 30 to determine the number of doses of RhIG required. A more precise determination can be made by calculating the actual total maternal blood volume based on height, weight, and body surface area. These formulas assume that all fetal cells will stain dark pink and that fetal RBCs are the same size as adult RBCs. Based on these assumptions and since the accuracy and precision of the Kleihauer-Betke test may be poor when performed by inexperienced personnel, it is recommended that 1 additional vial of RhIG be added to the calculated dose.[21]

Other Methods

Owing to the perceived lack of sensitivity and specificity of the various tests for detection and/or quantitation of FMH, some have advocated the use of postinjection RhIG titers to assess the need for administration of additional RhIG.[87,88] This has not been shown to be a reliable indicator of FMH since several days may be required before equilibration of plasma levels of RhIG occurs after IM injection.[89,90] In addition, the detection of circulating anti-D does not guarantee that Rh-positive fetal RBCs have been removed from the maternal circulation. The use of this testing method is limited and to be discouraged.

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This practice parameter was developed exclusively for the purposes set forth above and not for use in connection with matters involving reimbursement, credentialing, or utilization review.

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