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Prevention of Rh D alloimmunization

Background

This is a review of the patients at risk for sensitization to a red cell antibody. This is currently referred to as “alloimmunization.” The affected pregnancy poses the risk of hemolysis and anemia to the fetus, which can lead to hydrops and death. The infant is at risk of anemia and jaundice.

There is wide variation in prevalence rates of Rh D-negative individuals between regions, for example from 5% in India to 15% in North America. However, high birth rates in low prevalence areas means Rh hemolytic disease of the newborn is still an important cause of morbidity and mortality in countries without prophylaxis programs.

In such countries, 14% of affected fetuses are stillborn and one half of live born infants suffer neonatal death or brain injury. The routine use of Rh D immune globulin is responsible for the reduced rate of red cell alloimmunization in more economically developed countries.

First introduced in the 1970s, the postpartum administration of Rh D immune globulin reduced the rate of alloimmunization in at-risk pregnancies from approximately 13–16% to approximately 0.5–1.8%. The risk was further reduced to 0.14–0.2% with the addition of routine antepartum administration.

In general there is a low risk of alloimmunization, plus the fact that 40% of infants of Rh D-negative women will be Rh D negative. Despite considerable proof of efficacy, there are still a large number of cases of Rh D alloimmunization because of failure to follow established protocols.

Nomenclature

The Rhesus (Rh) blood system consists of the C, c, D, E, e, and G antigens (there is no d antigen). Variants include weak D and partial D antigens. In addition, the D gene may be present, but not translated or not expressed.

The standard obstetrical nomenclature for designating a pregnant woman’s blood type is the ABO type and either Rh positive or Rh negative. These terms are commonly used to describe a woman who has or does not have the Rh(D) antigen on her red cells (RBCs). However, this abbreviated nomenclature is an artificial designation and can be confusing because the Rhesus blood group system consists of 48 antigens; the most common antigens to which individuals make antibodies are D (there is no d antigen), C, c, E, e; C/c and E/e are alternate alleles with codominant expression.

Some combination of DCE is inherited as a haplotype from each parent. A woman who is "Rh-negative" (meaning no D antigen) can form anti-C, c, E, and/or e antibodies if exposed to fetal red cells with C, c, E, and/or e antigens inherited from the father that she does not share. Since she is Rh(D) negative, she may have received prophylactic anti-D immune globulin in previous pregnancies, but this would not prevent c alloimmunization.

Causes of Rh D Alloimmunization

Rh D alloimmunization occurs when a Rh D-negative woman is exposed to red cells expressing the Rh D antigen. Although the fetal and maternal circulations are separate, there is often some antenatal mixing of fetal and maternal blood, even in asymptomatic women. The volume of fetal–maternal hemorrhage leading to Rh D alloimmunization can be as small as 0.1 mL or as large as 30 mL.

Events such as miscarriage, ectopic pregnancy, antenatal bleeding, and delivery, as well as procedures such as chorionic villus sampling, amniocentesis, pregnancy-related uterine curettage, and surgical treatment of ectopic pregnancy can lead to maternal exposure to fetal red blood cells and, consequently, Rh D alloimmunization (Box 1).

Between 3% and 11% of women with threatened abortion in the first trimester, and approximately 45% giving birth in the third trimester, have a fetal–maternal hemorrhage. Until further evidence is available, expert advice continues to recommend administration of anti-D immune globulin within 72 hours of suspected breach of the choriodecidual space.

Risks

Spontaneous pregnancy loss (1st or 2nd trimester):	1.5-2.0%
Amniocentesis:	2-6%
External cephalic version (regardless of success):	2-6%
Threatened SAB, first trimester:	3-11%
Spontaneous pregnancy loss with uterine instrumentation:	4-5%
Chorionic villus sampling:	14%
Delivery, third trimester:	45%

Box 1. Potential Sensitizing Events in Rh D-Negative Women in Pregnancy

- Chorionic villus sampling, amniocentesis, cordocentesis
- Threatened miscarriage or miscarriage
- Ectopic pregnancy
- Evacuation of molar pregnancy
- Therapeutic termination of pregnancy
- Antepartum hemorrhage
- Abdominal trauma
- Intrauterine fetal death
- External cephalic version
- Delivery

Pathogenesis and consequences of Alloimmunization

The percentage of Rh (D)- negative individuals developing an immune response to infusion of Rh(D)-positive RBCs depends, in part, on the volume of blood infused: 0.3 mL RBCs stimulates an anti-D response in some subjects, whereas one unit (450 mL) of RBCs results in the

maximum percentage of responders (80 percent). The antibody response develops slowly, and is usually not detectable serologically until 5 to 15 weeks after exposure.

Whether a primary immune response occurs depends upon several factors besides the volume of fetal blood to which the mother was exposed. These variables include the frequency of fetomaternal transfusion and whether the mother and fetus are ABO compatible. Both the immunogenicity of the fetal RBCs and the immunogenic response capacity of the mother play a role in pathogenesis. Interestingly, individuals with acquired immune deficiency syndrome (AIDS), as well as some other patient populations, may not form alloantibodies to the D antigen.

Transplacental transfer of maternal antibody leads to hemolytic disease of the fetus/newborn. The severity of fetal anemia is influenced primarily by antibody concentration, but also by additional factors that are not fully understood. These include the subclass and glycosylation of maternal antibodies; the structure, site density, maturational development and tissue distribution of blood group antigens; the efficiency of transplacental IgG transport; the functional maturity of the fetal spleen; polymorphisms which affect Fc receptor function; and the presence of human leukocyte antigen (HLA)-related inhibitory antibodies.

Severe anemia leads to hydrops fetalis (two or more of the following: skin edema, ascites, pericardial effusion, pleural effusion). Hydrops occurs when the fetal hemoglobin deficit is at least 7 g/dL below the mean for gestational age (consistent with a hematocrit less than about 15 percent or hemoglobin <5g/dL).

Laboratory Evaluation

The test most commonly used for diagnostic purposes is the indirect Coombs test (ie, determination of antibodies in the plasma), which is the most accurate technique for determining antibody titers. The indirect Coombs titer is the value used to guide obstetrical management of alloimmunized pregnancies.

Incubation of known Rh(D)-positive red blood cells (RBCs) with maternal plasma is the first step in the indirect Coombs test. Any anti-Rh(D) antibody present will adhere to the RBCs. The RBCs are then washed and suspended in antihuman globulin (Coombs) serum. Red cells coated with maternal anti-Rh(D) will be agglutinated by the antihuman globulin, which is referred to as a positive indirect Coombs test.

Management

Screening

Rh(D) typing and an antibody screen should be performed at the first prenatal visit on all patients.

For Rh(D)-negative women with an initially negative antibody screen and uncomplicated pregnancy antibody screen is repeated:
-at 28 weeks of gestation
-at delivery

Diagnosis

The diagnosis of Rh(D) alloimmunization is based upon detection of anti-Rh(D) antibody in maternal serum.

A positive alloantibody identification should be interpreted as a screening test. A true positive alloantibody identification means that the fetus is at risk for hemolytic disease, not that it has occurred or will develop. (see False positive below)

False positive
Screening for anti-D antibodies may not be helpful in identifying alloimmunization if the patient has received anti-D immune globulin within the past 3 weeks.

The median half-life of anti-D immune globulin is 23 days in the third trimester. If delivery occurs within 12 weeks of the standard antenatal anti-D immune globulin administration, the postnatal dose may be withheld in the absence of excessive fetal–maternal hemorrhage*. The same is true when anti-D immune globulin is given for antenatal procedures, such as external cephalic version or amniocentesis, or for third-trimester bleeding.

Titration can be helpful: women who received anti-D immunoglobulin at 28 weeks will have a low (≤4) antibody titer at term; a high titer suggests the presence of alloanti-D. Also, new alloanti-D is associated with IgM antibodies, whereas exogenous anti-D is IgG.

*In Rh D-negative pregnant patients who have experienced abdominal trauma quantification of fetal–maternal hemorrhage should still be done with a Kleihauer Betke. (See Trauma in Pregnancy guideline) Please follow the instructions from the ANMC Blood Bank to determine how many additional vials of anti-D immune globulin to administer.

Weak D type
The ANMC Transfusion Service results Weak D patients as Rh positive. For the sake of caution, the ANMC Transfusion Service still administers a vial of anti-D immune globulin to all Weak D antenatal mothers in the peri-28 week period and another anti-D immune globulin vial at birth, if the infant is Rh positive.

Paternity
Reliable rates of nonpaternity are difficult to ascertain but a recent review indicates that the mean rate among population studies is approximately 3%. Strategies of selective administration of Rh D immune globulin depending on the partner's blood type have been shown to be cost equivalent to systematic prophylaxis.

If paternity is certain and the father is known to be Rh D negative, antenatal prophylaxis is unnecessary. If the Rh type of the partner is not known, and given that immunological typing of the father would probably not be carried out by most clinicians, routine antenatal prophylaxis remains the preferred option.

Treatment

A. Routine pregnancy:

28 weeks of gestation
Prophylactic anti-D immune globulin should be offered to unsensitized Rh D-negative women
-Obtain an antibody screen
-If the antibody screen test is negative, then a single dose of 300 micrograms of anti-D immunoglobulin should be administered.

Following birth:
-Obtain Rh status of infant
-If the infant is confirmed to be Rh D positive, then all Rh D-negative women who are not known to be sensitized should receive a single dose of 300 micrograms anti-D immune globulin within 72 hours of delivery.

Events during pregnancy

The following Rh D-negative women when the fetuses could be Rh D positive should receive prophylactic anti-D immune globulin:

-Abdominal trauma*
-Amniocentesis
-Antenatal hemorrhage
-Chorionic villus sampling
-Ectopic pregnancy
-External cephalic version (regardless of success)
-Fetal death in the 2nd / 3rd trimester*
-Pregnancy termination, either medical or surgical
-Spontaneous pregnancy loss (1st or 2nd trimester)
-Spontaneous pregnancy loss with uterine instrumentation
-Suspected of molar pregnancy and who undergo uterine evacuation
-Threatened SAB, first trimester

* consider excessive fetal–maternal hemorrhage, below

Excessive Blood Loss
In Rh D-negative pregnant patients who have experienced abdominal trauma quantification of fetal–maternal hemorrhage should be done with a Kleihauer Betke. (See Trauma in Pregnancy guideline) Please follow the instructions from the ANMC Blood Bank to determine how many additional vials of anti-D immune globulin to administer.

NB: This also applies to cases of excessive fetal–maternal hemorrhage, including some cases of abruptio placentae, placenta previa, intrauterine manipulation, persistent antepartum bleeding, or fetal death.

B. Treatment of red cell alloimmunization

1. When red cell alloimmunization is present, notify Blood Bank and Pediatrics at time of admission.

2. <u>D and Non-D (non-Rh) antibodies</u>

These are often referred to as “atypical” or “irregular. (See Appendix 1) Most cases of antibody sensitization to atypical antigens are caused by blood product transfusion.

a. If a patient has a positive antibody screen, confirm that the lab has identified the type and antibody level (titer). Titer values may be reported as 1:X or simply as X.

b. If the patient has had a previous pregnancy complicated by alloimmunization and the fetus/infant was affected (developed hydrops, required transfusion, etc), then serial titer assessment is inadequate. The patient should be referred to MFM for additional evaluation.
c. If the initial antibody titer is 8 or less, serial antibody titers should be obtained at four-week intervals.
d. If the antibody titer reaches a level of 16 or greater, additional evaluation is warranted. This is known as the critical titer, or the titer at which there is the potential for risk to the fetus of anemia and hydrops.
e. If the fetus is at increased risk of anemia (previous affected pregnancy, titer of 16 or greater, etc), the next step in evaluation is Doppler measurement of the peak systolic velocity in the middle cerebral artery (MCA). Generally, MCA Doppler is not performed prior to 18 weeks gestation.
f. If paternity is absolutely certain, the paternal genotype can be evaluated.
- If the father is negative for the red cell antigen in question, the fetus would be considered not at risk and no further testing would be necessary.
- If the father is homozygous for the red cell antigen, the fetus would be an obligate carrier and would be considered at risk.
-If the father is heterozygous, there is a 50% risk of inheritance to the fetus.
g. Amniocentesis can be offered to determine fetal genotype when paternity is uncertain, or when the father is a heterozygote for the red cell antigen in question. For Rh (D) and other antigens, cell-free fetal DNA may be an option, especially in the future.
h. If a patient has developed sensitization to anti-D (Rh), additional doses of RhIG (RhoGam) are unnecessary.
i. Timing of delivery depends on the estimated risk to the fetus. In general, the goal is to reach a gestational age of 37 – 38 weeks.

3. Kell (K-1) sensitization

a. Confirm paternity.
-If possible, the paternal antigen status should be obtained. The K-1 antigen is found in about 1 – 5% of individuals. Most individuals who are positive are heterozygotes.
b. If paternity is uncertain, or the father carries the antigen, fetal surveillance with MCA Doppler is indicated if the titer is 8 or higher.
c. Antibody titers are not appropriate for monitoring Kell-sensitized patients because Kell antibodies do not correlate with fetal status.

Quick tips

Do I need to give an additional dose of anti-D immune globulin if the patient goes post dates?

No. Anti-D immune globulin appears to persist for approximately 12 weeks in most patients, based on pharmacokinetic studies using modern assay methods. In the past, some authorities advised giving a second dose of Rh D immune globulin to women who have not given birth 12 weeks after receiving their antenatal dose. However, the vast majority of women who give birth more than 12 weeks after receiving antenatal Rh D immune globulin do not become alloimmunized.

Should anti-D immune globulin be withheld from a woman undergoing postpartum sterilization?

No. Although a primary reason to prevent alloimmunization is to reduce risk in future pregnancies, there are other indications as well. Pregnancies occur despite sterilization procedures, and most are intrauterine. In addition, alloimmunization complicates crossmatching of blood products in the future. Thus, Rh D-negative women who are undergoing postpartum tubal sterilization are candidates for treatment with anti-D immune globulin. If an Rh D-negative woman who has had a sterilization procedure does become pregnant later, even with a miscarriage or ectopic pregnancy, she should be offered anti-D immune globulin in a similar manner as women without sterilization.

What should be done if an Rh D-negative patient is discharged without receiving anti-D immune globulin after a potentially sensitizing event?

The ideal time to administer anti-D immune globulin is within 72 hours of a potentially sensitizing event. However, volunteers have received a range of partial to complete protection when anti-D immune globulin was given as late as 13 days after exposure. The longer prophylaxis is delayed the less it will be protective, but it has been suggested that a patient may still receive some benefit from anti-D immune globulin as late as 28 days postpartum.

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Appendix 1

Table 1. Atypical Antibodies and Their Relationship to Fetal Hemolytic Disease

Blood Group System	Antigens Related to Hemolytic Disease	Hemolytic Disease Severity	Proposed Management
Lewis	*		
I	*		
Kell	K	Mild to severe†	Fetal assessment
	k	Mild	Routine obstetric care
	Ko	Mild	Routine obstetric care
	Kp^a	Mild	Routine obstetric care
	Kp^b	Mild	Routine obstetric care
	Js^a	Mild	Routine obstetric care
	Js^b	Mild	Routine obstetric care
Rh (non-D)	E	Mild to severe†	Fetal assessment
	C	Mild to severe†	Fetal assessment
	C	Mild to severe†	Fetal assessment
Duffy	Fy^a	Mild to severe†	Fetal assessment
	Fy^b	‡	Routine obstetric care
	By^3	Mild	Routine obstetric care
Kidd	Jk^a	Mild to severe	Fetal assessment
	Jk^b	Mild	Routine obstetric care
	Jk^3	Mild	Routine obstetric care
MNSs	M	Mild to severe	Fetal assessment
	N	Mild	Routine obstetric care
	S	Mild to severe	Fetal assessment
	s	Mild to severe	Fetal assessment
	U	Mild to severe	Fetal assessment
	Mi^a	Moderate	Fetal assessment
MSSs	Mt^a	Moderate	Fetal assessment
	Vw	Mild	Routine obstetric care
	Mur	Mild	Routine obstetric care
	Hil	Mild	Routine obstetric care
	Hut	Mild	Routine obstetric care
Lutheran	Lu^a	Mild	Routine obstetric care
	Lu^b	Mild	Routine obstetric care

Diego	$D1^a$ Di^b	Mild to severe Mild to severe	Fetal assessment Fetal assessment
Xg	Xg^a	Mild	Routine obstetric care
P	$PP_{1pk}(Tj^a)$	Mild to severe	Fetal assessment
Public antigens	Yt^a Yt^b Lan En^a Ge Jr^a Co^a Co^{1-b-}	Moderate to severe Mild Mild Moderate Mild Mild Severe Mild	Fetal assessment Routine obstetric care Routine obstetric care Fetal assessment Routine obstetric care Routine obstetric care Fetal assessment Routine obstetric care
Private antigens	Batty Becker Berrens Biles Evans Gonzales Good Heibel Hunt Jobbins Radin Rm Ven $Wright^a$ $Wright^b$ Zd	Mild Mild Mild Moderate Mild Mild Severe Moderate Mild Mild Moderate Mild Mild Severe Mild Moderate	Routine obstetric care Routine obstetric care Routine obstetric care Fetal assessment Routine obstetric care Routine obstetric care Fetal assessment Fetal assessment Routine obstetric care Routine obstetric care Fetal assessment Routine obstetric care Routine obstetric care Fetal assessment Routine obstetric care Fetal assessment

*Not a proven cause of hemolytic disease of the newborn

[†]With hydrops fetalis

[‡]Not a cause of hemolytic disease of the newborn

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