Educational Case

Educational Case: Alloimmunization of pregnancy

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see https://www.journals.elsevier.com/academic-pathology/news/pathology-competencies-for-medical-education-pcme.[1]

Primary objective

Objective TM1.2: Transfusion Reactions: Compare and contrast the pathophysiology, presentations, prophylaxis, and acute management of the different types of transfusion reactions.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic: Transfusion Medicine (TM); Learning Goal 1: Concepts of Blood Transfusion.

Patient presentation

A 32-year-old gravida 2 para 1 (G2P1) woman presents for her first prenatal visit at 6 weeks' gestation. She has no medical comorbidities and has been taking a prenatal vitamin for the past 3 months. Her previous pregnancy two years ago was uncomplicated, and she delivered a healthy-term infant by Caesarean birth for breech presentation. Antibody screens were negative for the previous pregnancy and that child's blood type is A positive. Today, an ultrasound demonstrates a viable singleton pregnancy and routine labs are drawn.

Diagnostic findings, Part 1

Vital signs and physical exam are unremarkable.

Questions/discussion points, Part 1

What other labs would be appropriate to order at this time?

Prenatal labs include a complete blood count, urinalysis, urine culture, pap smear, screening for sexually transmitted infections, and immunity against vaccine-preventable diseases not discussed in this case. Additionally, blood typing (ABO and Rhesus (Rh)) and antibody screens are routinely ordered at the first prenatal visit of every pregnancy.

Diagnostic findings, Part 2

Results of the mother's blood type and antibody screen are shown in Table 1.

Questions/discussion points, Part 2

Interpret the mother's results. Is her antibody screen significant?

The mother has an A+ blood type and an antibody screen that shows an anti-c antibody. This indicates that she does not carry the c antigen but was exposed and sensitized to it in the past and now produces antibodies. This is a clinically significant antibody in pregnancy, as it may cause delayed hemolytic transfusion reactions (DHTRs) and hemolytic disease of the fetus and newborn.

Discuss the antigens of the Rh system

The Rh blood group system is a complex system that describes more than 45 independent antigens present on red blood cells (RBCs). Along with the ABO system, the Rh system is highly clinically significant in transfusion medicine. Despite its importance in this field, we can only speculate on the physiologic function of Rh proteins, which may aid in maintaining the integrity of the RBC membrane. The Rh antigens are highly immunogenic and capable of eliciting strong antibody-mediated immune responses. The most clinically relevant antigens are D, C, c, E,

Table 1
Mother's blood type and antibody screen.

Lab test	Mother – Age 32
Blood type	A+
Antibody screen	Positive: anti-c
Anti-c antibody titer	4

and e. This case uses the designated Rh antigen, D for example, to signify the RBC antigen. People who carry the Rh D antigen are identified as D positive.[2] The Rh genes are nearly identical and are located adjacent to one another on chromosome 1. Note that this results in predictable haplotypes, for example, the Rh haplotype DCe is most common in Caucasians (42%), Native Americans (44%), and Asians (70%).[3] This haplotype would indicate the presence of D, C, and e antigens.

Discuss the pathophysiology of hemolytic disease of the fetus and newborn. Is this fetus at risk?

In hemolytic disease of the fetus and newborn (HDFN), maternal antibodies enter the fetal circulation via the transplacental passage and destroy fetal RBCs resulting in hemolytic anemia. This immune hemolysis can be caused by naturally occurring maternal antibodies (against ABO antigens if the mother is type O and the fetus is not type O) or following sensitization after a blood transfusion or prior pregnancy. Among the five mentioned antigens, antibodies to D and c are the two most common causes of severe (intrauterine transfusion (IUT)-requiring) HDFN. The D antigen is most classically associated with HDFN.[4] Although this mother is D positive, she is still at risk of HDFN in this pregnancy due to sensitization to other Rh and non-Rh antigens, including the c antigen. The c antigen is present in approximately 80% of the U.S. population.[5]

During sensitization, maternal exposure to an RBC antigen that is not expressed on maternal RBCs results in alloantibody formation. These antibodies (IgG only) cross the placenta and result in fetal RBC opsonization and destruction by reticuloendothelial macrophages. Fetal hemolysis may result in anemia, hyperbilirubinemia, or both.[4]

The risk of alloimmunization increases with the volume of blood to which the mother is exposed. Common causes of feto-maternal hemorrhage leading to sensitization include miscarriage, pregnancy termination, maternal abdominal trauma, ectopic pregnancies, and external cephalic version. Transplacental feto-maternal bleeding occurs in nearly all pregnancies and is responsible for the vast majority of alloimmunization. A volume as low as 0.1 mL of allogeneic blood can illicit alloimmunization. After sensitization and the primary immune response, a repeat exposure during a subsequent pregnancy can trigger a secondary response.[6]

Describe IgG vs. IgM alloantibodies. How are they significant in HDFN?

The different classes of antibodies have specific functions. IgM antibodies have a lower affinity for antigens but form large pentamers with 10 binding sites. This can result in robust complement activation and higher avidity when binding multivalent antigens. In a humoral immune response, IgM antibodies are the first immunoglobulins produced, as rapid production of immunoglobulins and complement activation are crucial in controlling bloodstream infections.[7]

The large size of IgM pentamers confines them to the blood and limits entry into intercellular spaces. This is clinically important, as IgM antibodies are unable to cross the placenta and cause HDFN. IgG antibodies are smaller, capable of diffusing into tissues, and monomeric. They are the principal antibody class of the blood and are capable of crossing the placenta.[7]

Antibodies responsible for causing HDFN, including anti-c, are IgG antibodies.[4]

IgM non-ABO antibodies, such as those against Lewis, M, and N antigens, may be present during pregnancy but usually do not cause

clinically significant HDFN for many reasons. Two of the most important reasons are that they cannot enter the fetal circulation via transplacental passage and they do not bind at 37 °C.

What is the role of the ABO system in HDFN?

ABO antigens are also of significance, as blood type O individuals produce IgG and IgM antibodies against A and B antigens. However, A and B individuals generally produce IgM anti-B and anti-A antibodies, respectively, but not IgGs. Therefore, mothers with an O blood type are also at risk of HDFN during pregnancy due to ABO incompatibility. Although ABO incompatibility is the most common cause of HDFN in the United States, due to production of anti-A or B IgG antibodies in Group O patients, most cases are mild or undetectable and do not require intervention.[3]

What can be done to prevent alloimmunization during pregnancy?

As anti-D is the most common cause of severe HDFN in the United States, it is routine to administer anti-D immune globulin to D-negative women. In current practice, 300 μg of anti-D immunoglobulin are administered at 28 weeks' gestation (if repeat D antibody testing confirms that sensitization has not occurred) and within 72 hours after birth if the infant is confirmed to be D positive. This quantity of anti-D immune globulin can prevent alloimmunization after exposure to up to 30 mL of D-positive fetal whole blood or 15 mL of fetal RBCs. In cases where feto-maternal hemorrhage exceeds the volume covered by the standard dose, additional vials may be administered. This practice is associated with less than a 0.2% rate of Rh alloimmunization.[8] Of note, the prophylactic administration of D immune globulin only prevents anti-D formation and does not prevent alloimmunization against any other antigens such as the little c antigen.

How should this patient be monitored throughout her pregnancy?

ABO blood typing and antibody screening should be performed at every first prenatal visit. Once a clinically significant IgG alloantibody has been identified, close fetal monitoring and serial antibody titer testing are essential.[9] Although the titer of 4 is not significant enough to cause hemolysis, it indicates sensitization and the possibility for increased antibody production and resultant hemolysis with increased antigen exposure.

Because the endpoint is usually a visual interpretation of the magnitude of red cell agglutination (also known as grading reaction strength) and is inherently subjective to some degree, titer samples should always be checked in parallel with previously collected and frozen samples to standardize differences in test RBCs used.

As discussed, anti-c and other clinically significant antibodies can cause HDFN and DHTRs. Briefly describe the major transfusion reactions

Although not described in this clinical scenario, transfusion reactions are adverse events associated with the transfusion of any blood component and mechanistically related to HDFN. Transfusion reactions may be acute (presenting during or within 24 hours after transfusion) or delayed (days to weeks after transfusion). Severity ranges from minor to life threatening and therefore all suspected transfusion reactions should prompt immediate cessation of the transfusion and alert the blood bank and care team.

The major types of transfusion reactions include acute hemolytic transfusion reaction (AHTR), DHTR, transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), febrile non-hemolytic transfusion reactions (FNHTR), and allergic transfusion reactions.

Immune-mediated transfusion reactions are caused by incompatibilities between the recipient and blood product. AHTRs are usually caused by an ABO incompatibility between the recipient and donor. Naturally occurring IgM anti-A or anti-B antibodies in the recipient result in complement activation and rapid hemolysis of transfused cells. FNHTRs are present acutely but are a diagnosis of exclusion and include fever and/or chill without any signs of a more serious reaction. They are often caused by donor cytokines released by immune cells in the blood product during storage.

In allergic transfusion reactions, foreign antigens in donor blood products elicit a hypersensitivity reaction. Severe cases, termed anaphylactic transfusion reactions, are often due to a recipient selective IgA deficiency and a strong immune response to transfused IgA antibodies.

In TACO, the resultant increase in the recipient's intravascular volume after transfusion results in hypervolemia and circulatory overload. In TRALI, lung injury occurs due to donor antibodies against recipient human leukocyte antigens or human neutrophil antigens. The immune reaction ignited by donor antibodies results in pulmonary edema.

In DHTR, incompatibilities in minor antigens such as those of the Rh system result in hemolysis of transfused cells days to weeks after transfusion. This occurs in situations where recipients were previously exposed to an antigen but the level of antibody at the time of transfusion is undetectable. The second stimulus, that is, repeat exposure to the antigen from the transfusion, results in an increase in antibody production and hemolysis days to weeks after transfusion. This pathophysiology is similar to that of HDFN, as exposure to incompatible fetal RBCs in the first pregnancy results in antibody formation and then hemolysis in a subsequent pregnancy that has the same minor antigen incompatibility.[7]

Diagnostic findings, Part 3

The mother's anti-c antibody titers are measured every 4 weeks starting at 18 weeks' gestation. Previous blood samples are frozen and tested in parallel with each subsequent titer. Results are shown in Table 2.

Questions/discussion points, Part 3

How are antibody titers interpreted? How are they significant in HDFN?

Antibody titers are used to measure the amount of a specific antibody present in a patient's serum. Higher titers indicate a greater concentration of antibody and rising titers indicate ongoing exposure to the antigen and antibody production.

Although titers do not necessarily correlate linearly with HDFN risk or hemolysis, above a certain threshold called the critical titer, HDFN is likely, and the fetus should be monitored closely for anemia via middle cerebral artery (MCA) Doppler measurements.[5]

Should genotyping or paternal testing be done?

Not routinely and not for the reasons that are often cited, sometimes, the obstetrician orders genotyping or phenotyping for the antigen(s) in question on both the mother and father. A common rationale is that if the father does not have the cognate antigen to the mother's antibody, then there is no risk of HDFN and therefore no need to follow maternal antibody titers. While such testing may be clinically useful in certain

situations, it should not be done as a means to avoid following the titers or monitoring the fetus via MCA Doppler. Any testing of the father inherently includes the risk that paternity is not necessarily assured. That is, the person being tested may not, in fact, be the biological father.[10,11]

What additional antenatal testing is done after a critical titer is reached?

While the critical titer is often defined locally for a particular lab and OB group practice, it is common in most laboratories to consider 16 a critical titer, as published by the Association for the Advancement of Blood & Biotherapies.[12] If there is no Kell antigen incompatibility (anti-K antibodies, which cause severe hemolysis) and no history of HDFN, MCA Dopplers are usually initiated when the titer surpasses the critical titer or at 22–24 weeks' gestation. MCA Dopplers are also recommended starting at 18 weeks' gestation regardless of titer if the mother has an anti-K antibody or an antibody that caused HDFN in a previous pregnancy.[13]

MCA- Peak systolic velocity (PSV) is highly sensitive for fetal anemia. In HDFN and other causes of anemia, cerebral blood increases to preserve oxygen delivery to the brain. As RBCs are destroyed, hematocrit decreases resulting in decreased blood viscosity. Together with increased cardiac output associated with anemia, blood flow PSV increases.

In addition to anemia, ultrasound may indicate signs of hydrops fetalis. Though this is not seen until fetuses are severely anemic, ultrasound surveillance is important and may show edema, pleural and pericardial effusions, and/or ascites in a hydropic fetus.

A common trigger for IUT is when the PSV surpasses 1.5 multiples of the median (MoM) adjusted for gestational age.[9] At that trigger, cordocentesis is also often recommended to sample fetal blood and measure hemoglobin and hematocrit.[14]

Diagnostic findings, Part 4

The critical titer of 16 is reached at 22 weeks' gestation. The fetus is monitored for anemia via MCA-PSV. The hemoglobin of the fetus is measured via cordocentesis at 31 weeks' gestation. Results are shown in Table 3[15] and 4.[9]

Questions/discussion points, Part 4

Interpret the MCA-PSV results

The fetus shows an increasing MCA-PSV with gestational age. This is normal in a growing fetus. However, the rate of this increase can be measured in terms of MoM for gestational age. In this case, the increase is disproportionate. The PSV becomes >1.5 MoM at 30 weeks' gestation (see Table 4).

Additionally, cordocentesis showed hemoglobin greater than 2 standard deviations below the mean hemoglobin (or 0.84 MoM) for gestational age. This directly confirms fetal anemia.

What are the indications for IUT in alloimmunization of pregnancy?

The introduction of prophylactic anti-D immune globulin has drastically reduced the number of IUTs performed each year. However, it remains a crucial treatment for clinically significant fetal anemia.[9]

Table 2
Maternal anti-c titers.

Sample	Anti-c titer
6 weeks' gestation	4
18 weeks' gestation	4
22 weeks' gestation	16

Table 3
Fetal MCA-PSV Doppler studies.

Age	Fetal MCA-PSV	Median[15]	MOM
22 weeks' gestation	30 cm/sec	27.9 cm/sec	1.075
24 weeks' gestation	36 cm/sec	30.7 cm/sec	1.173
26 weeks' gestation	42 cm/sec	33.6 cm/sec	1.25
28 weeks' gestation	50 cm/sec	36.9 cm/sec	1.355
30 weeks' gestation	65 cm/sec	40.5 cm/sec	1.605

Table 4

Fetal cordocentesis hemoglobin results.

Age	Hemoglobin	Median[9]	MOM
31 weeks' gestation	10.9 g/dL	13.0 g/dL	0.84

When the MCA-PSV is > 1.5 MoM for gestational age, the risk of fetal anemia is high. However, Doppler flow alone does not prove fetal anemia, so fetal blood is usually sampled via cordocentesis to determine the hemoglobin directly prior to transfusion for both diagnosis and monitoring. In addition, signs of hydrops fetalis are also indications for cordocentesis.[9]

IUT may be performed if the fetus's hemoglobin is at least 2 standard deviations below the mean (or 0.84 MoM). A hematocrit of 30 may also be used as a threshold for IUT. Though anemia is considered relatively mild at this level, improved fetal outcomes have been seen with early transfusions as opposed to waiting until severe anemia develops.[16]

Similar to MCA-PSV, hemoglobin increases with gestational age. IUT is generally performed between 18 and 35 weeks' gestation. Before 18 weeks, the small anatomy poses significant technical challenges for the ultrasound operator. After 35 weeks, the risks of prematurity-related complications decrease to a point where delivery and postnatal transfusion are recommended.[17]

What kind of blood product would be transfused to this fetus?

Donor RBCs are crossmatched with maternal plasma to prevent delayed hemolysis of the transfused cells. That is, the RBCs should lack the cognates to mother's antibody. For example, the donor RBCs should lack c if the mother has anti-c.

Additionally, fresher blood (<7 days old) is often preferred, as the risk of hyperkalemia is minimized. Leukoreduction is nearly universally performed to decrease CMV transmission risk. The product is irradiated to prevent transfusion-associated graft-versus-host disease.[12]

In most cases of HDFN, O-negative blood is used for the IUT. However, in the specific cases of a mother with anti-c or anti-e, O-positive blood is nearly always transfused because donors with the "ideal" phenotype are extremely rare. This is due to characteristics of the genetic locus of the Rh system on chromosome 1, namely linkage disequilibrium. In other words, the Rh system genes for the D, C, c, E, and e antigens are not independently inherited.

The key point is that nearly all D-negative individuals are homozygous for the c and e genes. Said another way, individuals who are both D and c negative (that is, D negative and C homozygous) are vanishingly rare. The same is true of individuals who are both D negative and e negative (that is, D negative and E homozygous). Specifically, nearly all D negative haplotypes are ce. Haplotypes Ce and cE are very rare, and CE is case reportable.

Nearly all textbooks and guidelines state that IUTs must use O-RBCs. how can it be safe to use O + RBCs for an IUT when the mother has an anti-c (or anti-e) antibody? What about the risk of anti-D formation in the fetus/newborn?

While it may seem non-intuitive, it is safe to transfuse O-positive RBCs for an IUT when the mother has anti-c or anti-e antibodies. One reason is based on the prevalence of certain antigen combinations, and the other reason is an empirical fact of immune system maturation. First, if the mother makes anti-c, then she must be C homozygous. Based on the previous paragraph, she will almost certainly be homozygous for the D gene, which means that the fetus will have at least one D gene and will be D+. The same is true if the mother makes anti-e; she must be E homozygous, and she will almost certainly be homozygous for the D gene. In either case, the fetus will almost certainly be D+ and, as a result, would not be able to make anti-D.

More importantly, fetus/newborns do not make their own antibodies before 4–6 months of age. Thus, the risk of alloantibody formation is

essentially zero and is usually greatly outweighed by the risk of untreated fetal anemia.

This nuance is often overlooked as some well-intended guidelines and textbooks state or imply that all IUTs must always use O-negative RBCs. The case is usually that they are only considering the more common anti-D as a cause of HDFN.

Case conclusion

The fetus received two IUT transfusions at weeks 32 and 34 without progression in MCA-PSV and is delivered at 35 weeks' gestation. Following an uncomplicated induction of labor, delivery, and short NICU stay for prematurity, the neonate is discharged home without complications.

Teaching points

- Transfusion reactions are classified as acute or delayed and are important to recognize promptly as they can be life-threatening.
- The major transfusion reactions are AHTR, FNHTR, DHTR, TRALI, TACO, and allergic. These reactions are caused by pathophysiologic mechanisms involving donor-recipient Rh and ABO antigen incompatibilities, donor or recipient antibodies, cytokines, and circulatory overload. A DHTR has similar pathophysiology to that of HDFN.
- HDFN is most commonly caused by RBC antigens of the Rh, Kell, and ABO systems. With the introduction of routine Rh D immunoglobulin prophylaxis, ABO incompatibility is the most common cause of HDFN (of any severity) in the United States. Common causes of severe HDFN include antibodies to D, c, and K antigens.
- ABO typing and antibody screening should be performed at every first prenatal visit. After alloantibody detection, fetal monitoring and serial antibody titer testing are done.
- Fetuses should be monitored with MCA-PSV Doppler ultrasound. If values surpass 1.5 MoM for gestational age or ultrasound shows signs of hydrops fetalis, cordocentesis should be performed to confirm clinically significant anemia.
- IUT should be performed if the fetus is anemic, measured by a hemoglobin two standard deviations below the mean for gestational age.
- IUT should be done with fresh, leuko-reduced, and irradiated RBCs that are crossmatch compatible with maternal plasma.
- D-negative (specifically, O-negative) RBCs are used for IUTs in most situations with two important exceptions.
- If the patient has anti-c or anti-e, D-positive (specifically, O-positive) RBCs are used for IUTs due to the practical absence of D-negative/c negative and D-negative/e negative donors. Awareness of these two exceptions can help prevent surprises, misunderstandings, and delays from clinician demands that are impossible to fulfill.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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