53 Erythroblastosis fetalis

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INTRODUCTION

Red blood cell (RBC) alloimmunization, or isoimmunization, is the development of maternal antibodies to fetal RBC antigens. Hemolytic disease of the newborn/fetus is a hemolytic anemia that results from the lysis of the fetal RBCs by maternal antibodies. It is often characterized by excessive erythroblasts in the fetal bone marrow and circulation (erythroblastosis fetalis). Other features include generalized edema (hydrops fetalis) and hepatosplenomegaly. Alloimmunization and hemolytic disease of the newborn/fetus most commonly occur when maternal antibodies form against paternally derived Rhesus (D) antigens of the fetus, which is termed Rhesus (Rh) alloimmunization.

HISTORY

A midwife, Louyse Bourgeois, first described hemolytic disease of the newborn in 1609 after delivering a set of twins. One twin was a stillbirth with hydrops and the other with severe jaundice died a few days later. Sylvius, in 1679, reported cases of hydrops combined with severe jaundice, which was thought to be caused by a blockage of the "bile tract." Person and Cheyne, in 1799 and 1802, respectively, reported cases of infant fatalities with severe jaundice in which the autopsy reports showed patent bile ducts. In 1905, Auden described the features of hemolytic disease of the newborn. He noted that the first born was often unaffected and it was the subsequent pregnancies that showed hydrops and jaundice. While reporting a case of hydrops fetalis in 1912, Rautmann was the first to use the term "erythroblastosis." In 1923, Ottenburg wrote a paper in which he discussed "accidental placental transfusion of incompatible blood." This was followed by Darrow's hypothesis in 1938 that hemolysis was caused by the passage of anti-fetal hemoglobin from mother to fetus (1).

It was not until 1940 that the Rh antibody was discovered. Landsteiner and Wiener discovered the agglutination of human red cells when mixed with the antiserum of the *Macacus rhesus* and designated this property of human blood "Rh" (2). Wiener and Peters then demonstrated the transfusion reactions caused by giving Rh-positive blood to Rh-negative patients (1). It was in 1941 that Levine described several cases of infants with erythroblastosis fetalis in which the hemolytic disease of the fetus was caused by maternal Rh isoimmunization (3).

In the 1950s and 1960s, we began to see invasive testing take place to evaluate the fetus at risk for erythroblastosis fetalis. In 1956, Bevis, using spectrophotometry, measured blood pigments in amniotic fluid and suggested that the concentration of bilirubin in the amniotic fluid was an indicator of the severity of the hemolytic process in the baby (4). In 1961, Sir William Liley, using amniocentesis and spectrophotometric measurements, described the use of spectral absorption curves

to measure the degree of fetal anemia and suggested that these findings could be used to select the optimal time for delivery (5). He proposed a graph with three zones to indicate the severity of the disease from 27 weeks of gestation to term, which was known as the Liley method. In 1993, a modification of the Liley method by Queenan et al. expanded the normal spectrophotometric measurements to include 14 to 40 weeks of gestation and proposed four management zones (6). In 2000, Mari et al. proposed a noninvasive method to diagnose fetal anemia by ultrasonographic measurement of the middle cerebral artery peak systolic velocity (MCA-PSV) (7).

Before the 1960s, fetuses determined to be at significant risk for hemolytic disease could only be offered induction of labor at 34 weeks of gestation. Although perinatal mortality was greatly reduced by selective induction, there were no therapies for the severely affected fetus at less than 34 weeks of gestation (8). In 1963, after identifying an anemic fetus, Liley performed the first successful intraperitoneal fetal transfusion at 32 weeks of gestation and again at 33 weeks of gestation (8). At 34 weeks and 3 days of gestation, labor was induced and the mother eventually delivered via cesarean section a viable male infant weighing 2560g (8). The boy was named Grant Liley McLeod (9). In 1964, Freda and Adamson described the first intravascular exchange transfusion at 27 weeks of gestation via hysterotomy (10). A fetal leg was delivered through a small uterine incision, a catheter was placed in the femoral artery, and the transfusion was performed. Thirty-six hours later, preterm labor ensued and the infant died from complications of prematurity (10). In 1966, Asensio performed an intravascular exchange transfusion in Puerto Rico on a severely anemic fetus at 31 weeks of gestation. The technique was similar to that described by Freda and Adamson with a few modifications. The pregnancy continued for 3 weeks postoperatively and eventually delivered via cesarean section secondary to premature rupture of membranes. A viable female infant was born weighing 2034g and was discharged home 18 days after delivery (11). In 1973, Valenti used fetoscopy to perform the first aspiration of fetal blood from the umbilical cord and the blood samples were used to diagnose hemoglobinopathies (12). He proposed that the procedure could be used for intrauterine diagnosis and therapy. In 1978, Rodeck and Campbell described a technique to obtain pure fetal blood via fetoscopy in gestations ranging from 16 to 22 weeks (13). Rodeck proposed that since pure blood can be removed from the umbilical circulation via fetoscopy, transfusion should also be possible, and, in 1981, Rodeck described two cases of fetal blood transfusion directly into an umbilical vessel via fetoscopy (14). Both patients were initially transfused at 23 weeks of gestation: one fetus survived after cesarean delivery at 30 weeks of gestation, while the other underwent

Table 1 Risk of Red Blood Cell (RBC) Alloimmunization from Different Clinical Situations

Clinical situation	Risk of RBC alloimmunization (%)
Induced abortions	4–5
First-trimester losses	1–2
Chorionic villus sampling	14
Amniocentesis	7–15
External cephalic version	2–6
Threatened abortion	↑ (controversial)
Antepartum hemorrhage	↑↑
Placenta previa with bleeding	↑
Suspected abruption	↑
Blunt trauma to abdomen (including motor vehicle accidents)	↑
Fetal death	↑
Fetal blood sampling	↑
Fetal surgery	↑
Ectopic	↑
Partial molar pregnancy	↑

Source: From Ref. 17.

an intrauterine demise at 26 weeks of gestation (14). And in 1982, Bang described a case of ultrasound-guided intravenous fetal transfusion in a fetus severely affected by Rh isoimmunization at 29 weeks of gestation. The patient delivered via cesarean section at 32 weeks of gestation and the neonatal period was unremarkable (15).

PATHOPHYSIOLOGY

Maternal alloimmunization occurs when the gravid patient develops an immune response to a paternally derived antigen found on the surface of the fetal RBC. The most common antigen to cause RBC alloimmunization is Rh (D). The majority of Rh (D) alloimmunization is the result of fetomaternal hemorrhage. Fetomaternal hemorrhage has been shown to affect 75% of pregnancies during or shortly after delivery (16). Less than 0.1 mL of fetal blood entering the maternal circulation causes most cases of alloimmunization (16). There are several clinical scenarios when a pregnancy is at risk for fetomaternal hemorrhage, the most significant of which is at the time of delivery. Other clinical scenarios that have demonstrated fetomaternal hemorrhage and the risk of RBC alloimmunization are found in Table 1.

Once Rh (D) alloimmunization has occurred, it is then possible for the maternal IgG antibodies to cross the placenta, attach to the fetal RBC Rh (D) antigen, and cause RBC destruction in the fetal spleen resulting in a hemolytic anemia. This fetal anemia stimulates erythropoietin and the bone marrow produces more RBCs. If the anemia is severe enough, the bone marrow alone may not be able to produce enough RBCs. In this case, erythropoiesis will take place outside of the bone marrow in sites such as the liver and spleen, causing hepatosplenomegaly. Erythropoiesis outside of the fetal bone marrow also will cause immature erythroblasts to appear in the circulation.

NOMENCLATURE

There are several systems of nomenclature used to classify the Rh antigens. The Fisher–Race is perhaps the most commonly used nomenclature system in obstetrics. In this system, there

are five major antigens: C, c, D, E, and e (note that there is no d antigen). A group of three antigens form one gene complex, such as CDe. One gene complex is inherited from each parent, thereby forming a person's genotype (such as CDe/cDE). The most common gene complexes are CDe, cde, and cDE, which are found in 41%, 39%, and 16% of the Caucasian population, respectively. The terms “Rh (D) positive” and “Rh (D) negative” describe the presence or absence of the D antigen. When a genotype includes the letter “d” (e.g., Cde), this is used to indicate the absence of the D antigen, which results in an Rh (D)-negative phenotype (18). The Rh genes are encoded on chromosome 1p34–p36. Rh (D)-negative status in Caucasians is due to a deletion of the Rh (D) gene on chromosome 1 (18).

In addition to the five major antigens in the Rh system, there are several variants. Of note is the antigen called weak D or Du. Among patients with weak D, alloimmunization is rare and anti-D immunoglobulin is not routinely indicated for prophylaxis (19).

Other antibodies outside of the Rh system have been identified, such as Kell, Duffy, and Kidd. Anti-Lewis antibodies do not cause hemolytic disease of the fetus/newborn because the Lewis antigens do not develop on the fetal RBC until after birth and the anti-Lewis antibody is of the IgM class, making it too large to cross the placenta into the fetal circulation. Table 2 displays a more complete list of antigens known to cause alloimmunization and the associated risks of hemolytic disease.

EPIDEMIOLOGY

The Rh (D)-negative blood type is found in 15% of Caucasians, 8% of African-Americans, and less than 1% of Asian populations. The highest known incidence is in the Basque population, where the rate of Rh (D)-negative status is 30%. In 2003, the incidence of Rh alloimmunization was 6.8 per 1000 live births in the United States (21). Fetal anemia from RBC alloimmunization occurs less than 1% of the time, and only 10% of those neonates will require transfusions.

DIAGNOSIS

All pregnant females should be tested for ABO blood group type, Rh (D) status, and the presence of erythrocyte antibodies at the time of their initial prenatal visit (19). This battery of tests should be repeated at the onset of every pregnancy. The intent of routine screening is to identify Rh (D)-negative females prior to the development of alloimmunization in order to provide Rh (D) immunoglobulin. Anti-D immunoglobulin prophylaxis, when properly administered, prevents more than 99% of cases of alloimmunization. The American Association of Blood Banks recommends reevaluation of Rh antibody status prior to the administration of Rh (D) immunoglobulin, postpartum, and after any traumatic events during the antepartum period. The advantage of a second screening test prior to administration of Rh (D) immunoglobulin at 28 weeks of gestation is the detection of those rare cases in which immunization occurs early in pregnancy. Unfortunately, there is no immunoglobulin available for the prevention of an RBC antigen reaction other than for Rh (D).

Rh (D)-positive females do not require further evaluation. As mentioned previously, mothers who are D positive, but

Table 2 Atypical Antibodies and Their Relationship to Fetal Hemolytic Disease

Blood group system	Antigens related to hemolytic disease	Hemolytic disease severity	Proposed management
Lewis	*		
L	*		
Kell	K	Mild to severe [†]	Fetal assessment
	K	Mild	Routine obstetric care
	K ₀	Mild	Routine obstetric care
	Kp ^a	Mild	Routine obstetric care
	Kp ^b	Mild	Routine obstetric care
	Jsa	Mild	Routine obstetric care
	Jsb	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
MSSs	Mi ^a	Moderate	Fetal assessment
	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	Mild to severe	Fetal assessment
	Di ^b	Mild to severe	Fetal assessment
Xg	Xg ^a	Mild	Routine obstetric care
P	PP _{1pk} (Tj ^a)	Mild to severe	Fetal assessment
Public antigens	Yt ^a	Moderate to severe	Fetal assessment
	Yt ^b	Mild	Routine obstetric care
	Lan	Mild	Routine obstetric care
	En ^a	Moderate	Fetal assessment
	Ge	Mild	Routine obstetric care
	Jr ^a	Mild	Routine obstetric care
	Co ^a	Severe	Fetal assessment
	Co ^{1-b-}	Mild	Routine obstetric care
Private antigens	Batty	Mild	Routine obstetric care
	Becker	Mild	Routine obstetric care
	Berrens	Mild	Routine obstetric care
	Biles	Moderate	Fetal assessment
	Evans	Mild	Routine obstetric care
	Gonzales	Mild	Routine obstetric care
	Good	Severe	Fetal assessment
	Heibel	Moderate	Fetal assessment
	Hunt	Mild	Routine obstetric care
	Jobbins	Mild	Routine obstetric care
	Radin	Moderate	Fetal assessment
	Rm	Mild	Routine obstetric care
	Ven	Mild	Routine obstetric care
	Wright ^a	Severe	Fetal assessment
	Wright ^b	Mild	Routine obstetric care
	Zd	Moderate	Fetal assessment

* Not a proven cause of hemolytic disease of the newborn. [†] With hydrops fetalis. [‡] Not a cause of hemolytic disease of the newborn. Source: From Ref. 20.

with D present in reduced quantities, formerly called Du, do not need anti-D prophylaxis. Mothers who are partial D positive, that is, lacking some epitopes of D, should receive anti-D immunoglobulin, since they are at risk for hemolytic disease (19). Rh (D)-negative females without alloantibodies require administration of Rh (D) immunoglobulin at defined intervals during pregnancy (see Management section). Rh (D)-negative females with preformed alloantibodies require further investigation to determine the potential for impact upon the fetus. The least invasive approach involves paternal testing utilizing antisera or DNA (PCR). If the father is homozygous for the D antigen, all his offspring will be at risk for anemia; if he is heterozygous, then 50% of the offspring will be at risk. If the father of the fetus/neonate is definitely known to be Rh (D) negative, neither antepartum nor postpartum anti-D prophylaxis needs to be administered.

Analysis of the fetal genotype is appropriate when the paternal genotype is heterozygous or uncertain. Amniocentesis with PCR of uncultured amniocytes is the most common method utilized, with a sensitivity and specificity of 98.7% and 100%, respectively, and positive and negative predictive values of 100% and 96.9%, respectively (22). Chorionic villus sampling (CVS) also has been utilized for this purpose, but is discouraged due to the increased risk of fetomaternal hemorrhage and alloimmunization (23). Another option is the analysis of maternal plasma or serum for fetal DNA. Greater than 90% accuracy exists in the second trimester due to the high concentrations of fetal DNA in maternal plasma (24–26).

MANAGEMENT OF UNAFFECTED PREGNANCIES

During the 1960s, efforts were made to prevent the formation of maternal antibodies. In 1961, Dr. Ronald Finn proposed that administration of antibodies to Rh (D)-negative mothers could prevent a maternal immune response to Rh (D)-positive fetal blood cells (27). In 1964, Dr. Vincent Freda, at Sing Sing Prison in Ossining, New York, conducted an experiment on Rh (D)-negative male prisoners in which he injected radiolabeled Rh (D)-positive blood cells. The men were then given anti-D serum and it was noted that the radiolabeled cells were removed rapidly from their systems (28). In 1966, RhoGAM[®] was first developed and found to be successful at preventing alloimmunization. It eventually was introduced in North America in 1968.

Anti-D immunoglobulin is routinely administered during pregnancy in Rh (D)-negative women without evidence of alloimmunization. The American Association of Blood Banks recommends dosing at 28 weeks of gestation, postpartum, and after any suspected episodes of fetomaternal hemorrhage. The majority of cases of fetomaternal hemorrhage occur during uncomplicated vaginal deliveries, with an average of 0.1 mL of fetal blood entering the maternal circulation and causing alloimmunization (29–32). Antepartum fetomaternal hemorrhage accounts for 1% to 2% of Rh alloimmunization (33). Common scenarios leading to alloimmunization include spontaneous, threatened, and induced abortions, ectopic pregnancy, CVS, amniocentesis, and external cephalic version.

While a variety of Rh immunoglobulin is available in the United States, RhoGAM was the first approved for clinical use and is the most commonly used. Other products that are available include HyperRHO[®] S/D, Rhophylac[®], and WinRho[®]

SDF. The primary difference in these products is the method of purification and subsequent mode of delivery. RhoGAM and HyperRHO S/D are processed by cold ethanol fractionation, resulting in a less purified product that requires intramuscular administration. By contrast, Rhophylac and WinRho SDF are prepared by chromatography, allowing for intravenous and intramuscular administration routes. Anti-D immunoglobulin is extracted from plasma of individuals with high-titer D IgG antibodies. The risk of transmission of viral infections or side effects is minimal to absent and is not a clinically significant factor.

In the United States, standard dosing of anti-D immunoglobulin for Rh (D)-negative women without evidence of alloimmunization is 300 µg given at 28 weeks of gestation, and again in the postpartum period if the neonate is Rh (D)-positive and delivery occurs at least 3 weeks after the first administration. This practice reduces the incidence of antenatal alloimmunization from 2% to 0.1% (16,34).

An alternate anti-D immunoglobulin prophylaxis regimen, used primarily in the United Kingdom, is 100 µg at 28 and 34 weeks of gestation, as well as after delivery if the neonate is Rh (D) positive. There is a trend for less immunization during pregnancy for all women [relative risk (RR) = 0.42, 95% confidence interval (CI): 0.15–1.17] and for women giving birth to an Rh (D)-positive infant (RR = 0.41, 95% CI: 0.16–1.04) compared with no prophylaxis. Overall risk was lowered to about 0.2% without any adverse effects (35–37). Although trials have shown a nonsignificant reduction in immunization at 2 to 12 months following the birth of an Rh-positive infant in women who had received anti-D (RR = 0.14, 95% CI: 0.02–1.15), they were significantly less likely to have a positive Kleihauer-Betke (KB) test during pregnancy (RR = 0.60, 95% CI: 0.41–0.88) and at the birth of an Rh (D)-positive infant (RR = 0.60, 95% CI: 0.46–0.79) (36). Similarly, in Canada, 100 to 120 µg are administered at 28 and 34 weeks of gestation.

Alloimmunization can infrequently occur prior to 28 weeks of gestation, leading to the recommendation that an antibody screen be performed at the time of immunoglobulin administration to detect preexisting maternal sensitization. One dose of Rh (D) immunoglobulin is effective for 12 weeks, with a half-life of 16 to 24 days, and should be re-dosed after that interval in antepartum patients. The anti-D immunoglobulin affects the antibody screen (38). Usually, the effect of the immunoglobulin is not present until 12 weeks after its administration.

Excessive maternal-fetal hemorrhage may require more than the typical dose of Rh (D) immunoglobulin. The standard dose of 300 µg can protect from maternal sensitization caused by up to 30 mL of fetal whole blood or 15 mL of fetal RBCs in the maternal circulation. Occurrence of maternal-fetal hemorrhage can be determined by the rosette test. The standard dose of Rh (D) immunoglobulin is given for negative results, while a positive result requires further testing. A KB test determines the percentage of fetal blood cells in maternal circulation by utilizing flow cytometry. This value is then multiplied by 50 to determine the volume of hemorrhage, and one unit (300 µg) of immunoglobulin is provided for every 30 mL of hemorrhaged volume. A maximum of five units may be given by intramuscular administration in a 24-hour period; alternatively, 600 µg may be provided intravenously

Figure 1 The steps to correctly sample the middle cerebral artery peak systolic velocity (MCA-PSV). (A) An axial section of the head is obtained at the level of the sphenoid bones; (B) color Doppler evidences the circle of Willis; (C) the circle of Willis is enlarged; (D) the color box is placed around the MCA; (E) the MCA is zoomed; and (F) the MCA flow velocity waveforms are displayed and the PSV is measured at its proximal point, after the origin from the internal carotid artery, and at a 0° angle, thus avoiding angle corrections. *Source:* From Ref. 45.

every 8 hours until the entire dose is given. Some clinicians have advocated the KB test for all Rh (D)-negative women at delivery, since 50% of cases requiring more than the standard postpartum dose of anti-D immunoglobulin can be missed by high-risk situation screening only (39). The risk of fetomaternal hemorrhage greater than 30mL is approximately 0.1% to 0.2%.

Anti-D immunoglobulin given within 72 hours after childbirth is associated with a 96% decreased incidence of Rh (D) alloimmunization 6 months after birth and an 88% decreased incidence of Rh (D) alloimmunization in a subsequent pregnancy in Rh (D)-negative women who have given birth to an Rh (D)-positive infant (40). These benefits

are seen regardless of the ABO status of the mother and baby. Anti-D immunoglobulin can be given as late as 28 days postpartum if indicated but previously not given. Anti-D immunoglobulin is given to all Rh (D)-negative women after confirmation from cord blood of the Rh (D)-positive status of the neonate. Even when immunoglobulin is correctly administered, alloimmunization can still occur in the antepartum period in up to 2% of women with postpartum anti-D administration only. The risk of Rh (D) alloimmunization remains because of inadvertent antepartum or postpartum omission, failure to use the drug due to other antenatal complications, and insufficient dosing at delivery in cases of large volume fetomaternal hemorrhage.

Table 3 Reference Ranges for Fetal Hemoglobin Concentrations as a Function of Gestational Age^a

Week of gestation	Multiples of the median (MoM) grams per deciliter				
	1.16	1.00 (median)	0.84	0.65	0.55
18	12.3	10.6	8.9	6.9	5.8
20	12.9	11.1	9.3	7.2	6.1
22	13.4	11.6	9.7	7.5	6.4
24	13.9	12.0	10.1	7.8	6.6
26	14.3	12.3	10.3	8.0	6.8
28	14.6	12.6	10.6	8.2	6.9
30	14.8	12.8	10.8	8.3	7.1
32	15.2	13.1	10.9	8.5	7.2
34	15.4	13.3	11.2	8.6	7.3
36	15.6	13.5	11.3	8.7	7.4
38	15.8	13.6	11.4	8.9	7.5
40	16.0	13.8	11.6	9.0	7.6

^aThe hemoglobin values at 0.65 and 0.55 multiples of the median (cutoff points for mild and moderate anemia, respectively) are also shown. The values at 1.16 and 0.84 multiples of the median correspond to the 95th and 5th percentiles, respectively (the normal range). *Source:* From Ref. 7.

Table 4 Expected Peak Velocity of Systolic Blood Flow in the Middle Cerebral Artery as a Function of Gestational Age

Week of gestation	Multiples of the median (MoM) centimeters per second			
	1.00 (median)	1.29	1.50	1.55
18	23.2	29.9	34.8	36.0
20	25.5	32.8	38.2	39.5
22	27.9	36.0	41.9	43.3
24	30.7	39.5	46.0	47.5
26	33.6	43.3	50.4	52.1
28	36.9	47.6	55.4	57.2
30	40.5	52.2	60.7	62.8
32	44.4	57.3	66.6	68.9
34	48.7	62.9	73.1	75.6
36	53.5	69.0	80.2	82.9
38	58.7	75.7	88.0	91.0
40	64.4	83.0	96.6	99.8

Source: From Ref. 7.

MANAGEMENT OF RBC-ALLOIMMUNIZED PREGNANCIES

If Rh (D) antibodies are detected in the maternal circulation on the antibody screen, for example, by a positive indirect Coombs' test, the patient is considered alloimmunized. Among Rh (D)-alloimmunized pregnancies, mild-to-moderate hemolytic anemia and hyperbilirubinemia occur in 25% to 30% of fetuses/neonates, and 25% of those can develop hydrops (41). With correct management, the perinatal survival rate in cases of anemia is >90%; when fetal hydrops is present, survival is often >80%. There is no trial that has assessed the best management for RBC-alloimmunized pregnancies, but fetal transfusion is probably the most beneficial of all fetal therapies available. Although it is reported that the risk of fetal demise is between 1% and 2% for each fetal blood sampling (FBS), there are situations in which the risk is much higher, for example, when cordocenteses and transfusions are performed at gestational ages as early as 15 to 18 weeks.

Once the fetal D antigen is known to be present in an alloimmunized pregnancy, antenatal management centers on monitoring the situation and preventing fetal hemolysis. In patients at risk, it is important to perform a first-trimester ultrasound to establish the gestational age. Assessment for the risk of fetal anemia depends on the history of Rh complications in previous pregnancies, the titer of RBC antibodies, and MCA-PSV values (22). Determination of the maternal titer using an indirect Coombs' test is the most common screening tool used to determine the degree of alloimmunization. Titer values represent the highest level of dilution that can produce an agglutination reaction. Critical titers correspond to the level of antibodies that place a fetus at significant risk for anemia/hydrops, with 1:16 = 10%, 1:32 = 25%, 1:64 = 50%, and 1:128 = 75% risk of anemia. The critical titer should be determined in each laboratory due to large differences in measurements between laboratories. In most laboratories, the critical titer for anti-D is approximately 1:16 in albumin or 1:32 using an indirect Coombs' test. For anti-Kell, the critical titer is 1:8. Maternal titers are typically drawn monthly until 24 weeks of gestation and then are repeated biweekly to detect worsening alloimmunization. Antenatal testing from 32 weeks of gestation forward and scheduled induction of labor by 38 weeks of gestation are routinely performed.

Traditionally, spectral analysis of amniotic fluid at 450 nm (ΔOD_{450}) to detect bilirubin levels has been used as a measure of hemolytic activity. Due to its dependence upon gestational age, the ΔOD_{450} is plotted on a Queenan curve (6) (14–40 weeks of gestation) or a Liley graph (8) (27 weeks of gestation to term).

Contemporary management of alloimmunized pregnancies emphasizes the noninvasive detection of worsening fetal anemia through the use of ultrasound. With fetal anemia, decreased blood viscosity leads to increased venous return, consequently increasing cardiac output with increased blood flow velocity in all vessels. Degrees of anemia correlate with corresponding changes in blood velocity. Mari et al. have previously shown that Doppler ultrasound measurement of the MCA-PSV can reliably identify fetal anemia. Compared with amniocentesis for ΔOD_{450} assessment, the MCA-PSV is associated with a 70% to 80% reduction in the number of invasive tests (22). The MCA-PSV is also more accurate than amniocentesis in detecting fetal anemia (7,42–44).

Screening with the MCA-PSV can be started as early as 15 weeks of gestation (8). The main advantage of the MCA is that it is easy to measure at a 0° angle, which is essential for an accurate measurement of the true blood flow. The steps for the correct measurement of the MCA-PSV are (i) an axial section of the head is obtained at the level of the sphenoid bones; (ii) color Doppler evidences the circle of Willis; (iii) the circle of Willis is enlarged; (iv) the color box is placed around the MCA; (v) the MCA is zoomed; and (vi) the MCA flow velocity waveforms are displayed and the PSV is measured at its proximal point, after the origin from the internal carotid artery, and at a 0° angle, thus avoiding angle corrections (Fig. 1). The waveforms should be similar. The above sequence is repeated at least three times in each fetus. Measurement at this point allows the lowest intra- and interobserver variability, as well as standardization of the measurement (46). Multiples of the median (MoM) for the hemoglobin concentration (Table 3) and the MCA-PSV (Table 4 and Fig. 2) correct for

Figure 2 Middle cerebral artery peak systolic velocity (MCA-PSV) values correlated with gestational age. Abbreviation: MoM, multiples of the median. Source: From Ref. 7.

the effect of gestational age on the measurements (22). The MCA-PSV has a sensitivity of 100% (95% CI: 0.86–1.0) for the detection of significant fetal anemia with a false positive rate of 12% at 1.50 MoM (22). The number of false positive findings increases following 35 weeks of gestation (47). The number of false positive cases after 35 weeks of gestation may be decreased by looking at the overall trend of the MCA-PSV, rather than single values (48). An MoM > 1.5 indicates moderate-to-severe anemia and the need for invasive management.

The MCA-PSV may also be used to diagnose anemia of other etiologies, including parvovirus infection, nonimmune hydrops, fetomaternal hemorrhage, and twin-to-twin transfusion syndrome. Severe intrauterine growth restriction (IUGR) is also associated with an increased MCA-PSV (49). This should be taken into account when the MCA-PSV is used diagnostically; however, it is very unlikely that an anemic fetus is also a severe IUGR fetus. Moderate-to-severe fetal anemia may also be suggested by hydropic signs (at least two out of the following: pericardial or pleural effusion, ascites, or skin edema), an increase in size of the fetal liver or placental thickness, or by tricuspid regurgitation.

Once conservative measurements document a worsening hemolysis consistent with moderate-to-severe anemia (MCA-PSV > 1.5 MoM), therapeutic intervention involves intrauterine fetal assessment and transfusion. This process begins with administration of a paralytic [vecuronium bromide 0.1 mg/kg of estimated fetal weight (EFW)] to stop fetal movement, followed by the acquisition of venous blood from the base of the umbilical cord by the placental insertion site. The baseline fetal hematocrit determines the volume of transfusion to yield a target hematocrit of 40% to 50%. This target value takes into

account an expected decline of approximately 1% per day until a subsequent fetal transfusion. Transfusion is performed at the umbilical vein at either the placental insertion or inside the abdomen. Intraperitoneal transfusion is rarely performed and is contraindicated in the hydropic fetus because of poor absorption of blood. Corticosteroids for fetal maturation should be given before the procedure when FBS is performed after 24 weeks of gestation. Type O, Rh (D)-negative, cytomegalovirus-negative, washed, leukoreduced, irradiated packed RBCs cross-matched against maternal blood should be used. The blood usually contains 75% to 85% RBCs, allowing minimal blood volume for the transfusion (50). Transfusions are provided every 14 days until suppression of fetal erythropoiesis is documented; thereafter, the rate of declining hematocrit levels can be used to time further transfusions. Intrauterine transfusions are performed until 35 weeks of gestation, with delivery planned for 38 weeks of gestation (51).

The FBS is performed under continuous ultrasound guidance. Prophylactic antibiotics can be given, although no trial has evaluated their efficacy. Following 24 weeks of gestation, the procedure should be performed after alerting anesthesiology and in a location close to an operating room should an emergency occur. Tubing and syringes should be heparinized. Maternal skin can be anesthetized with 1% lidocaine at the point of needle entry. A 20- or 22-gauge needle is typically used. After entering the umbilical vein, a sample of fetal blood is withdrawn and the hemoglobin concentration is immediately (within 1 or 2 minutes) determined. Fetal anemia is confirmed by a mean corpuscular volume (MCV) > 110 μm^3 . If the hematocrit is below the fifth percentile (0.84 MoM) for gestational age, blood is transfused in a sterile fashion. A computer

program is used to estimate the amount of blood to transfuse based on the initial fetal hematocrit, the EFW, and the concentration of the blood transfused (52,53). A final fetal blood sample is taken a few seconds after the transfusion has been completed. If the fetus is hydropic, it is better to perform another transfusion 3 to 5 days later to increase the hematocrit to the median value for the gestational age. At 24 weeks of gestation or later, the fetal heart rate should be monitored for 2 to 3 hours after the transfusion until fetal movements resume. The risk of fetal death is 1% to 2% per procedure, even with ultrasound guidance, expert operators, and accurate management (54).

Hematocrit decreases about 1% per day post-transfusion in the anemic alloimmunized fetus, and this knowledge helps to assess when to repeat the transfusion. If the fetus is non-hydropic, the second transfusion is often necessary 14 days after the first. After the third transfusion, longer intervals, such as 3 weeks, may be possible, as the fetal RBCs are replaced by donated RBCs. Following three transfusions, 99% of the fetal blood is replaced with the adult transfused blood. The value of a maternal dose of 30mg of phenobarbital three times per day for 7 to 10 days to enhance fetal liver maturity and the ability to conjugate bilirubin has yet to be confirmed by large studies (55).

NEONATOLOGY MANAGEMENT

Neonatology management of an infant with hemolytic disease of the newborn focuses on monitoring the level of anemia and hyperbilirubinemia. Symptoms of anemia, such as tachycardia, usually warrant RBC transfusions. In severe cases, exchange transfusions may be necessary to remove the maternal antibodies from the circulation of the infant. Hyperbilirubinemia clinically presents as jaundice. Bilirubin levels are monitored and controlled with phototherapy and IV hydration.

REFERENCES

- Gold ER, Butler NR. ABO haemolytic disease of the newborn. Baltimore: The Williams and Wilkins Company, 1972.
- Landsteiner K, Weiner AS. An agglutinable factor in human blood recognized by immune sera for Rhesus blood. *Proc Soc Exp Biol Med* 1940; 43: 223.
- Levine P. Isoimmunization in pregnancy: its possible bearing on etiology of erythroblastosis fetalis. *JAMA* 1941; 116: 825.
- Bevis DC. Blood pigments in haemolytic disease of the newborn. *J Obstet Gynaecol Br Emp* 1956; 63: 68–75.
- Liley AW. Liquor amni analysis in the management of the pregnancy complicated by resus sensitization. *Am J Obstet Gynecol* 1961; 82: 1359–70.
- Queenan JT, Tomai TP, Ural SH, King JC. Deviation in amniotic fluid optical density at a wavelength of 450 nm in Rh-immunized pregnancies from 14 to 40 weeks of gestation: a proposal for clinical management. *Am J Obstet Gynecol* 1993; 168: 1370–6.
- Mari G, Deter RL, Carpenter RL, et al. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. *N Engl J Med* 2000; 342: 9–14.
- Liley AW. Intrauterine transfusion of foetus in haemolytic disease. *Br Med J* 1963; 2: 1107–9.
- Casper MJ. The making of the unborn patient: a social anatomy of fetal surgery. New Brunswick, NJ: Rutgers University Press, 1998.
- Adamsons K Jr, Freda VJ, James LS, Towell ME. Prenatal treatment of erythroblastosis fetalis following hysterotomy. *Pediatrics* 1965; 35: 848–55.
- Asensio SH, Figueroa-Longo JG, Pelegrina IA. Intrauterine exchange transfusion. *Am J Obstet Gynecol* 1966; 95: 1129–33.
- Valenti C. Antenatal detection of hemoglobinopathies. A preliminary report. *Am J Obstet Gynecol* 1973; 115: 851–3.
- Rodeck CH, Campbell S. Sampling pure fetal blood by fetoscopy in second trimester of pregnancy. *Br Med J* 1978; 2: 728–30.
- Rodeck CH, Kemp JR, Holman CA, et al. Direct intravascular fetal blood transfusion by fetoscopy in severe Rhesus isoimmunisation. *Lancet* 1981; 1: 625–7.
- Bang J, Bock JE, Trolle D. Ultrasound-guided fetal intravenous transfusion for severe rhesus haemolytic disease. *Br Med J (Clin Res Ed)* 1982; 284: 373–4.
- Bowman JM, Pollock JM, Penston LE. Fetomaternal transplacental hemorrhage during pregnancy and after delivery. *Vox Sang* 1986; 51: 117–21.
- Mari G, Hanif F, Drennan K. Hemolytic disease of the fetus/neonate. In: Berghella V, ed. *Maternal-Fetal Evidence Based Guidelines*. UK: Informa Healthcare, 2007: 327–36.
- Hoffman R. *Hematology: basic principles and practice*, 5th edn. Philadelphia, PA: Churchill Livingstone/Elsevier, 2009.
- ACOG practice bulletin no. 75: management of alloimmunization. *Obstet Gynecol* 2006; 108: 457–64.
- Weinstein L. Irregular antibodies causing hemolytic disease of the newborn: a continuing problem. *Clin Obstet Gynecol* 1982; 25: 321–32.
- Hamilton BE, Martin JA, Ventura SJ, et al. Births: preliminary data for 2004. *Natl Vital Stat Rep* 2005; 54: 1–17.
- Van den Veyver IB, Moise KJ Jr. Fetal RhD typing by polymerase chain reaction in pregnancies complicated by rhesus alloimmunization. *Obstet Gynecol* 1996; 88: 1061–7.
- Moise KJ, Whitecar PW. Antenatal therapy for haemolytic disease of the fetus and newborn. In: Hadley A, Soothill P, eds. *Alloimmune disorders in pregnancy: anaemia, thrombocytopenia and neutropenia in the fetus and newborn*. Cambridge, UK: Cambridge University Press, 2002: 173–201.
- Pertl B, Bianchi DW. Fetal DNA in maternal plasma: emerging clinical applications. *Obstet Gynecol* 2001; 98: 483–90.
- Lo YM, Hjelm NM, Fidler C, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. *N Engl J Med* 1998; 339: 1734–8.
- Gautier E, Benachi A, Giovangrandi Y, et al. Fetal RhD genotyping by maternal serum analysis: a two-year experience. *Am J Obstet Gynecol* 2005; 192: 666–9.
- Finn R, Clarke CA, Donohoe WT, et al. Experimental studies on the prevention of Rh haemolytic disease. *Br Med J* 1961; 1: 1486–90.
- Freda VJ, Gorman JG, Pollack W. Successful prevention of experimental Rh sensitization in man with an anti-Rh gamma2-globulin antibody preparation: a preliminary report. *Transfusion* 1964; 4: 26–32.
- Zipursky A, Israels LG. The pathogenesis and prevention of Rh immunization. *Can Med Assoc J* 1967; 97: 1245–57.
- Stedman CM, Baudin JC, White CA, Cooper ES. Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women. *Am J Obstet Gynecol* 1986; 154: 1363–9.
- Ness PM, Baldwin ML, Niebyl JR. Clinical high-risk designation does not predict excess fetal-maternal hemorrhage. *Am J Obstet Gynecol* 1987; 156: 154–8.
- Bowman JM. Suppression of Rh isoimmunization. A review. *Obstet Gynecol* 1978; 52: 385–93.
- Davey M. The prevention of rhesus-isoimmunization. *Clin Obstet Gynaecol* 1979; 6: 509–30.
- Siberil S, de Romeuf C, Bihoreau N, et al. Selection of a human anti-RhD monoclonal antibody for therapeutic use: impact of IgG glycosylation on activating and inhibitory Fc gamma R functions. *Clin Immunol* 2006; 118: 170–9.
- Hughes RG, Craig JL, Murphy WG, Greer IA. Causes and clinical consequences of Rhesus (D) haemolytic disease of the newborn: a study of a Scottish population, 1985–1990. *Br J Obstet Gynaecol* 1994; 101: 297–300.
- Cherif-Zahar B, Mattei MG, Le Van Kim C, et al. Localization of the human Rh blood group gene structure to chromosome region 1p34.3-1p36.1 by in situ hybridization. *Hum Genet* 1991; 86: 398–400.
- Carritt B, Kemp TJ, Poulter M. Evolution of the human RH (rhesus) blood group genes: a 50 year old prediction (partially) fulfilled. *Hum Mol Genet* 1997; 6: 843–50.
- Bowman JM. The prevention of Rh immunization. *Transfus Med Rev* 1988; 2: 129–50.
- Avent N. *Fetal genotyping*. Cambridge, UK: Cambridge University Press, 2002.
- Bowman JM. Controversies in Rh prophylaxis. Who needs Rh immune globulin and when should it be given? *Am J Obstet Gynecol* 1985; 151: 289–94.

41. Pirelli K, Pietz B, Johnson S, et al. Molecular determination of RhD zygosity. *Am J Obstet Gynecol* 2006; 195: S172.
42. Oepkes D, Seaward PG, Vandenbussche FP, et al. Doppler ultrasonography versus amniocentesis to predict fetal anemia. *N Engl J Med* 2006; 355: 156–64.
43. Divakaran TG, Waugh J, Clark TJ, et al. Noninvasive techniques to detect fetal anemia due to red blood cell alloimmunization: a systematic review. *Obstet Gynecol* 2001; 98: 509–17.
44. Abel DE, Grambow SC, Brancazio LR, Hertzberg BS. Ultrasound assessment of the fetal middle cerebral artery peak systolic velocity: a comparison of the near-field versus far-field vessel. *Am J Obstet Gynecol* 2003; 189: 986–9.
45. Mari G, Abuhamad AZ, Cosmi E, et al. Middle cerebral artery peak systolic velocity: technique and variability. *J Ultrasound Med* 2005; 24: 425–30.
46. Nicolaides KH, Rodeck CH. Maternal serum anti-D antibody concentration and assessment of rhesus isoimmunisation. *BMJ* 1992; 304: 1155–6.
47. Soothill PW, Nicolaides KH, Rodeck CH, et al. Relationship of fetal hemoglobin and oxygen content to lactate concentration in Rh isoimmunized pregnancies. *Obstet Gynecol* 1987; 69: 268–71.
48. Geifman-Holtzman O, Grotegut CA, Gaughan JP. Diagnostic accuracy of noninvasive fetal Rh genotyping from maternal blood—a meta-analysis. *Am J Obstet Gynecol* 2006; 195: 1163–73.
49. Giannina G, Moise KJ Jr, Dorman K. A simple method to estimate volume for fetal intravascular transfusions. *Fetal Diagn Ther* 1998; 13: 94–7.
50. Scheier M, Hernandez-Andrade E, Fonseca EB, Nicolaides KH. Prediction of severe fetal anemia in red blood cell alloimmunization after previous intrauterine transfusions. *Am J Obstet Gynecol* 2006; 195: 1550–6.
51. Moise KJ Jr. Management of rhesus alloimmunization in pregnancy. *Obstet Gynecol* 2008; 112: 164–76.
52. Klumper FJ, van Kamp IL, Vandenbussche FP, et al. Benefits and risks of fetal red-cell transfusion after 32 weeks gestation. *Eur J Obstet Gynecol Reprod Biol* 2000; 92: 91–6.
53. Howe DT, Michailidis GD. Intraperitoneal transfusion in severe, early-onset Rh isoimmunization. *Obstet Gynecol* 2007; 110: 880–4.
54. Oepkes D, Adama van Scheltema P. Intrauterine fetal transfusions in the management of fetal anemia and fetal thrombocytopenia. *Semin Fetal Neonatal Med* 2007; 12: 432–8.
55. Ruma MS, Moise KJ Jr, Kim E, et al. Combined plasmapheresis and intravenous immune globulin for the treatment of severe maternal red cell alloimmunization. *Am J Obstet Gynecol* 2007; 196: 138 e1–6.