Rh Isoimmunization - Update

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Abstract

Rh isoimmunization continues to occur because some susceptible women are not identified and are not given Rh immunoglobin (RhIG) prophylaxis, or because routine postpartum prophylaxis fails due to an unrecognized excessive fetomaternal hemorrhage. Methods of identifying fetomaternal hemorrhage are discussed. Administration of RhIG after early pregnancy losses, after amniocentesis, after other selected obstetric events, and to all Rh-negative, unsensitized women at 28 to 29 weeks of gestation will minimize isoimmunization during pregnancy. The protocol for identifying new or developing cases of isoimmunization, and for treating identified patients is presented. Women whose fetuses are severely affected should be referred to perinatal centers for specialized care. This care includes repeated ultrasound examinations, amniocentesis, and may also involve intrauterine transfusions, plasmapheresis, and preterm delivery.

Key Words: *Rh-isoimmunization, prevention, ultrasound, intrauterine transfusions, plasmapheresis.*

The incidence of Rh (anti-D) isoimmunization has decreased dramatically since the introduction of Rh immune globulin (RhIG) postpartum prophylaxis in 1968.[1] However, isoimmuniztion has not been eradicated as was hoped. There are several reasons for the continuation of Rh-isoimmunization (Table 1).

1. Under-utilization:

Some candidates for RhIG postpartum prophylaxis are not identified. Fortunately this is becoming rare, as Rh typing of the parturient and her neonate is accepted routine practice. However, it will continue as a problem when delivery occurs at home, or in the emergency room of a small hospital while en route to the hospital in which the patients' records are kept. Additionally, a few cases will continue to be isoimmunized after early pregancy terminations (e.g. abortion, ectopic) when routine Rh typng is less strictly practiced. Also, some patients abort spontaneously at home and do not seek medical care. Obviously these patients do not receive RhIG.

2. Failure of postpartum prophylaxis:

The standard dose of RhIG (300 μg) purportedly protects the mother against isoimmunization by as much as 15 ml of Rh positive fetal red blood cells. If the fetomaternal hemorrhage (FMH) - at the time of separation of the placenta - is excessive i.e. > 15 ml red blood cells, the standard dose may not be completely protective, and isoimmunization can ocur. The incidence of excessive FMH has varied in different series (0.2-1%).[2,3] Failure to identify these patients and to give them correspondingly larger doses, is a cause for continuing Rh isoimmunization.

There are several methods to detect excessive FMH.[4] The most commonly used is the Kliehauer-Betke staining of a peripheral smear from Maternal blood.* The maternal red blood cells will appear as ghost cells (HbA is eluted by the acid) whereas fetal red blood cells stain pink, (HbF being acid-resistant). The ratio of fetal/maternal cells gives an estimate of the volume of FMH. In some hands this test has been very useful, while in other hands there has been an unacceptably high incidence of false positive results.[5] A basic disadvantage of this test is that it detects HbF rather than fetal cells and this may be falsely elevated in patients who have high levels of HbF (not related to FMH) e.g. hemoglobinopathies including hereditary persistence of HbF.[6]

In our institution we have used a rosette screening test** which detects Rh positive (fetal) cells in the maternal circulation. It is more sensitive and appears to be a superior test. We have also used an indirect antiglobulin test to detect excessive FMH. An indirect Coombs' test is performed 24-48 hours after RhIG administration. If all Rh positive antigenic sites have been covered and there remains free circulating anti-D, we consider that the RhIG dose was sufficient. However, if the indirect Coombs' test is negative we assume that some of the RH positive an-

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Table 1. Causes of Continuing Development of Rh Sensitization

Under-Utilization:	
Postpartum, or more commonly after early pregnancy termination.	
Antepartum Sensitization:	
Amniocentesis	
Placental separation	
? threatened abortion	
External cephalic version	
No known predisposing factor	
Failure of postpartum prophylaxis:	
Excessive fetal-maternal hemorrhage	

tigenic sites are still uncovered and give additional doses of RhIG until the indirect Coombs' test is positive.[5] A much more sensitive test for the quantitation of FMH has been introduced i.e. enzyme-linked antiglobulin test (ELAT).[7] Unfortunately, it is still unavailable in most hospitals.

3. Antepartum sensitization:

Although most FMH(s), specially those of a significant volume, occur at the time of separation of the placenta at delivery, it has been documented that FMH may occur throughout pregnancy, especially in the third trimester. Antepartum FMH is responsible for isoimmunization in 1.8% of women (Table 2).[2] Any condition which involves separation of, or injury to placental tissue will predispose to FMH. Amniocentesis, especially when the needle traverses placental tissue can be associated with FMH. Placenta previa, placental abruption, and threatened abortion are all suspected of causing FMH. External cephalic version may also be a predisposing factor. Moreover, antepartum FMH has been known to occur in the absence of any of the above cited conditions.[4]

The administration of RhIG to all RH negative unsensitized women who undergo amniocentesis, and to all those who had any of the predisposing conditions, if FMH has been demonstrated (e.g. by Klihauer-Betke test of the maternal blood) has been recommended.[8]

Bowman has demonstrated that the antepartum administration of RhIG to all Rh negative unsensitized women at 28 weeks will significantly reduce (by 97%) the risk of isoimmunization during pregnancy and within three days of delivery. The reason that the time of administration was chosen to be 28 weeks is that only 8% of the women who were sensitized during pregnancy, became sensitized prior to 28 weeks (Table 2).

*Fetaldex, Ortho Diagnostics, Raritan, NJ.

**Fetalscreen, Ortho Diagnostics, Raritan, NJ.

Table 2. Sensitization During Pregnancy Manitoba 1967-1974

Immunization during pregnancy or within three days of delivery occurred in 62 of 3533 (1.8%) Rh negative women.

< 28 weeks	5	8%
29-34 weeks	10	16%
35 weeks - three days postpartum	47	76%
TOTAL	62	

Modified from Bowman.[2]

In spite of these excellent results, considerable disagreement has been voiced in British and American literature concerning the advisability of the antenatal prophylaxis program. These concerns have dealt less with the safety and efficacy of the program than with the cost-effectiveness and the risk to donor volunteers needed for the production of the greatly increased quantities of RhIG.[8]

Recently, the American College of Obstetricians and Gynecologists (ACOG) recommended the routine administration of RhIG to all unsensitized pregnant women at 28 weeks in addition to the antepartum administration of RhIG in the special cases cited previously (Table 3).[9] It should be obvious that the antepartum administration of RhIG under any of these circumstances does not replace or exclude postpartum RhIG prophylaxis. Postpartum prophylaxis is indicated only if the neonate is Rh positive (D or D^u positive). D^u is a variant that is still capable of producing sensitization in the Rh negative mother.[10]

Notwithstanding these best efforts, it has been estimated that there will be an irreducible minimum of Rh isoimmunization of 0.5-1/1000 pregancies.[2] In Manitoba, where universal antenatal prophylaxis was begun in July 1975, 44 sensitized women were seen in 1977. The causes of sensitization of these women are listed in Table 4.

Diagnosis:

There will be a group of women who present with a history of previous isoimmunized pregnancies and/or affected babies. However, in a regular (non-referral) practice, the physician must be alert to identify new or developing cases of isoimmunization. All pregnant women should have Rh typing and antibody screening done at their first prenatal visit. If Rh antibody is detected, the patient is isoimmunized. If negative, the antibody testing should be repeated at 28 weeks and if still negative RhIG (300 ug) should be administered. It is a good idea to obtain another antibody screen at 35-36 weeks. It may be positive because of the administered RhIG. If so, the an-

Table 3. Antepartum Administration of RHIG*

RHIG is to be given to Rh negative unsensitized women in the following circumstances:

A. After amniocentesis for any indication, at any period of gestation.
B. Routinely at 28 weeks of gestation.
C. A positive K-B test in patients with:
   a. antepartum bleeding not necessitating immediate delivery.
   b. unexplained fetal death.
   c. after external cephalic version.
   d. threatened abortion**

*This does not preclude its postpartum administration. If the newborn is D or D^u positive, RHIG should be given.
**This is not an official recommendation at present.

tibody titer should be 1:1 or 1:2. However, if it is $\geq$ 1:4 or higher, active immunization must be suspected. These patients represent either previously undetected sensitization, sensitization before 28 weeks, or prophylaxis failure.

In sensitized pregnancies, only Rh positive fetuses will be affected by the disease. It is not possible to determine the fetal Rh type in utero and indirect methods are used to diagnose fetal involvement. Antibody titers are usuallly obtained serially. However, antibody titers do not usually give an accurate indication of the degree of fetal involvement (if any). The most generally used method is measurement of ΔOD at 450 nm of the amniotic fluid (AF). This test estimates the bilirubin content and therefore indirectly reflects the degree of fetal blood hemolysis. One has to perform amniocentesis if there is a significant increase in antibody titer, or if it reaches a threshold that should be identified for each blood bank. Usually the amniocentesis is performed after 28 weeks. However, if the titer is very high or there is history of severely affected previous offspring, amniocentesis should be done earlier i.e. 22-24 weeks. Amniocentesis should always be done with realtime ultrasonic guidance. The ΔOD_{450} is plotted on Liley's chart (or a similar graph) to indicate mild (Zone I), moderate (Zone II), or severe (Zone III) fetal involvement.

Hydrops fetalis can be easily diagnosed by sonography (Figure 1). However, there are no clear cut criteria to sonographically diagnose early or mild fetal involvement. Progressive dilation of umbilical vein, increased thickness of the placenta, increased size of liver, increased volume of AF all suggest fetal involvement.

Table 4. Causes of Sensitization in 44 Pregnant Women Seen in Manitoba 1977

Immunization prior to Rh immunoglobulin availability	17
Failure to give Rh immune globulin	13
Sensitization during pregnancy*	13
Unsuspected massive fetomaternal hemorrhage (postpartum prophylaxis failure).	1

*Universal antenatal prophylaxis started July 1975. Modified from Bowman.[2]

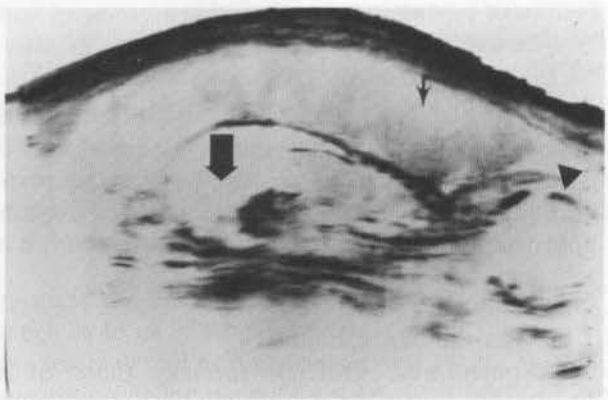

Figure 1. Sonogram showing fetal hydrops secondary to severe Rh sensitization. Note the thick placenta (arrow), the halo around the skull (arrowhead), and fetal ascites (broad arrow).

Management:

The management plan is summarized in Table 5. The management depends on the severity of the fetal involvement.

Mild Cases:

Amniocentesis is repeated every 2-3 weeks as long as there is downward trend of ΔOD_{450}. At 38 weeks, the fetal lung maturity should be determined by measuring the lecithin/sphingomyelin (L/S) ratio and/or phosphatidylglycerol (PG) in the AF. If mature, labor is induced when the cervix becomes favorable. If ΔOD_{450} continues to decline significantly, the amniocentesis may be further spaced (3-4 weeks). If ΔOD_{450} approaches zero, the fetus is most probably unaffected (Rh negative) and spontaneous delivery could be awaited.

If ΔOD_{450} increases at anytime the patient should be managed as a moderate case.

Moderate Cases:

Amniocentesis is repeated in 7-10 days, if ΔOD_{450} has further increased, specially if it is in the upper mid zone, the amniocentesis is repeated in 3-7 days. If it has further increased or reached Zone III, the patient should be treated as a severe case.

If ΔOD_{450} continues to be in midzone (Zone II), the amniocentesis is repeated every 10 days to 2 or more weeks depending on the trend (the greater the decline, the longer could be the interval). If it decreases to Zone I the patient is treated as a mild case.

At 36-37 weeks, fetal lung maturity should be determined and the patient delivered once lung maturity has been demonstrated.

These fetuses should be thoroughly evaluated by sonography prior to each amniocentesis looking for signs of hydrops. They should also be monitored with weekly Non-Stress tests (NSTs).

Severe Cases:

These patients should be managed in perinatal centers where there is a Maternal-Fetal Medicine subspecialist with expertise in the management of this problem, including intrauterine fetal transfusion (IUT), level III neonatal intensive care unit, as well as optimal sonographic and blood banking services.

Severe cases diagnosed after 32 weeks of gestation or after there is evidence of fetal lung maturity are delivered immediately usually by cesarean section. If the diagnosis is made at 30-32 weeks, or if the lung was found not to be mature, delivery 48 hours after the administration of a glucocorticoid to enhance fetal lung maturation may be considered. In gestations < 30 weeks, IUT is probably the treatment of choice. Plasmapheresis can be used in selected cases. Promethazine therapy and oral desensitization have been also used in the management of severe cases.

A. Intrauterine transfusion:

The fetus can absorb 70-80% of the red blood cells deposited in its peritoneal cavity through lymphatics, specially in the diaphragmatic area. We use fresh, Rh negative group O packed(75-85%) red blood cells that are cross matched with the maternal blood. The cells are irradiated and then thoroughly washed to remove the lymphocytes. This is done to avoid the graft-versus-host reaction that may otherwise occasionally happen. In the past, transfusions were carried out with fluoroscopic guidance four hours after amniography (to delineate the fetal bowel). Since 1978, we and others,[11,12] have utilized realtime ultrasonic guidance with markedly improved results. After confirmation of the position of the fetus and selection of the needle insertion site, the patient is given 50 mg of meperidine hydrochloride and 5-10 mg of diazepam slowly intravenously. The abdomen is prepared and draped. The ultrasound transducer is wrapped in a sterile plastic bag and a sterile lubricant jelly is used as coupling medium. Local anesthesia is administered at the preselected insertion site and a 17-guage Touhy needle is inserted under vision (ultrasonic). The needle is advanced until the fetal abdominal wall is touched, and then a thrust is made through it. The needle tip can usually be seen in the fetal peritoneal cavity. One to two ml of normal saline is injected and the flow of fluid into the peritoneal cavity is seen (as bubbles). In case of doubt, 3 mls of diatrizoate sodium (Hypaque) are injected and a plain radiograph is obtained. Multiple crescent shaped radio-opacities indicate correct intraperitoneal placement. The blood cells may be transfused directly through the Touhy needle (Figure 2), or through a catheter that is threaded through the needle which is then removed. The blood cells are transfused at a rate of 1-2 ml/minute. The volume of blood cells to be transfused is determined by the formula: volume = (gestational age (GA) in weeks - 20) X 10. For example, 60 ml at 26 weeks. Throughout the procedure, the ultrasound transducer is intermittently aimed at the heart and its rate counted. After the procedure the fetal heart is monitored continuously and the patient is closely observed for the presence of uterine contractions, rupture of membrances and/or vaginal bleeding for at least four hours.

Figure 2. Ultrasonic guided intrauterine transfusion. The needle is advanced while watching on the TV monitor. Packed red blood cells are injected directly through the Touhy needle and the flow of blood into the fetal peritoneal cavity is seen on the monitor.

Complications of IUT:

1. Maternal:
   Preterm labor, preterm premature rupture of the membranes (PROM), placental abruption, and amnionitis.
2. Fetal:
   Prematurity, trauma, and cardiovascular collapse. Severely anemic and sick fetuses sometimes do not tolerate the procedure. Fetal death within 48 hours of the procedure is considered causally related. Using this definition, the fetal death rate has been estimated to be 5% per transfuison,[13] the majority being secondary to trauma.

Follow-up:

After IUT, some blood seeps into the AF and makes the ΔOD_{450} measurement unreliable as a guide to the fetal condition or to the need for further IUTs. IUTs are therefore emperically repeated every 2-3 weeks. In the intervals, the fetal condition is closely evaluated by NSTs and repeated ultrasound examinations.

More recently, direct intravascular transfusion has been proposed as being superior to the "conventional" intraperitoneal transfusion. The procedure was originally performed through a fetoscope.[14] However, with improved ultrasound resolution it is now possible to perform the intravascular transfusion directly with a 22-gauge needle inserted in the umbilical vein either in the cord[15] or in its hepatic portion.

B. Plasmapheresis:

The exchange of the anti-D rich maternal plasma with saline will - at least temporarily - reduce the antibody titer. This may reduce fetal hemolysis and thus ameliorate the fetal condition.[17] However, plasmapheresis has its disadvantages:

1. The salutory effect is - at best - temporary.
   a) The anti-D (IgG) is distributed between the intravascular and extravascular components and when the antibody concentration is reduced in the circulating plasma, IgG difuses back into the circulation. For the procedure to be effective, it has to be repeated frequently.
   b) The fetus continues to shower the mother with additional Rh positive cells, thus activating the production of new antibody.
2. It reduces the concentration of the "useful" antibodies as well, thus increasing the risk of infection of the mother (and her fetus).
3. It is expensive, time consuming, and inconvenient.
4. It has not been proven effective in well controlled studies.

I have used plasmapheresis in selected cases of isoimmunized pregnancies as an adjunct to IUTs and found it quite useful.[19] I have used plasmapheresis in the following circumstances:

1. Women with very poor obstetric history (prior fetal losses in early pregnancy; < 26 weeks). It is known that erythroblastosis fetalis becomes more severe with each succeeding pregnancy.
2. Women with very high antibody titers discovered early in pregnancy.

In both situations, the fetus may be lost before the fetal condition can be evaluated and/or before it is possible to perform IUT. Under these circumstances, plasmapheresis is started at 8-10 weeks of gestation and repeated weekly, or more frequently, to keep the antibody titer at a low level. At 22-24 weeks, amniocentesis is performed and the principles of management as outlined above are followed until it is shown that the fetus is not affected by the disease or until IUT can be performed.

3. Rarely when IUT is indicated, but for technical reasons it could not be performed; a small fetus < 24 weeks or persistent unfavorable fetal position, plasmapheresis is done daily or every other day until the conditions permit the performance of IUT.

This protocol was followed in eight patients during the last few years. There were no complications associated with plasmapheresis in these cases, and I believe that it may have contributed to the salvage of some severely affected fetuses.

Table 5. Management of Rh Isoimmunization

Detection of cases, serial antibody titers.
Serial amniocenteses, with determination of ΔOD_{450} and fetal lung maturity.
Ultrasonic evaluation.
Evaluation of fetal well-being; NST(s), CST(s), biophysical profile.
Delivery: Awaiting spontaneous labor in the mildest cases.
Induction of labor in mild and some of the moderate cases.
Elective cesarean sections in some moderate and most severe cases.
Intrauterine transfusions in severe cases.
Plasmapheresis in selected cases.

C. Other modalities of therapy:

1. Promethazine in large doses has been given to sensitized women beginning in early pregnancy with the hope of minimizing the antigen-antibody interaction and thus fetal hemolysis. However, this may inhibit other useful immunologic responses. Further, the large doses necessary for this action cause drowsiness in the mother. Although successful results have been reported,[20] it has not gained wide acceptance.
2. Desensitization by oral ingestion of Rh antigen prepared from red cell membranes. This is still experimental with few reported successes.[21]

D. Delivery - by cesarean section - is indicated when:

1. GA > 33 weeks.
2. The appearance of PG in AF (indicating fetal lung maturity). L/S ratios are unreliable when AF is contaminated with

blood.

3. Ultrasonography reveals no signs of improvement or actual signs of deterioration i.e. appearance of hydrops fetalis.
4. Abnormal fetal heart rate testing: sinusoidal pattern, and/or non-reactive NST followed by a positive contraction stress test.

Summary:

Although Rh isoimmunization is now very rare, vigorous attempts to further minimize its occurence must be continued. All Rh negative women should be identified. They should be given RhIG if they threaten to abort, after early termination of pregnancy, or whenever they undergo amniocentesis. RhIG should be also administered to Rh negative patients with placental separation, unexplained fetal death, or after external cephalic version especially if FMH was demonstrated. All Rh negative unsensitized women should receive RhIG at 28 weeks. Rh negative women (including those who are D^u positive) who deliver Rh positive or D^u positive neonates should receive postpartum RhIG (in addition to the antepartum dose).

In spite of optimal care, occasional cases of isoimmunization will continue to occur. Isoimmunized pregnant women should be followed carefully with frequent determinations of the antibody titer and amniocentesis when indicated. Moderately and/or severely affected pregnancies whose management could include IUTs, plasmapheresis, etc. and the majority of whom will be delivered prematurely and whose neonates will require exchange transfusion, are best followed in perinatal centers. The provision of optimal care to these patients requires the presence of a team consisting of a Maternal-Fetal Medicine specialist,, Sonographer, Neonatologist, and experienced blood banking personnel. This team approach, and the utilization of the principles of management outlined here resulted in marked improvement of outcome in even the most severe cases.[12] The average survival rate in the recent series is 80%. This is to be compared with a survival rate of 34% reported in the 1969 cooperative study[13].

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