EDUCATIONAL COMMENTARY – WARM AUTOANTIBODIES AND TRANSFUSION

Educational commentary is provided through our affiliation with the American Society for Clinical Pathology (ASCP). To obtain FREE CME/CMLE credits see the Continuing Education menu on the left side of the screen.

Learning Outcomes

Upon completion of this exercise, the participant should be able to:

- explain the etiology and pathogenesis of warm autoantibodies.
- describe the complications of pretransfusion testing.
- communicate transfusion recommendations to clinicians.

Warm autoantibodies are antibodies that react with intrinsic antigens present on autologous red blood cells (RBCs) at body temperature. However, the relationship of an autoantibody and its hemolytic potential is not well defined, and some may never have a pathologic effect in the patient. Therefore, the presentation of patients with warm autoantibodies is highly variable and may range from patients who are asymptomatic without RBC destruction to severe and life-threatening anemia. Patients who are symptomatic seek treatment for fatigue, dyspnea, and or cardiac tachycardia or palpitations. The degree of anemia a patient experiences is dependent on the ability of the patient's marrow to compensate for the increased RBC destruction.

Etiology and Pathophysiology of Warm Autoantibodies

Warm autoantibodies develop when there is an immunoregulation breakdown, which may be determined by genetic factors. As part of the immunoregulation breakdown, the loss of T-cell regulation combined with overactive B cells leads to the production and emergence of autoantibodies. Several known triggers initiate this process, including viral infections, inflammatory processes, malignancies, and medications. Epstein-Barr virus (EBV) and Mycoplasma pneumoniae infections are commonly associated with cold reacting autoantibody formation. Infections increase the macrophage’s ability to phagocytose antibody-coated RBCs to initiate this process. Chronic inflammatory processes such as systemic lupus erythematosus, biliary cirrhosis, and ulcerative colitis are autoimmune processes that are commonly associated with the formation of warm autoantibodies. Other lymphoproliferative disorders such as Hodgkin lymphoma, chronic lymphocytic leukemia, hairy cell leukemia, and angioimmunoblastic lymphoma are lymphoproliferative processes associated with the production of autoantibodies. Medications through several mechanisms also serve as triggers that enhance immunoregulation breakdown for the production of warm autoantibodies.

The Role of Blood Transfusion

Blood transfusion is a trigger for alloantibody formation but is also capable of stimulating the formation of autoantibodies. This phenomenon was first recognized 60 years ago when RBC autoimmunization was first described in a patient who developed anti-D antibody as well as a severe autoimmune hemolytic anemia due to formation of a warm autoantibody. Autoantibodies have been noted after transfusion and can present in
patients weeks to months later as a delayed transfusion reaction. Two separate studies have looked at the formation of autoantibodies in transfused patients and have found that autoantibody production due to allogeneic transfusion is higher than previously reported and that autoimmunization should be recognized as a complication of allogeneic transfusion.

The pathophysiology of warm autoantibodies results in the destruction of RBCs by intravascular, extravascular, or cell-mediated processes. The IgG binds and coats the RBC membrane with or without the presence of complement. The coated RBCs then travel to the organs of the reticuloendothelial system where they are sequestered within the microvasculature allowing for the coated RBC to interact closely with macrophages. The macrophages have membrane receptors for the Fc portion of the IgG antibodies, and the cell may be completely or partially phagocytized. If the RBC is partially phagocytized, the cell membrane is altered and is released back into circulation as a smaller RBC known as a microspherocyte, which has a loss of cellular elasticity, increased osmotic fragility, and a shortened life span (see Figure 1).

**Figure 1.** Pathophysiology of warm autoantibodies. WAIHA indicates warm autoimmune hemolytic anemia.

The quantity and subclass of IgG influences the development and degree of hemolysis as macrophages have receptors specifically for IgG1 and IgG3 subclasses. Removal of RBCs is further accelerated when complement
is also bound to the RBC membrane because the macrophages also have receptors for the complement component, C3b. The IgG and complement receptors act together to enhance the binding of the coated RBC. Removal of these cells occurs primarily within the spleen, although if large amounts of IgG are present on the RBC membrane then the liver can assist with the clearance of these coated RBCs.

**Pretransfusion Testing**

Warm autoantibodies are typically panreactive in the antibody screens and panels as well as in the autocontrol (patient plasma incubated with autologous RBC). Panreactivity precludes identification of underlying alloantibodies. Because this requires further investigation, a polyspecific direct antiglobulin test (DAT) is performed. If the DAT positive, the monospecific IgG and C3d DAT are performed. For warm autoantibodies, the IgG monospecific DAT is positive with or without the presence of C3d. An elution procedure is performed on all samples with a positive IgG DAT to remove IgG antibody coating the RBCs. Elution methods include heat, the Lui freeze-thaw method, acid, or Digitonin acid elution, which dissociate the antibody from the RBC and concentrate it in the remaining suspension (see Figure 2). The eluted antibody suspension is then reacted against a panel of RBCs. If the eluate demonstrates specificity to an antigen, this indicates the presence of an alloantibody that formed as a result of exposure to foreign RBC antigens during transfusion or pregnancy. When the eluate is panreactive, then an autoantibody is the most likely explanation, particularly if the patient has no exposure history.

**Figure 2.** Elution techniques. Ab indicates antibody.
EDUCATIONAL COMMENTARY – WARM AUTOANTIBODIES AND TRANSFUSION (cont.)

Patient samples that are panreactive in the antibody panel and the eluate must have an adsorption procedure performed to rule out the presence of an alloantibody inasmuch as increased reactivity is not always demonstrated with underlying alloantibodies. An autoadsorption may be performed in patients who have not been transfused within the previous three months. The patient’s autologous cells are first pretreated with enzymes or heat to disrupt the bonds between the autoantibodies and RBC membrane so binding sites are exposed. The treated cells are then incubated with the patient’s plasma to adsorb free floating autoantibody. In patients with high titers of autoantibodies, this may require sequential autoadsorptions with a new aliquot of treated autologous cells until the adsorbed plasma is no longer reactive with autologous cells. After this is accomplished, the adsorbed plasma is reacted against a panel of RBCs to detect the presence of an underlying alloantibody.

Autoadsorptions cannot be performed in patients who have been transfused within three months because donor cells are still potentially in circulation. The options are to perform a reticulocyte harvest by gradient centrifugation to isolate autologous RBCs for autoadsorption or perform an allogeneic adsorption. The allogeneic adsorption requires using at least three different donor cells with known phenotypes to differentially perform the adsorption procedure. The alloadsorption can be performed up to three times, but adsorbing >3 times may potentially dilute the plasma and prevent detection of the underlying alloantibody. Because the phenotype of the adsorbing cells is known, adsorbing the plasma with Kidd a (JK^a) antigen-negative cells that are then reactive with a panel cell that is positive for Jk^a antigen is interpreted as an underlying anti-Jk^a alloantibody. The most important consideration when transfusing a patient with warm autoantibodies is to rule out any underlying alloantibodies capable of causing a hemolytic transfusion reaction masked by broadly reactive autoantibodies. Therefore, prior to transfusing a patient with a panreactive autoantibody, an adsorption technique should be performed to rule out an underlying alloantibody.

If an underlying alloantibody is detected, then antigen-negative blood is provided. If the autoantibody demonstrates specificity to one of the Rh, Kell, or Kidd antigen systems and the patient has active hemolysis, then donor blood lacking this antigen is also recommended. In the absence of hemolysis, providing antigen-negative blood is debatable. Also prior to providing antigen-negative blood, the zygosity of the patient must be considered. For example, if the autoantibody demonstrates anti-E specificity but the patient is negative for the high-incidence e antigen, providing this patient with e antigen-positive blood places the patient at risk for developing an alloanti-e antibody, thereby decreasing the rate of compatibility to <1% with the donor population. Providing phenotypically matched blood is another option and should be considered in those patients who are chronically transfused, such as patients with hemoglobinopathies, to decrease the risk of alloimmunization. In patients who are rarely transfused, providing phenotypically matched blood is not required. An autocontrol should be performed with each crossmatch to select allogenic units that are equal or less reactive than the autocontrol. This is commonly referred to as selecting units that are “least incompatible.”
EDUCATIONAL COMMENTARY – WARM AUTOANTIBODIES AND TRANSFUSION (cont.)

Transfusion
Direct communication should occur between the blood bank physician and the patient’s clinician. The blood bank physician must gather the patient’s transfusion and pregnancy history to assess the risk of alloimmunization and the need for transfusion. Conservative transfusion practices must be encouraged with the clinician, and transfusion should be reserved for patients with life-threatening anemia, ongoing bleeding, or underlying comorbidities that place the patients at risk, such as cardiovascular disease or cerebrovascular ischemia. The clinician should be reassured that a thorough investigation for clinically significant underlying alloantibodies has been performed but that the search for compatible blood is futile. The clinician and blood bank physician should together decide to transfuse the patient with the least amount of blood to provide adequate oxygenation after assessing the transfusion risk versus benefit to the patient.

The patient should be slowly transfused while the medical team is available. Vital signs should be monitored closely for signs of hemolysis. In patients without in vivo hemolysis, transfusions are generally well tolerated because transfused RBCs have the same rate of survival as autologous RBCs. Sequelae to transfusion include suppression of erythropoiesis of autologous cells, possible rise in autoantibody titer, and increased risk of hemolysis, as well as increased risk of alloimmunization. The risk of alloimmunization in patients with autoantibodies is 12% to 40% per unit, compared to 2% to 10% in patients without autoantibodies, and the incidence of autoantibodies coexisting with newly detected antibodies is up to 50%. Therefore, a thorough investigation of underlying alloantibodies prior to each transfusion is required, and conservative transfusion practices are strongly encouraged in these high-risk patients.

Summary
Warm autoantibodies are antibodies that react with intrinsic antigens on autologous RBCs at body temperature. Therefore, they are considered clinically significant, but the relationship between detection and pathologic effect is not well defined. The presence of these autoantibodies complicates pretransfusion and compatibility testing by panreactivity that precludes detection of underlying alloantibodies by routine testing. Performing a thorough laboratory investigation to detect the presence of underlying alloantibodies is vital to prevent hemolytic transfusion reactions during or after transfusion of allogenic blood. The decision to transfuse these high-risk patients should be made after establishing communication with the patient’s clinician to weigh the risk versus benefit of transfusion, and should only occur in patients with life-threatening anemia or to improve oxygen-carrying capacity in patients with underlying cardiovascular or cerebrovascular comorbidities. Patients should be slowly transfused while the medical team is available, and vital signs should be carefully monitored for signs and symptoms of a hemolytic transfusion reaction. Transfusion with allogenic blood suppresses the patient’s erythropoiesis of autologous RBCs and increases the risk of alloimmunization, thereby increasing the complexity of serologic testing. In patients without signs and symptoms of hemolytic anemia, transfused allogeneic RBCs have life expectancy similar to that of autologous RBCs.
EDUCATIONAL COMMENTARY – WARM AUTOANTIBODIES AND TRANSFUSION (cont.)

Suggested Reading


Ness PM. How do I encourage clinicians to transfuse mismatched blood to patients with autoimmune hemolytic anemia in urgent situations. Transfusion. 2006;46:1859-1862.

© ASCP 2010