Lesson S25: Preanesthetic Assessment of the Patient with Serum Antibodies – Part 1

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REVIEW DATE: May, 2012

Read this article, reflect on the information presented, then go online and complete the lesson post-test and course evaluation before the termination date below. (CME credit is not valid past this date.) You must achieve a score of 80% or better to earn CME credit.

TIME TO COMPLETE ACTIVITY: 2 hours
RELEASE DATE: July 1, 2012
TERMINATION DATE: July 31, 2013

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This is a 2 part series. Part 1 presents an update on blood transfusion and its complications. Methods of blood cross matching and identification of antibodies are described. In Part 2, the incidence and pathophysiology of sickle cell disease is reviewed. Antibody formation is particularly common in this group of patients and appropriate blood replacement and anesthetic management are outlined.

Needs Statement
The presence of antibodies in the blood of patients who require perioperative transfusion is not uncommon. Many anesthesiologists are unaware of which antibodies are significant and when a blood transfusion may cause complications that outweigh the benefit for the patient. This is especially true in patients with sickle cell disease.

Learning Objectives
At the end of this activity, the participant should be able to:

1. Cite the incidence of antibodies found on routine cross matching
2. Describe the structure of an antibody
3. Explain how blood is typed and cross matched
4. Cite the incidence of blood transfusion worldwide
5. Tabulate commonly occurring antibodies in blood
6. Identify common problems in cross matching
7. Present a brief history of blood transfusion
8. List means by which a blood type may be changed
9. Know the current protocol tests for donated blood
10. Distinguish between type and screen and type and cross match
Case History

A 25 year old African-American woman with sickle cell disease (SCD) presented for an emergency appendectomy. She had frequent hospitalizations due to crisis episodes requiring red cell transfusions. She also had 4 pregnancies. Her hematocrit was 22%. The decision was made to transfuse the patient prior to surgery. The blood bank technician reported that the blood sample tested positive for antibodies.

Introduction

Approximately 24 million units of blood components are transfused annually in the United States. Transfusion related adverse events occur in about 20% of all transfusions, with serious adverse complications in 0.1% after red cell replacement and 0.04% after platelet infusion. Section 606.170(b) of Title 21, Code of Federal Regulations (21 CFR 606.170(b)), requires that facilities notify the Food and Drug Administration (FDA), Center for Biologics Evaluation and Research (CBER), Office of Compliance and Biologics Quality (OCBQ), as soon as possible after confirming a fatality associated with a complication of blood collection or transfusion. The collecting facility should report donor fatalities, and the compatibility testing facility is to report recipient fatalities. The regulation also requires the reporting facility to submit a full report of the investigation within 7 days after the fatality. According to the FDA, 54 deaths were reported in 2008, 66 in 2009, 64 in 2010 and 58 in 2011. These numbers may not represent the true incidence because of inherent variation and inaccuracy in reporting. Also, these numbers do not include fatalities from disease transmission, patients who suffer a severe injury but survive, and others who have less serious but still disabling complications.

Complications are generally categorized as immunological or infectious. Complications can also arise directly or indirectly from potential quality degradation during storage. Overall, the financial toll of adverse events from blood transfusions in the US is estimated at 17 billion dollars which adds more to the cost of each transfusion than acquisition and procedure costs combined. While some risks of complication are specifically related to patient status or quantity of transfusion, the overall risk of an adverse outcome increases in direct proportion to the frequency and volume of transfusion.

History

The two most significant blood group systems were discovered by Karl Landsteiner during early experiments with blood transfusion: (1) the ABO group, in 1901 and (2) the Rhesus group, in cooperation with Alexander S. Wiener, in 1937. Early transfusions were made directly from donor to receiver. Around 1910, it was found that blood could be stored by adding anticoagulant and refrigeration. Blood banks were established. Albert Huston, a Belgian doctor, and Luis Agote, from Argentina, successfully performed a non-direct transfusion of diluted blood in 1914. Both used sodium citrate. The first blood transfusion using blood that had been stored and cooled was performed in 1916. Oswald Hope Robertson, a medical researcher and U.S. Army officer, is credited with establishing the first blood bank while serving in France during World War I. The first academic institution devoted to the science of blood transfusion was founded by Alexander Bogdanov in Moscow in 1925. Bogdanov was partially motivated by a search for eternal youth, and delighted in the improvement of his eyesight, suspension of balding, and other positive symptoms after receiving 11 transfusions of whole blood.
Development of the Coombs test in 1945, the advent of transfusion medicine, and the understanding of ABO hemolytic disease of the newborn led to discovery of more blood groups, and now 30 human blood group systems are recognized by the International Society of Blood Transfusion (ISBT). Within the 30 blood groups, over 600 different blood group antigens have been found; many of these are very rare or are mainly found in certain ethnic groups.

Today, red blood cells (RBC) are stored for up to 42 days or 6 weeks from the time of collection, assuming proper storage solutions and conditions. While this particular shelf life has little evidentiary basis and persists primarily for historical reasons, it remains the default metric in the absence of any direct means for measuring actual quality degradation of product units. Many controversies surround the extent to which this practice is reliable.

**Blood Typing**

Blood types are classified on the presence or absence of inherited antigenic substances on the surface of red blood cells. (Figure 1) These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of other tissues. Several red blood cell surface antigens can stem from one allele (or very closely linked genes) and collectively form a blood group system. Blood types are inherited from both parents.

**Fig 1: ABO blood group system**

![ABO blood group system](image)

*ABO blood group system: the carbohydrate chains that determine the ABO blood group*
The same blood group remains for life in most individuals, but very rarely may be changed through addition or suppression of an antigen in infection, malignancy, or autoimmune disease. Another more common cause in blood type change is bone marrow transplantation as performed for diseases such as leukemia or lymphomas. If a person receives bone marrow from someone with another ABO type, the patient's blood type will eventually convert to the donor's type.

Other blood types are associated with inheritance of some diseases; for example, the Kell antigen is sometimes associated with McLeod syndrome. Certain blood types may affect susceptibility to infections. For example resistance to specific malaria species is seen in individuals lacking the Duffy antigen.

The Rh system is the second most significant blood-group system in human-blood transfusion with about 50 antigens. The most important Rh antigen is the D antigen, because it is the most likely to provoke an immune system response among the five main Rh antigens. It is common for D-negative individuals not to have any anti-D IgG or IgM antibodies, because anti-D antibodies are not usually produced by sensitization against environmental substances. However, D-negative individuals can produce IgG anti-D antibodies following a sensitizing event such as a fetomaternal transfusion of blood from a fetus in pregnancy or a blood transfusion with D positive red blood cells. Rh negative blood types are rare in Asian populations (0.3%) and more common in Caucasians (15%). Of note, newborns do not form antibodies to ABO blood group antigens (anti-A, B, or AB) during the first few months of life (i.e., infants do not form anti-A or anti-B until 3-4 months after birth).

All donated blood is tested for HIV-1, HIV-2, HTLV-1, HTLV-2, Hepatitis B, Hepatitis C, Syphilis (*T. pallidum*), Chagas disease (*T. cruzi*), and West Nile Virus. Platelet products that are stored at room temperature are also tested for bacterial infections due to the higher risk for contamination. Blood products may also be tested for cytomegalovirus (CMV) because of the risk it presents to certain immunocompromised recipients, such as those with HIV or those undergoing organ transplantation. In the United Kingdom, all blood is tested for CMV. In contrast, blood banks in the United States perform testing sufficient to maintain an available supply of CMV-negative blood. The majority of the population in the United States is CMV positive. Other than positivity for CMV, any products tested positive for an infectious disease are discarded.

**Antibodies**

The ABO system is the most important blood-group system. The associated anti-A and anti-B antibodies are usually immunoglobulin M, abbreviated IgM, antibody. The terms antibody and immunoglobulin are often used interchangeably. ABO IgM antibodies are produced in the first years of life during gestation and by sensitization to environmental substances such as food, bacteria, and viruses.

Antibodies are large Y-shaped proteins produced by B-cells. Within the immune system, antibodies identify and neutralize foreign objects (antigens) by recognizing a unique part of the antigen. Each tip of the "Y" antibody contains a paratope that is specific to an epitope on an antigen. With the paratope functioning like a lock and the epitope function like a key, these two structures can precisely bind together and then tag a substance for attack by other parts of the immune system, or can neutralize it by destroying a critical part of its function.
Antibodies in blood are produced by plasma cells and can occur in two physical forms: a soluble form secreted from the cell, and a membrane-bound form attached to the surface of a B cell known as the B cell receptor or BCR. The BCR facilitates the activation of B cells which can differentiate into either plasma cells which produce antibodies, or memory B cells that survive and recognize specific antigens on subsequent exposure to allow faster response. Interaction of the B cell with a T helper cell is necessary to fully activate the B cell and generate the antibody following antigen binding.

**Typing and screening or cross matching**

At the blood bank, a specimen is first checked against the requisition slip. Small discrepancies are sufficient to result in rejection of the sample, a requirement necessary to comply with the rules of the American Association of Blood Banks (AABB), national regulations and local policies. Even small errors are associated with an increased risk of a transfusion reaction.

The “type” is performed in about 15 minutes and determines the ABO-Rh status of the patient. The test is performed by incubating the recipient’s red cells with commercial anti-A and anti-B antibodies. Incubation determines if the patient has A or B antigens on the surface of the red cells. For example, if there is agglutination during the mixing of the recipient’s blood and the anti-A antibody, then the recipient has surface A antigens. Similarly, incubation with commercial anti-D antibodies determines RhD status. While there are approximately 50 Rh antigens, the D antigen is considered the most immunogenic and is the only Rh antigen routinely tested. The second part of the type is called “reverse” typing, and requires commercially available A and B reagent cells to test if the donor’s serum has naturally preformed antibodies to these antigens.

The “screen” portion tests for non-A, non-B antibodies, and takes at least 30 minutes. There are approximately 29 discrete blood group systems that are currently identified, including ABO and Rh, encompassing over 250 antigens that coat the red cell surface. Approximately 18 antigens, those deemed most clinically significant, are required by the FDA to be present on reagent red cells used in the antibody screen. About 3-10% of persons who have had multiple blood transfusions have antibodies to these “unexpected” antigens. For pregnant women, the estimate is that 1 in 100 women will have “unexpected” IgG antibodies not of AB or RhD specificity. Transfusion of ABO-Rh type specific, unscreened red cells could be deleterious for these patients.

The antibody screen significantly reduces the chance of incompatibility due to alloantibodies (Figure 2). The probability of this screening test missing an antibody that is potentially dangerous in the general population has been estimated to be no more than 1 in 10,000. Oberman et al studied 31,320 samples from 8,969 patients and, after complete cross match, found nine "clinically significant" antibodies that were not detected during the screen. These antibodies were too low in titer to be detected by the antibody screen but may have been clinically significant in large numbers. A recent review of 22,463 cases, positive results for antibody screening were found in 243 patients or 1.52%. Lewis, Rh, Xga and mixed antibodies were identified in 123 patients. The specifics of the antibodies could not be determined in the rest but 52% had a history of pregnancy and 20% had been transfused previously.
Screening is done by incubating the recipient's serum with commercially available type O reagent cells which contain the most clinically significant antibodies (approximately 18) causing hemolytic transfusion reactions. Agglutination or hemolysis of reagent cells simply means that there are unexpected antibodies. The blood bank technician then identifies which of the 18 antibodies is expressed in the patient's serum and tries to locate donors that do not express these antigens. This latter portion is called the cross match.

If a patient has a negative antibody screen and no history of antibodies in the past, an "immediate spin" cross match to issue blood can be done. In this procedure to detect ABO incompatibility, the recipient's serum is added to ABO-Rh compatible donor red cells at room temperature, centrifuged,
and then graded for macroscopic agglutination. This process takes 1 to 5 minutes and reduces the risk of serious hemolytic reactions resulting from ABO mismatch. Unexpected antibodies in the MNS, P, and Lewis systems are also detected. If there are no unexpected antibodies on screening and/or no history of antibodies, a computer cross match can be performed in less than 5 minutes. Here, the ABO and Rh status of the patient is matched with the confirmed ABO type of the unit. Using LASER wand and bar code technology, the risk of a hemolytic reaction is less than 0.1%.

If clinically significant antibodies are present, antigen-negative units need to be located and a serologic cross match, including an antiglobulin test is required. Multiplying the negative frequency of each antigen allows calculation of the approximate number of units that need to be screened to find an antigen negative donor (e.g., .91 (K antigen) x .48(S antigen) = .436 x 100 = 44% = approximately 1 in 3 units will lack K and S antigens). The hospital inventory is first checked for this antigen negative blood. If not available, phenotyped donors from limited donor programs, frozen inventories, and rare donor registries may be examined to find a match.

In a serologic cross match, donor red cells and the recipient’s plasma are incubated and examined for agglutination/hemolysis at 37 degrees C followed by re-examination after the indirect antiglobulin test (IAT). The IAT uses anti-human IgG to detect antibody-antigen reactions not seen with 37 degree incubation alone.

Some blood banks perform the serologic crossmatch even if there are no antibodies on screening, because the screen is imperfect. Not all clinically significant antigens are represented on the screening cells, antibody screening RBCs may have weak expression of the offending antigen if they are not homozygous for the antigen, weak antibodies may react with fresh donor units but not older screening cells, and antibodies might be missed if there is an error in the technique or reading of the screening test. This serologic test can take up to 30 minutes.

For a patient without a significant transfusion history, ABO-Rh typing alone results in a 99.8% chance of a compatible transfusion, an antibody screen increases this chance to 99.94%, and a serologic cross match increases that number further to 99.95%. The chance of the screen missing a significant antibody is estimated to be 1 in 10,000. Antibodies to the Rh antigens C and E, Kell (K), Duffy (Fy), Kidd (Jk), and S are most likely to cause hemolysis. Alloantibodies which become serologically undetectable over time are implicated in delayed hemolytic transfusion reactions (DHTRs). Thus a prior transfusion history should be obtained.

**Types of Transfusion Reactions**

Overall, transfusion carries an incidence of severe reactions determined to be about 0.61% per unit when an online transfusion reaction reporting system is used. Mild symptoms such as fever and chills usually go unreported. Several countries have transfusion surveillance systems in place and report frequently.

Some of the reactions described include the following:

- Acute hemolytic reactions occur in about 0.016 percent of transfusions, with about 0.003 percent being fatal. Donor erythrocytes are destroyed by preformed recipient antibodies usually due to clerical errors or improper typing and cross matching. Symptoms include fever,
chills, chest pain, back pain, hemorrhage, tachycardia, dyspnea, and hypotension. Treatment is supportive. Kidney injury may occur due to the effects of the hemolytic reaction.

- Delayed hemolytic reactions occur in about 0.025 percent of transfusions and are due to the same mechanism. However, the consequences are generally mild and a many patients may have no symptoms. However, evidence of hemolysis and lowered hemoglobin levels may still occur. Treatment is generally not needed, but due to the presence of recipient antibodies, future compatibility may be altered.

- Febrile nonhemolytic reactions are due to recipient antibodies to donor white blood cells, and occur in about 7% of transfusions and are often related to prior transfusion exposure.

- Allergic reactions may occur when the recipient has preformed antibodies to certain chemicals in the donor blood. They do not require prior exposure to transfusions. Symptoms include urticaria, pruritus, and may even result in anaphylactic shock. A small number of patients (about 0.13%) lack immunoglobin IgA, and upon exposure to IgA-containing blood, may develop an anaphylactic reaction.

- Post-transfusion purpura is a rare complication that occurs after transfusion containing platelets that express a surface protein HPA-1a. Recipients who lack this protein develop sensitization to this protein from prior transfusions, and develop thrombocytopenia about 7–10 days after subsequent transfusions. Treatment is with intravenous immunoglobulin, and recipients should only receive future transfusions with washed cells or HPA-1a negative cells.

- Transfusion-associated acute lung injury (TRALI) is an increasingly recognized adverse reaction. It is a syndrome of acute hypoxia occurring within 6 hours of transfusion with 70% requiring mechanical ventilation. It may occur as often as 1 in 5000 transfusions. TRALI is the leading cause of transfusion-related fatalities in the United States (around 50% of cases). Mortality is around 10%. TRALI has been consistently associated with anti-HLA antibodies, antibodies commonly formed during pregnancy. The complication is typically associated with plasma products but can also occur in recipients of packed red blood cells, presumably due to the residual plasma present in the unit. Plasma infusions should be from male donors. In 2006, the AABB (formerly the American Association of Blood Banks) recommended that blood banks use high plasma volume components from female donors for further manufacturing instead of transfusion due to the higher risk.

- Transfusion-transmitted bacterial infection is estimated at about 1 in 50,000 platelet transfusions, and 1 in 500,000 red blood cell transfusions. Platelets are stored at room temperature for short periods of time and thus more likely to become contaminated. Contamination is also more common with longer duration of storage, especially when exceeding 5 days.

- HIV contamination is now rare. The development of a nucleic acid test for the HIV-1 RNA has dramatically lowered the rate of donor blood seropositivity to about 1 in 3 million units.

- Hepatitis C via transfusion risk is about 1 in 2 million units. As with HIV, this low rate has been attributed to the ability to screen for both antibodies as well as viral RNA nucleic acid testing in
donor blood.

- Other rare transmissible infections include hepatitis B, syphilis, Chagas disease, cytomegalovirus infections (in immunocompromised recipients), HTLV, and Babesia.

- Transfusion-associated volume overload occurs especially in recipients with underlying cardiac or kidney disease. Plasma transfusion is especially prone to causing volume overload due to its hypertonicity.

- Hypothermia can occur with transfusions with large quantities of blood products which normally are stored at cold temperatures.

- Coagulopathies (disseminated intravascular coagulation, dilution of recipient platelets and coagulation factors, hypothermia) can occur with large volume transfusions.

- Metabolic alkalosis is due to the breakdown of citrate stored in blood into bicarbonate.

In Part 2 of this 2 part series, the management of an anemic patient with sickle cell disease and antibodies is outlined.

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REFERENCES


Post-test

1. The number of blood component units transfused in the United States is estimated to be:
   a. Increasing at an alarming rate
   b. About 24 million
   c. Close to 100 million
   d. Largely unknown

2. The proportion of adverse complications of red cell and platelet transfusions are estimated to be:
   a. 0.1% and 10% respectively
   b. 5% and 0.4% respectively
   c. Variable, depending on the sex of the donor
   d. 0.1% and 0.04% respectively

3. A true statement regarding TRALI:
   a. It occurs most commonly after red cell transfusion
   b. It is associated with transfusions of plasma from female donors
   c. Fatality is about 50%
   d. Mechanical ventilation is usually not required

4. With no significant transfusion history:
   a. ABO-Rh typing allows a compatibility transfusion in almost 100% of cases
   b. Addition of an antibody screen adds nothing further
   c. Serologic cross matching decreases the accuracy
   d. The chance of the screen missing a significant antibody is about 1:1,000,000

5. During serologic cross matching:
   a. Donor plasma and recipient red cells are incubated
   b. Donor red cells and recipient plasma are examined for agglutination
   c. A temperature of 55 degrees is required
   d. The IAT test is not involved
6. Complications of blood transfusions are due to:

   a. Immunologic compromise
   b. Infectious contamination
   c. Degradation during storage
   d. All of the above

7. Regarding the development of blood typing:

   a. A blood group system was described by Karl Landsteiner in 1801
   b. Weiner contributed to the discovery of the Rh system in 1937
   c. The addition of refrigeration and anticoagulation was widespread by 1900
   d. Diluted blood transfusion always resulted in fatalities

8. The typing:

   a. Determines the ABO-Rh status
   b. Takes less than 5 minutes
   c. Requires incubation of commercial anti A and B antibodies with the recipient’s plasma
   d. None of the above

9. Screening:

   a. Tests for Rh incompatibility
   b. Determines A and B antibodies
   c. Takes at least 30 minutes
   d. Requires that at least 29 antigens be present on reagent red cells

10. Clinically significant antibodies have been discovered in a patient’s blood:

    a. Antigen negative blood must be located
    b. Computer cross match will solve the problem
    c. An antiglobulin test is not useful
    d. Phenotyping will reveal appropriate recipients