Human Platelet and Red Blood Cell Antigen Genotyping

Introduction

Molecular testing of red blood cell or human platelet antigens in individuals to determine alloimmunization status or risk is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan’s procedure code list for management requirements.

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<thead>
<tr>
<th>Procedure addressed by this guideline</th>
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<td>Navigator DI Sequencing</td>
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What are tissue antigens

Definition

An antigen is a substance (protein, sugar, or lipid) that is on the surface of a cell. Red blood cell antigens are on the surface of red blood cells (RBC), while human platelet antigens (HPA) are on the surface of platelets.

Individuals can be exposed to red blood cell or human platelet antigens that they do not have on their cells through blood transfusion or pregnancy. Once exposed, they
may become alloimmunized to these antigens and mount an immune response to them if they are presented again (e.g., during future transfusions).\(^1\)\(^2\)

If subsequent antigen exposure occurs during pregnancy, the fetus/newborn is at risk for serious disease.

- **Red Blood Cell Antigens**: Fetuses and newborns of alloimmunized mothers are at risk for developing Hemolytic Disease of the Fetus and Newborn (HDFN). Symptoms include high output cardiac failure and kernicterus.\(^3\)\(^4\)
- **Human Platelet Antigens**: Fetuses and newborns of alloimmunized mothers are at risk for developing Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT). Symptoms include thrombocytopenia and intracranial, gastrointestinal, or genitourinary hemorrhage.\(^5\)\(^6\) Unlike HDFN, FNAIT can occur in a first pregnancy.\(^6\)

**Test information**

**Introduction**

Laboratory work-up of alloimmunization may include serology (antibody and/or antigen analysis) and molecular analysis.

**Human Platelet Antigen (HPA) Genotyping**

Molecular testing for human platelet antigens is typically performed in specialized reference laboratories via laboratory developed tests. Testing typically consists of targeted genotyping for specific, well-described gene variants.

**Red Blood Cell (RBC) Antigen Genotyping**

Molecular testing for red blood cell antigens is typically performed in specialized reference laboratories via laboratory developed tests, but RBC antigen panels may also be performed on FDA-approved instrument platforms. Testing may consist of targeted genotyping for specific gene variants, gene sequencing, or deletion analysis.

**Table: Selected Red Blood Cell Antigens and Corresponding Genes**

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<th>Red Blood Cell Blood Group Name</th>
<th>Antigen Abbreviation</th>
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<td>RH</td>
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Guidelines and evidence

Introduction

This section includes relevant guidelines and evidence pertaining to human platelet and red blood cell antigen genotyping.

American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2018) Practice Bulletin 192 Management of Alloimmunization During Pregnancy makes the following recommendations after maternal antibodies are identified:3

- “The initial management of a pregnancy involving an alloimmunized patient is determination of the paternal erythrocyte antigen status.”
• “The fetal genotype should be assessed when the paternal genotype is thought to be heterozygous or is unknown.”

ACOG Practice Bulletin 181 Prevention of Rh D Alloimmunization (2017) states: 4

• “All pregnant women should be tested at the time of the first prenatal visit for ABO blood group and the Rh D type and screened for the presence of erythrocyte antibodies.”

• “If Rh D antibodies are present because of sensitization, anti-D immune globulin is not beneficial, and management should proceed in accordance with protocols for Rh D-alloimmunized pregnancies.”

• “If paternity is certain and the father is known to be Rh D negative, antenatal prophylaxis is unnecessary.”

• “Despite the improved accuracies noted with noninvasive fetal RHD genotyping, cost comparisons with current routine prophylaxis of anti-D Immunoglobulin at 28 weeks of gestation have not shown a consistent benefit and, thus, this test is not routinely recommended.”

Regarding maternal weak D phenotype on serology, ACOG Bulletin 181 (2017) states: 4

• “An attractive solution to this problem [maternal weak D phenotype] is to perform molecular genetic RHD typing in weak D phenotype individuals as suggested by the Work Group on RHD Genotyping.”

• “Clinicians are advised to administer Rh D immune globulin to patients with weak D blood type in appropriate clinical situations, by the same rationale as that for Rh D typing blood donors, until further scientific and economic studies are available.”

American Society of Hematology

The American Society of Hematology (ASH, 2020) state the following in their guidelines for transfusion support for sickle cell disease: 1

• “The ASH guideline panel recommends prophylactic red cell antigen matching for Rh (C, E or C/c, E/e) and K antigens over only ABO/RhD matching for patients with SCD (all genotypes) receiving transfusions (strong recommendation based on moderate certainty in the evidence about effects).”

• “The ASH guideline panel suggests an extended red cell antigen profile by genotype or serology over only ABO/RhD typing for all patients with SCD (all genotypes) at the earliest opportunity (optimally before the first transfusion) (conditional recommendation based on very low certainty in the evidence about effects).”

In a 2014 Mini Review, the ASH states: 2

• “One to two percent of all patients who receive transfusions develop antibodies to RBC antigens.”
• Between 10 and 30% of patients receiving chronic transfusions are alloimmunized, typically before the 15\textsuperscript{th} transfusion.

• “Once alloimmunization occurs, the likelihood of additional antibody responses is also relatively high. In surgical, pregnant, and non–hematologic malignancy patients, once RBC antibodies have been induced, 20 percent to 25 percent of patients form additional antibodies after subsequent transfusions and thus become multiply alloimmunized.”

• In this review, ASH lists the following scenarios in which red blood cell antigen genotyping may be helpful:
  o Hemoglobinopathy patients at baseline,
  o Alloimmunized patients who are expected to need additional transfusions,
  o Alloimmunized patients with a co-existing autoantibody,
  o Patients who have been recently transfused,
  o Prenatal diagnosis in pregnancies at risk for hemolytic disease of the newborn.

\textbf{British Committee for Standards in Haematology}

In a 2017 guideline on red cell transfusion in sickle cell disease, the British Committee for Standards in Haematology states:\textsuperscript{7}

• “An extended phenotype (or genotype) including C, c, E, e, K, k, Jka, Jkb, Fya, Fyb, S, s should be performed on all patients at baseline. If the patient is S- s-, then U typing should be performed (Milkins et al, 2013). If the patient has not been transfused within 3 months then this can be undertaken serologically, otherwise the genotype needs determination by molecular techniques (Chou & Westhoff, 2011; Milkins et al, 2013) through an appropriate reference laboratory.”

• “Select ABO extended Rh and K matched units negative for the relevant antigen(s) to which there are current or historical antibodies.”

• “If the identity of the new alloantibody is in doubt despite further specialist testing, consider providing extended antigen matched blood (if serological phenotyping cannot be used because of the presence of transfused donor red blood cells, the sample should be sent to an appropriate reference laboratory for molecular red cell genotyping).”

In a 2017 guideline on the use of platelet transfusions the British Committee for Standards in Haematology states:\textsuperscript{8}

• Post-transfusion purpura (PTP) is “a rare condition associated with severe thrombocytopenia following blood transfusion and caused by antibodies against platelet-specific antigens. Bleeding can be serious and fatal”. The condition usually occurs 5-10 days after transfusion.

• “Management is based on individual case reports and case series.”
• “Current practice is to transfuse high dose intravenous immunoglobulin without waiting for the results of laboratory investigations, with random donor platelets reserved to control severe bleeding.”

**College of American Pathologists and AABB**

A College of American Pathologists (CAP) and AABB Work Group on RHD Genotyping (2015) makes the following recommendation regarding genotyping individuals with a weak D phenotype on serology:

- “The Work Group recommends that RHD genotyping be performed whenever a discordant RhD typing result and/or a serological weak D phenotype is detected in patients, including pregnant women, newborns and potential transfusion recipients. It is anticipated that the immediate benefit will be fewer unnecessary injections of RhIG and increased availability of RhD-negative RBCs for transfusion.”

The AABB reiterates on their website:

- “RHD genotyping is recommended whenever a weak D phenotype is detected by routine Rh blood typing of pregnant women and other females of childbearing potential. The Work Group rates this as strong recommendation, based on high-quality evidence from observational studies (1A).”

**Newborn Services Clinical Guideline: Auckland District Health Board**

In a clinical clinical management guideline (2015), the Auckland, New Zealand health Board states the following regarding FNAIT:

- “Neonatal Alloimmune Thrombocytopenia (NAIT) results from maternal human platelet antibodies (HPA) against fetal platelet antigens inherited from the father. In contrast to rhesus haemolytic disease, platelet allo-immunization can occur during the first pregnancy.”
- “Definitive diagnosis of NAIT depends on parental testing.”
- Maternal and paternal genotyping is recommended in this clinical guideline. If paternity is uncertain or no paternal sample is available, fetal genotyping is recommended.

**Royal College of Obstetricians and Gynaecologists**

In a 2019 guideline addressing pregnancies at risk for alloimmune thrombocytopenia, the Royal College of Obstetricians and Gynaecologists states:

- There is no evidence to support routine screening for pregnancies at risk of FNAIT (Fetal and Neonatal Alloimmune Thrombocytopenia).
- “IVIg in pregnancy is safe and likely to be effective. It seems reasonable to start therapy at 16–18 weeks of gestation in an at-risk pregnancy.”
Selected Relevant Publications

Multiple review articles have addressed human platelet antigen genotyping, specifically with regard to Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT).

A review by Winklehorst and colleagues (2017) states:\(^\text{12}\)

- “When FNAIT is suspected, or in case of a family member with FNAIT, diagnostic work-up should ideally include HPA genotyping of mother, father, and child to establish possible HPA incompatibilities, as well as serological testing (maternal–paternal serum crossmatch, and a maternal platelet antibody screening).”
- “If, in case of suspicion due to an affected family member, after the HPA-typing, the pregnant woman turns out to be HPA-1a negative, the HPA-1a type of father and, in case of paternal heterozygosity, consequently fetus can be determined.”
- “Adequate diagnosis does not only contribute to adequate management in the index cases, but is just as important for taking adequate measures in subsequent pregnancies to prevent bleeding complications.”
- “When the the father is homozygous, every consecutive pregnancy is incompatible and therefore the fetus is at risk. When the father is heterozygous, fetal genotyping has to be performed.”

A review by Mella and colleagues (2015) states:\(^\text{13}\)

- “Approximately 80% of pregnancies affected by NAIT have maternal antibodies that are directed against platelet antigen HPA-1a with the remaining 20% being affected by the other HPA types. Studies have shown that approximately 98% of Caucasian women express HPA-1a (genotype HPA-1a/HPA-1a or HPA1a/HPA1b) and about 2% of Caucasian women are HPA-1a negative (genotype HPA-1b/HPA-1b). The second most common platelet antigen causing NAIT in Caucasians is HPA-5b antigen, followed by HPA-1b and HPA-15.”
- “In at-risk pregnancies, mothers are antigen negative (most commonly HPA-1b) and fathers are either antigen-positive homozygous (genotype HPA-1a/1a) or heterozygous (genotype HPA-1a/1b).”
- “If the parental genotypes are different and the mother has specific antibodies to the putative antigen, then the pregnancy is at risk for NAIT and fetal/neonatal antigen typing would then be indicated.”

A review by Peterson and colleagues (2013) states:\(^\text{14}\)

- “Some have argued that it may be cost-effective to perform such screening routinely and offer special case management to the 10% of HPA-1a-negative women who produce antibody (Husebekk et al, 2009) but at the present time this is not practiced in the absence of a family history of NAIT, e.g., in a sister.”
- “A first affected neonate with NAIT in a family is normally identified when clinical signs of bleeding are evident at or shortly after birth and a platelet count confirms isolated thrombocytopenia.”
Criteria
Introduction
Requests for molecular testing for tissue antigen typing are reviewed using these criteria.

Human Platelet Antigen (HPA) Genotyping

Testing for human platelet antigens through molecular genotyping is considered medically necessary for individuals with clinical indications as outlined here.

• Member has at least one of the following:
  o Post-transfusion purpura 5-10 days after a blood transfusion, or
  o Suspected Neonatal Alloimmune Thrombocytopenia (NAIT)/ Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT) based on clinical presentation during pregnancy or neonatal period, or
  o Pregnancy or newborn with suspected or diagnosed NAIT/FNAIT, or
  o Female partner had a previous child with NAIT/FNAIT and is known to be alloimmunized, or
  o Fetus with suspected NAIT/FNAIT based on clinical presentation (ie: intracranial bleeding on ultrasound), and fetal diagnostic testing is medically necessary, or
  o Previous child with NAIT/FNAIT and there is a risk for this disorder in a current pregnancy based on parental HPA genotypes, and prenatal risk assessment is desired, AND

• Rendering laboratory is a qualified provider of service per the Health Plan policy.

Exclusions and other considerations

Targeted HPA genotyping is not considered medically necessary when assessed as part of a pharmacogenomics or coagulopathy workup. The procedure codes billed for HPA genotyping (including, but not limited to ITGB3 and ITGA2B) have no coverable indications outside of those outlined above, including for use in pharmacogenomics panels or to assess other cardiovascular disease states. For information on pharmacogenomics panels, please see the guideline titled Pharmacogenomic Testing for Drug Toxicity and Response.

Red Blood Cell (RBC) Antigen Genotyping

Testing for red blood cell antigens through molecular genotyping is considered medically necessary when the member has a documented risk for red blood cell alloimmunization as outlined here.

• One of the following criteria must be met:
  o Member has weak D antigen on serology, or
o Member is pregnant and has erythrocyte antibodies identified, or
o Member is the parent of a pregnancy or newborn suspected of having or at risk for Hemolytic Disease of the Fetus and Newborn (HDFN), or
o Pregnancy or newborn is suspected of having or at risk for Hemolytic Disease of the Fetus and Newborn (HDFN), or
o Member has warm autoantibodies, or
o Member is receiving certain monoclonal antibody therapies (such as anti CD38 therapy), or
o Member has a blood disorder requiring frequent transfusions (such as sickle cell disease, some forms of thalassemia, autoimmune hemolytic anemia, or myelodysplasia), or
o Member has a result from a traditional serology (hemagglutination) assay that is not consistent with the antibody that they are expressing, or
o Member has evidence of an antigen that cannot be detected, or is not easily detected, by traditional hemagglutination (including the Dombrock antigen, complex Rh phenotypes, Fy silencing mutations, and MNS system mutations), AND

• Rendering laboratory is a qualified provider of service per the Health Plan policy.

Billing and reimbursement considerations

Although most genotyping tests should only be performed once per lifetime, it may be medically necessary to repeat RBC antigen genotyping in some individuals. These requests will be reviewed on a case by case basis.

References

Introduction

These references are cited in this guideline.


