Rhesus (Rh) D-negative individuals who are exposed to RhD-positive red blood cells can develop anti-Rh antibodies, which can cross the placenta and cause fetal anemia. If undiagnosed and untreated, alloimmunization can cause significant perinatal morbidity and mortality. Determining the Rh status of the fetus may guide subsequent management of the pregnancy. The use of cell-free fetal DNA in maternal blood has been proposed as a noninvasive method to determine fetal RhD genotype.

Background

Alloimmunization refers to the development of antibodies in a patient whose blood type is Rh-negative and who is exposed to Rh-positive red blood cells (RBCs). This most commonly occurs from fetal-placental hemorrhage and entry of fetal blood cells into maternal circulation. The management of an Rh-negative pregnant patient who is not alloimmunized and is carrying a known Rh-positive fetus, or if fetal Rh status is unknown, involves administration of Rh immune globulin at standardized times during the pregnancy to prevent formation of anti-Rh antibodies. If the patient is already alloimmunized, monitoring the levels of anti-Rh antibody titers and for the development of fetal anemia is performed. Both noninvasive and invasive tests to determine fetal Rh status exist.

Rh blood groups

The (Rhesus) Rh system includes more than 100 antigen varieties found on RBCs. RhD is the most common and the most immunogenic. When people have the RhD antigen on their RBCs, they are considered to be RhD-positive; if their RBCs lack the antigen, they are considered to be RhD-negative. The RhD-antigen is inherited in an autosomally dominant fashion, and a person may be heterozygous (Dd) (~60% of Rh-positive people) or homozygous (DD) (~40% of Rh-positive people). Homozygotes always pass the RhD antigen to their offspring, whereas heterozygotes have a 50% chance of passing the antigen to their offspring. A person who is RhD-negative does not have the Rh antigen. Although nomenclature refers to RhD-negative as dd, there is no small d antigen (i.e., they lack the RHD gene and the corresponding RhD antigen).

RhD-negative status varies among ethnic group and is 15% in whites, 5 to 8% in African Americans, and 1 to 2% in Asians and Native Americans, respectively.

In the white population, almost all RhD-negative individuals are homozygous for a deletion of the RHD gene. However, in the African-American population, only 18% of RhD-negative individuals are homozygous for an RHD deletion, and 66% of RhD-negative African Americans have an inactive RHBy. There are also numerous rare variants of the D antigen, which are recognized by weakness of expression of D and/or by absence of some of the epitopes of D.
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Some individuals with variant D antigens, if exposed to RhD-positive RBCs, can make antibodies to one or more epitopes of the D antigen. RhD-negative individuals can have a fetus that is RhD-positive if the fetus inherits the RhD-positive antigen from the paternal father.

Causes of alloimmunization

By 30 days of gestation, the RhD antigen is expressed on the red blood cell (RBC) membrane, and alloimmunization can be caused when fetal Rh-positive RBCs enter maternal circulation, and the Rh-negative mother develops anti-D antibodies. Once anti-D antibodies are present in a pregnant individual’s circulation, they can cross the placenta and cause destruction of fetal RBCs.

The production of anti-D antibodies in RhD-negative individuals is highly variable and significantly affected by several factors, including the volume of fetomaternal hemorrhage, the degree of maternal immune response, concurrent ABO incompatibility, and fetal homozygosity versus heterozygosity for the D antigen. Therefore, although ~10% of pregnancies are Rh-incompatible, <20% of Rh-incompatible pregnancies actually lead to maternal alloimmunization.

Small feto-maternal hemorrhages of RhD-positive fetal RBCs into the circulation of an RhD-negative individuals occurs in nearly all pregnancies, and percentages of fetomaternal hemorrhage increase as the pregnancy progresses: 7% in the first trimester, 16% in the second trimester, and 29% in the third trimester, with the greatest risk of RhD alloimmunization occurring at birth (15 to 50%). Transplacental hemorrhage accounts for almost all cases of maternal RhD alloimmunization.

Fetomaternal hemorrhage can also be associated with miscarriage, pregnancy termination, ectopic pregnancy, invasive in-utero procedures (e.g., amniocentesis), in utero fetal death, maternal abdominal trauma, antepartum maternal hemorrhage, and external cephalic version. Other causes of alloimmunization include inadvertent transfusion of RhD-positive blood and RhD-mismatched allogeneic hematopoietic stem-cell transplantation.

Consequences of alloimmunization

IgG antibody–mediated hemolysis of fetal RBCs, known as hemolytic disease of the fetus and newborn, varies in severity and can have a variety of manifestations. The anemia can range from mild to severe with associated hyperbilirubinemia and jaundice. In severe cases, hemolysis may lead to extramedullary hematopoiesis and reticuloendothelial clearance of fetal RBCs, which may result in hepatosplenomegaly, decreased liver function, hypoproteinemia, ascites, and anasarca. When accompanied by high-output cardiac failure and pericardial effusion, this condition is known as hydrops fetalis, which without intervention, is often fatal. Intensive neonatal care, including emergent exchange transfusion, is required.

Cases of hemolysis in the newborn that do not result in fetal hydrops can still lead to kernicterus, a neurologic condition observed in infants with severe hyperbilirubinemia due to the deposition of unconjugated bilirubin in the brain. Symptoms that manifest several days after delivery can include poor feeding, inactivity, loss of the Moro reflex, bulging fontanelle, and seizures. The 10% of infants who survive may develop spastic choreoathetosis, deafness, and/or mental retardation.

The result of disease from alloimmunization, hemolytic disease of the fetus or newborn, was once a major contributor to perinatal morbidity and mortality. However, with the widespread adoption of antenatal and postpartum use of Rh immune globulin in developed countries, the result has been a major decrease in frequency of this disease. In developing countries without prophylaxis programs, stillbirth occurs in 14% of affected pregnancies, and 50% of pregnancy survivors either die in the neonatal period or develop cerebral injury.
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**Prevention of alloimmunization**

There are four currently in use Rh immune globulin products available in the U.S., all of which undergo micropore filtration to eliminate viral transmission. To date, no reported cases of viral infection related to Rh immune globulin administration have been reported in the U.S. Theoretically, the Creutzfeldt-Jakob disease (CJD) agent could be transmitted by use of Rh immune globulin. Local adverse reactions may occur, including redness, swelling, and mild pain at the site of injection, and hypersensitivity reactions have been reported.

The American College of Obstetricians and Gynecologists (ACOG) and the American Association of Blood Banks (AABB) recommend the first dose of Rho(D) immune globulin (e.g., RhoGAM®) be given at 28 weeks’ gestation, (or earlier if there’s been an invasive event), followed by a postpartum dose given within 72 hours of delivery.

**Diagnosis of alloimmunization**

The diagnosis of alloimmunization is based on detection of anti-RhD antibodies in the maternal serum.

The most common test for determining antibodies in serum is the indirect Coombs test. Maternal serum is incubated with known RhD-positive RBCs. Any anti-RhD antibody present in the maternal serum will adhere to the RBCs. The RBCs are then washed and suspended in Coombs serum, which is antihuman globulin. RBCs coated with maternal anti-RhD will agglutinate, which is referred to as a positive indirect Coombs test. The indirect Coombs titer is the value used to direct management of pregnant alloimmunized individuals.

**Management of alloimmunization during pregnancy**

A patient’s first alloimmunized pregnancy involves minimal fetal or neonatal disease. Subsequent pregnancies are associated with more severe degrees of fetal anemia. Treatment of an alloimmunized pregnancy requires monitoring of maternal anti-D antibody titers and serial ultrasound assessment of middle cerebral artery peak systolic velocity of the fetus.

If severe fetal anemia is present near term, delivery is performed. If severe anemia is detected remote from term, intrauterine fetal blood transfusions may be performed.

**Determining fetal RhD status**

ACOG recommends that all pregnant individuals should be tested at the time of their first prenatal visit for ABO blood group typing and Rh-D type and be screened for the presence of anti-RBC antibodies. These laboratory tests should be repeated for each subsequent pregnancy. The AABB also recommends that antibody screening be repeated before administration of anti-D immune globulin at 28 weeks’ gestation, postpartum, and at the time of any event during pregnancy.

If the mother is determined to be Rh-negative, the paternal Rh status should also be determined at the initial management of a pregnancy. If paternity is certain and the father is Rh-negative, the fetus will be Rh-negative, and further assessment and intervention are unnecessary. If the father is RhD-positive, he can be either homozygous or heterozygous for the D allele. If he is homozygous for the D allele (i.e., D/D), then the fetus is RhD-positive. If the paternal genotype is heterozygous for Rh status or is unknown, determination of the Rh-status of the fetus is the next step.

Invasive and noninvasive testing methods to determine the Rh status of a fetus are available.
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Invasive procedures use polymerase chain reaction (PCR) assays to assess the fetal cellular elements in amniotic fluid by amniocentesis or by chorionic villus sampling (CVS). Although CVS can be performed earlier in a pregnancy, amniocentesis is the preferred method because CVS is associated with disruption of the villi and the potential for larger fetomaternal hemorrhage and worsening alloimmunization of the fetus if RhD-positive. The sensitivity and specificity of fetal RHD typing by PCR are reported as 98.7% and 100%, respectively, with positive and negative predictive values of 100% and 96.9%, respectively.

Noninvasive testing involves molecular analysis of cell-free fetal DNA (cffDNA) in the maternal plasma or serum. In 1998, Lo et al. showed that about 3% of cffDNA in the plasma of first trimester pregnant individuals is of fetal origin, with this percentage rising to 6% in the third trimester. Fetal DNA cannot be separated from maternal DNA, but if the pregnant individual is RhD-negative, the presence of specific exons of the RHD gene, which are not normally present in the circulation of an RhD-negative patient, predicts an RhD-positive fetus. cffDNA has been proposed as a noninvasive alternative to obtaining fetal tissue by invasive methods, which are associated with a risk of miscarriage.

The large quantity of maternal DNA compared to fetal DNA in the maternal circulation complicates the inclusion of satisfactory internal controls to test for successful amplification of fetal DNA. Therefore, reactions to detect Y chromosome-linked gene(s) can be included in the test, which will be positive when the fetus is a male. When Y chromosome-linked genes are not detected, tests for polymorphisms may be performed to determine whether the result is derived from fetal but not maternal DNA. cffDNA testing to determine the fetal RHD genotype is standard of practice in many European countries.

Regulatory Status

Sequenom offers SensiGene™ Fetal RHD Genotyping test, performed by proprietary SEQureDx™ technology. The assay targets exons 4, 5, and 7 of the RHD gene located on chromosome 1, psi (y) pseudogene in exon 4, and assay controls which are 3 targets on the Y chromosome (SRY, TTTY, DBY).

The company claims that the uses of its test include:

- Clarify fetal RHD status without testing the father
  Avoiding the cost of paternity testing and paternal genotyping
- Clarify fetal RHD status when maternal anti-D titers are unclear
- Identify the RHD (-) fetus in mothers who are opposed to immunization(s) and vaccines
- RhD (-) sensitized patients
- Avoid invasive testing by CVS or genetic amniocentesis

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). No genotyping tests were found. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test

***Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.

Policy

Fetal RHD genotyping using maternal plasma is considered investigational. BCBSNC does not provide coverage for investigational services or procedures.
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Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When fetal RHD genotyping using maternal plasma is covered

Not applicable.

When fetal RHD genotyping using maternal plasma is not covered

Fetal RHD genotyping using maternal plasma is considered investigational.

Policy Guidelines

The evidence for the use of fetal RHD genotyping using cell-free DNA in individuals who are pregnant and have RhD-negative blood type includes: for clinical validity, a meta-analysis and additional prospective studies; and for analytic validity, no direct evidence. Relevant outcomes are test accuracy and validity, morbid events, medication use, and treatment-related morbidity. Clinical validity studies have demonstrated that the sensitivity and specificity of the test are high; however, the false-negative rate of the test, which is low, is not zero, potentially leading to alloimmunization of the Rh-negative mothers in these cases. It is uncertain whether RHD genotyping using cell-free fetal DNA will lead to improved health outcomes. The evidence is insufficient to determine the effects of the technology on health outcomes.

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Effective July 1, 2014, this testing is included in code 81403.

Prior to July 2014, the unlisted molecular pathology code 81479 would have been used.

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources


Fetal RHD Genotyping Using Cell-Free Fetal DNA


Policy Implementation/Update Information

1/28/14  New policy developed. Fetal RHD genotyping using maternal plasma is considered investigational. Medical Director review 1/2014. (sk)

10/14/14  Specialty Matched Consultant Advisory Panel review 9/30/14. No change to Policy statement. (sk)

2/10/15  Reference added. Code 81403 added to Billing/Coding section. No change to Policy statement. (sk)

10/30/15  Specialty Matched Consultant Advisory Panel review 9/30/15. (sk)


Medical policy is not an authorization, certification, explanation of benefits or a contract. Benefits and eligibility are determined before medical guidelines and payment guidelines are applied. Benefits are determined by the group contract and subscriber certificate that is in effect at the time services are rendered. This document is solely provided for informational purposes only and is based on research of current medical literature and review of common medical practices in the treatment and diagnosis of disease. Medical practices and knowledge are constantly changing and BCBSNC reserves the right to review and revise its medical policies periodically.