A Case of Weak D Serologic Phenotype

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ABSTRACT

Introduction: Rh D alloimmunization is the serologic response that occurs when Rh D-negative patients are exposed to Rh D-positive blood. Rh D blood typing is recommended in pregnancy to prevent alloimmunization.

Case Presentation: A 27-year-old gravida 3, para 2012 (G3P2012) previously Rh D-negative female presented with discordant and weakly positive Rh D blood typing results. Confirmatory genetic testing revealed weak D phenotype that can be treated clinically as Rh D-positive.

Discussion: Genetic variants of Rh D can cause varied blood typing results depending on the hospital reporting protocol utilized. If labeled as Rh D-negative, this could lead to unnecessary administration of Rh D immunoglobulin in pregnancy. Genetic variants should be suspected when patients are noted to have blood typing results that are discordant or weakly positive.

Conclusions: Rh D genotyping should be considered when discordant or weakly positive Rh D blood type results are noted in order to confirm and classify genetic subtype.

INTRODUCTION

Antigens on the surface of red blood cells determine blood type and are designated as ABO and Rh, with specific subtypes including Rh D. Patients with the Rh D antigen on the surface of their red blood cells are considered Rh D-positive. Patients without the Rh D antigen on the surface of their red blood cells are considered Rh D-negative. The US Preventive Services Task Force (USPSTF) recommends Rh D blood typing and antibody screening for all pregnant women during their first prenatal visit (Grade A recommendation).[1] Maternal antibody screening is an indirect antibody test evaluating for maternal antibodies to red blood cell antigens (indirect Coombs). Further, the USPSTF recommends repeat Rh D antibody screening at 24 to 28 weeks for Rh D-negative women unless the biological father is also Rh D-negative (Grade B recommendation).[1]

Evidence supports the USPSTF recommendations for performing routine blood typing during pregnancy in order to prevent maternal Rh D alloimmunization, which is an immune response resulting from exposure to foreign red blood cell antigens.[2] Antenatal bleeding, miscarriage, ectopic pregnancy, procedures including chorionic villus sampling, and delivery all can result in fetal-maternal hemorrhage.[2] During a fetal-maternal hemorrhage, small amounts of fetal blood cells can be introduced into maternal circulation. If the fetus is Rh D-positive, Rh D-negative mothers can subsequently form antibodies against fetal blood cells. Maternal IgG antibodies created to combat foreign red blood cell antigens can subsequently cross the placenta, attach to fetal red blood cell antigens, and cause destruction of the red blood cells by macrophages in the spleen.[3] This can have serious implications for fetal well-being, often resulting in hemolytic disease of the fetus and newborn (HDFN). This can be identified with direct antibody testing of the newborn, evaluating for maternal antibodies attached to the surface of neonatal red blood cells (direct Coombs). HDFN can lead to severe hemolysis, anemia, hydrops fetalis, stillbirth, postnatal jaundice, and multiorgan failure. More than 50 red blood cell antigens have been identified to cause HDFN; however, one of the most severe forms is caused by Rh D alloimmunization.[3,4]

Prophylactic use of passive Rh D (anti-D) immunoglobulin (RhIG) given to Rh D-negative women can prevent alloimmu-

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Table. Patient Blood Type Results

Specimen	Historical Red Cross Blood Type	May 2018 Status Post Spontaneous Abortion	July 2018 Prenatal Labs	Feb 2019 Delivery	Nov 2020 Prenatal Labs	June 2021 Delivery in New Hospital System
Blood Type	A Rh D-positive	A Rh D-negative	A Rh D-negative	A Rh D-negative	A Rh D-negative	A Rh D (weak positive)
Genotype						Weak D Serologic Phenotype with a Type 1 Allele

nization. In the United States, a dose is administered routinely at 28 weeks gestation to prevent alloimmunization during the third trimester prior to delivery. A second dose is given within 72 hours after delivery for Rh D-negative mothers with Rh D-positive babies.[2] Rh D-negative prevalence varies widely based on geographic location, with rates as high as 15% in North America and Europe, 5% in India, and 0.1% to 0.3% in Asia.[2] The prevention of sensitization and, thus, prevention of Rh HDFN has saved millions of lives, though inequitable access to screening and treatment globally contributes to ongoing disparities in perinatal morbidity and mortality.[3]

CASE PRESENTATION

A 27-year-old woman with no pertinent past medical history transferred to our family medicine clinic for prenatal care. Her first pregnancy in May 2018 resulted in early spontaneous abortion. She had no previous documentation of blood type in the electronic medical record; however, she had donated blood prior and had documentation from the Red Cross that her blood type was A Rh D-positive. At the time of her miscarriage, type and screen demonstrated A Rh D-negative with indirect Coombs negative. Due to the discordant results, the type and screen was repeated and confirmed. She subsequently received her first dose of RhIG.

Pregnancy was achieved shortly thereafter in July 2018. Her prenatal labs noted her to be A Rh D-negative with indirect Coombs negative. At approximately 28 weeks gestation, she received her second dose of RhIG. In February 2019, she delivered a term newborn via normal spontaneous vaginal delivery. RhIG evaluation after birth again noted blood type A Rh D-negative with indirect Coombs negative, and her newborn infant was similarly noted to be Rh D-negative with direct Coombs negative. She did not receive RhIG at that time.

In November 2020, she achieved pregnancy again and transferred care to our family medicine clinic. Prenatal labs demonstrated A Rh D-negative with indirect Coombs negative. She received her third dose of RhIG at approximately 28 weeks gestation. In June 2021, she delivered a term newborn via normal spontaneous vaginal delivery in a new hospital system with a different blood typing protocol. She developed a postpartum hemorrhage. A type and screen evaluation was obtained after birth with noted blood type A Rh D (weak positive) with indirect Coombs negative. Her newborn infant was noted to be A Rh D-positive, direct Coombs negative. Fetal cell stain was negative. Because of both discordant and weak positive testing, confirmatory genetic weak D testing was recommended by the blood bank. She received her fourth precautionary dose of RhIG while confirmatory genetic testing was pending.

Ultimately, the patient was found to have weak D serologic phenotype with a type 1 allele. She is not a candidate for RhIG in future pregnancies, and she can receive Rh D-positive blood should red blood cell transfusion be required in the future.

DISCUSSION

Weak D serologic phenotype is a genetic variant of the Rh D antigen most commonly affecting White patients at a rate of approximately 0.2% to 1% of the population. In many cases, one or more amino acid substitutions occur in the Rh D protein, which results in reduced antigen expression on the surface of red blood cells.[2,5] This results in weak or no reactivity to anti-D reagent initially but moderate or strong agglutination with antihuman globulin.[5] Clinically, some subtypes of weak D can be managed safely as Rh D-positive with minimal risk of alloimmunization and some cannot. Other Rh D antigen genetic variants beyond weak D exist as well but are beyond the scope of this case report.

Interestingly, serologic typing methods and Rh D interpretation vary by lab. In the case of patients with weak D phenotype, this can result in discordant findings. In addition, current standards for transfusion medicine require blood donors and newborns to undergo more thorough analysis and confirmation of weak D phenotype. Generally, this results in the inclusion of the weak D phenotype into an undifferentiated Rh D-positive category. This prevents administration of weak D phenotype blood to a Rh D-negative patient and ensures that a Rh D-negative mother appropriately receives RhIG after giving birth to an infant with weak D phenotype. While hospital protocols vary as noted in the case presented, more thorough testing often is not a requirement for transfusion recipients or pregnant women, and weak D phenotype patients in this case are generally categorized as Rh D-negative.[2,5] These management protocols aim to prevent alloimmunization but result in confusion for both patients and clinicians, as exemplified in the case presented. Prior to receiving care from our clinic, discordant blood typing results were noted, yet the patient was managed as Rh D-negative. She received RhIG several times before confirmatory genotyping occurred.

Testing for agglutination with antihuman globulin will identify

weak D phenotype; however, further delineation with genotyping is necessary to impact management decisions. Types 1, 2, and 3 weak D antigens do not form antibodies when exposed to Rh D-positive red blood cells, so they can be managed safely as Rh D-positive. These subtypes encompass approximately 80% of all weak D phenotypes identified. There has been alloimmunization demonstrated with some other weak D subtypes, thus demonstrating the value of genotyping.[5] The work group convened by Association for the Advancement of Blood and Biotherapies and College of American Pathologists estimates that if Rh D genotyping were performed in women with childbearing potential who are noted to have discordant blood typing results, approximately 24 700 doses of RhIG could be avoided annually in the United States.[5] Similarly, if transfusion recipients with discordant blood typing results underwent Rh D genotyping, 47 700 units of Rh D-negative red blood cells could be spared annually.[5]

The potential social impacts of routinely utilizing Rh D genotyping are vast. Rh D-negative blood type is less common than Rh D-positive blood type. Its prevalence varies based on geographic location but is less than or equal to 15% of the population.[2] Rh D-negative blood is utilized disproportionally when emergencies preclude the use of blood typing prior to red blood cell administration. This results in risk for a shortage of Rh D-negative blood, particularly in low resource areas of the world. Additionally, RhIG is manufactured by intentionally alloimmunizing Rh D-negative male donors (by injection of Rh D-positive red blood cells) and utilizing their plasma.[5] This poses theoretical risk to the donor who becomes alloimmunized through the process.[5] In the last 50 years, Rh disease-related morbidity and mortality have only dropped by approximately 50% despite the emergence of RhIG. While the specific burden of Rh disease in lower income countries is not well known, it is known that the biggest shortfalls occur in South Asia and sub-Saharan Africa where there is a high incidence of neonatal deaths due to complications of HDFN.[6] Shortages of RhIG play a role. Reducing the use of Rh D-negative blood and RhIG in situations where weak D genotyping deems it appropriate could mean more availability where shortages currently exist.

The work group supports performing Rh D genotyping for all discordant blood typing results.[5] The American College of Obstetricians and Gynecologists has identified that genotyping is a management option but recognizes that there is an overall lack of comprehensive cost-benefit data to strongly support a change in current recommendations.[2] However, a simulated financial analysis published in 2015 suggested that Rh D genotyping may provide clinical value without significantly increasing costs.[7]

CONCLUSIONS

Rh D genotyping should be considered when patients are found to have discordant or weakly positive Rh D blood typing results. Since blood type cannot change with time, discordant results should increase suspicion of Rh D variants, including weak D. Doing so could reduce risk to individuals by avoiding unnecessary medical interventions. Additionally, as we strive to better understand and address health care disparities, Rh D genotyping has the potential for social impacts on a larger scale by increasing availability of precious resources. Additional cost-benefit analysis of Rh D genotyping may be useful in better defining the role of Rh D genotyping in clinical care.

Funding/Support: None declared.

Financial Disclosures: None declared.

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