

Autoimmune Hemolytic Anemia and Red Blood Cell Autoantibodies

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• **Autoimmune hemolytic anemia is a rare disorder caused by autoreactive red blood cell (RBC) antibodies that destroy RBCs. Although autoimmune hemolytic anemia is rare, RBC autoantibodies are encountered frequently and can complicate transfusion workups, impede RBC alloantibody identification, delay distribution of compatible units, have variable clinical significance that ranges from benign to life-threatening, and may signal an underlying disease or disorder. In this review, we discuss the common presenting features of RBC autoantibodies, laboratory findings, ancillary studies that help the pathologist investigate the clinical significance of autoantibodies, and how to provide appropriate patient care and consultation for clinical colleagues. Pathologists must be mindful of, and knowledgeable about, this entity because it not only allows for direct clinical management but also can afford an opportunity to preemptively treat an otherwise silent malignancy or disorder.**

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Autoimmune hemolytic anemia (AIHA), caused by autoreactive red blood cell (RBC) antibodies along with clinical and laboratory evidence of hemolysis, is estimated to occur in approximately 1 in 80 000 patients annually.¹ Although AIHA is rare, RBC autoantibodies that bind to RBCs are frequently encountered in the blood bank laboratory. For example, 7.6% of all antibody workups at our institution involve RBC autoantibodies, which is similar to the 7% incidence reported elsewhere.² Although RBC autoantibodies are seen in patients of all ages, a general increase in incidence is observed with age, most dramatically in patients older than 50 years.³ The RBC autoantibodies are generally classified as either *warm RBC autoantibodies*, if optimum reactivity with RBCs occurs at 37°C, or *cold RBC autoantibodies*, if optimum reactivity with RBCs occurs at less than 30°C.

The RBC autoantibodies can have varied clinical importance, ranging from insignificant to life-threatening AIHA. Because RBC autoantibodies impede RBC alloantibody identification and crossmatching of compatible units, timely blood-product distribution can be delayed, which becomes substantiated during urgent RBC transfusion situations, such as bleeding and life-threatening anemia. As transfusion-medicine consultants, pathologists often have to integrate both laboratory and clinical findings and available resources to evaluate the risk of RBC transfusions and to recommend a course of action.

CLINICAL FEATURES

Clinically, AIHA and RBC autoantibodies present with signs and symptoms that reflect the activity and thermal reactivity of the autoantibody. For many patients, the presence of an RBC autoantibody alone may have no clinically apparent sequelae and will be detected only by an irregular antibody screen performed for another reason, such as during a preoperative workup or during testing of blood after donation.

Patients with AIHA associated with cold RBC autoantibodies have symptoms that reflect pathologic antibody characteristics. Cold RBC autoantibodies with broad thermal amplitude can fix complement when patients are exposed to cold temperatures, such as those occurring during winter months at northern latitudes, or when patients undergo cooling during a cardiopulmonary bypass procedure. This phenomena most often occurs in a patient's extremities, resulting in hemolysis and agglutination, and presents clinically as pallor, tachycardia, syncope, hypotension, and dyspnea (secondary to anemia), with red-brown urine and icterus (secondary to hemolysis), and cyanotic extremities (secondary to RBC sludging in the peripheral microvasculature).

Patients with AIHA and warm RBC autoantibodies do not have the spontaneous agglutination of RBCs but can still have significant hemolysis as the RBCs coated with autoantibodies are removed by the reticuloendothelial system, which also presents with signs and symptoms of anemia and hemolysis.

In children, a special type of AIHA and autoantibody occurs, which clinically results in paroxysmal cold hemoglobinuria (PCH), a condition where a sudden presence of hemoglobin in the patient's urine is noted, often associated with a viral illness. This clinical presentation results from the causative biphasic immunoglobulin (Ig) G RBC autoantibody, which is a unique cold-activating G-class immunoglobulin

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that binds to the P antigen on RBCs in low temperatures in the extremities and fixes complement, resulting in hemolysis when RBCs are rewarmed in the patient's core.

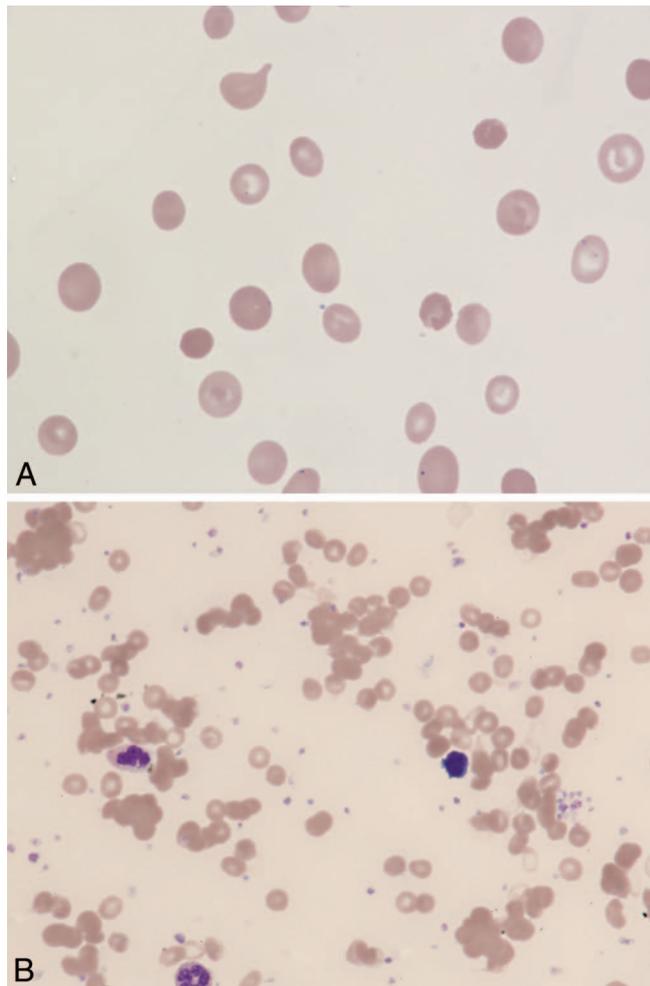
Although nearly one-half of RBC autoantibodies arise in an idiopathic fashion, the remainder present in patients with underlying clinical conditions. For example, cold RBC autoantibodies may arise in patients with bacterial (*Mycoplasma pneumoniae*) and viral (mononucleosis, Epstein-Barr virus) illnesses. Warm RBC autoantibodies have been found more frequently in patients with B-cell lymphomas (particularly chronic lymphocytic leukemia), macroglobulinemias, and autoimmune disorders like systemic lupus erythematosus. In a study categorizing 100 patients with warm autoantibodies, the most frequently associated clinical conditions were hematologic malignancies and autoimmune disorders followed by cardiac disease and non-hematologic malignancies.²

LABORATORY FINDINGS

Screening for RBC autoantibodies is performed with the direct antiglobulin test (DAT). The DAT is often performed initially with a polyspecific antihuman globulin (AHG) with reactivity for cells coated with sufficient immunoglobulins or complement. If that test is positive with the polyspecific reagent, then the patient's RBCs can be tested with monospecific anti-IgG and anti-C3d to elicit the causative agent(s). The DAT reagent is incubated with the patient's RBCs, followed by examination for agglutination. In general, warm RBC autoantibodies result in a positive test when monospecific anti-IgG reagent is used, whereas cold RBC autoantibodies result in a positive test when monospecific anticomplement reagent is used because IgM is able to fix complement. The sensitivity of a positive DAT for RBC autoantibodies can depend on the reagents used as well as the technical expertise of the personnel performing the test.⁴ Rare IgA RBC autoantibodies will not be detected by routine DAT testing.

True RBC autoantibodies have additional serologic characteristics. First, an eluate prepared from the patient's own RBCs should rereact with the patient's RBCs. Plasma or serum from patients with a cold RBC autoantibody may have specificity for a carbohydrate antigen, such as i or I, and can react more or less strongly with group O panel cells that contain or lack such antigens. Warm RBC autoantibodies tend to have reactivity with common antigens on Rh proteins, and eluates prepared from patient cells containing a warm RBC autoantibody tend to be broadly reactive with all cells except the rare Rh-null cell.

Both warm and cold RBC autoantibodies can cause AIHA, with the level of hemolysis reported to correlate with the strength of the DAT reaction, as well as reactivity with complement.³ Several other laboratory findings can be useful in determining the presence of hemolysis. For example, peripheral tests and complete blood cell counts typically reveal a normochromic, normocytic anemia; polychromasia; and reticulocytosis. The RBC autoantibodies that result in a predominately extravascular hemolysis can cause spherocytosis, predominately composed of microspherocytes with a diameter of 5 μm or less (Figure, A). Cold RBC autoantibodies can cause spontaneous agglutination, observed as clumping of the RBCs on the test (Figure, B). Immune-mediated hemolysis can result in hemoglobinemia, decreased serum haptoglobin, increased serum total and unconjugated (indirect) bilirubin, as well as



A, The peripheral blood test demonstrates features of extravascular hemolysis, including microspherocytes scattered among unremarkable red blood cells. The test also shows features of a normochromic, normocytic anemia with anisocytosis. B, The peripheral blood test shows findings commonly observed in a cold autoantibody including red blood cell agglutination (Wright-Giemsa, original magnifications $\times 100$ [A] and $\times 60$ [B]).

increased lactate dehydrogenase. Urinalysis may reveal hemoglobinuria and urobilinogen. Both clinicians and pathologists should understand that cold autoantibodies have the ability to interfere with hematology-based testing, rendering the only reliable component in a complete blood cell count to be the hemoglobin.

In the blood bank, during screening for irregular RBC antibodies, RBC autoantibodies can cause the autocontrol consisting of the patient's plasma and his or her own RBCs to agglutinate. Cold-reacting IgM RBC autoantibodies cause the autocontrol RBCs and the affected panel cells to agglutinate at room temperature and below when incubated with the patient's plasma or serum. The agglutination usually occurs without the addition of AHG because of the relatively large IgM pentamer, which cross-links with, and agglutinates, RBCs. In some cases, prewarming the patient's specimen will abrogate the autoantibody-induced agglutination, allowing the RBC alloantibodies to be identified so blood can be crossmatched for transfusion. Aside from prewarming the specimens, circumventing an offending cold antibody can be done by using 22% bovine serum

albumin instead of low ionic-strength solutions because some cold RBC autoantibodies are enhanced by low ionic-strength solutions.⁵

Warm-reacting IgG RBC autoantibodies tend to cause all panel and autocontrol RBCs to agglutinate after incubation at 37°C and the addition of AHG. Because all panel cells tend to agglutinate in the presence of warm RBC autoantibodies and AHG, underlying RBC alloantibodies cannot be reliably detected. In addition, RBC units intended for transfusion are not crossmatch-compatible in the presence of AHG because the warm RBC autoantibodies bind to antigens of the donor unit.

Several techniques are used to sequester the interfering autoantibody and thereby to look for the underlying alloantibodies. The optimal means of removing the autoantibody is by adsorption. In this procedure, chloroquine or enzymes are used to remove bound autoantibody from the patient's RBCs, which are then incubated with a different sample of the patient's serum. This causes absorption of the autoantibody onto the patient's treated RBCs, leaving behind alloantibodies for further identification. If the patient has recently undergone transfusion, autologous adsorption cannot be performed, and instead, allogeneic RBCs with known antigen profiles can be used to adsorb the autoantibody. Allogeneic RBCs also adsorb any underlying alloantibodies if the RBCs contain the antigen recognized by the alloantibody. Therefore, only alloantibodies that react with antigens not on the adsorbing RBCs can be detected using this technique.

ANCILLARY STUDIES

Molecular immunohematology, in which the genetic determinants of the common clinically significant antigens of blood groups are probed to predict the patient's RBC phenotype, can help manage the transfusion needs of patients with RBC autoantibodies. By using this method to predict the patient's RBC phenotype, the ability of the patient to generate an RBC alloantibody can be inferred. For example, patients with a predicted Kell protein containing the K antigen would not make an anti-K RBC alloantibody. Similarly, by knowing the patient's predicted phenotype, a physician can sometimes obtain antigen-compatible RBC units for transfusion in less time than required to perform adsorption procedures to find the most compatible blood, a potential benefit when urgent transfusion is needed. Potential pitfalls occur if the patient has an unusual or rare genetic determinant not detected by the molecular assay. However, given the difficulty in phenotyping a patient's RBCs serologically, molecular methods have been suggested to be more accurate.⁶

In suspected cases of PCH, the DAT may be reactive with anti-C3 reagent alone. A Donath-Landsteiner test can be used to detect the presence of the implicated biphasic hemolysin.⁷ Normal human serum for a source of complement is incubated on ice with type O RBCs known to contain the P antigen along with the test serum for 30 minutes. Auto anti-P will bind to the RBCs and fix complement. The specimen is then warmed to 37°C, where the fixed complement will lyse the RBCs, indicated by pink-tinted serum.

DIFFERENTIAL DIAGNOSIS

The clinical differential diagnoses associated with the detection of RBC autoantibodies include determining

whether the autoantibodies are significant, resulting in AIHA. The strength of DAT agglutination can help because increased agglutination and the presence of complement has been suggested to correlate with the hemolysis.² The presence of both hemolysis and RBC autoantibodies does not always mean AIHA, and other causes of hemolysis, such as medications, microangiopathic hemolysis, or parasitemia, need to be considered. Because of the association of RBC autoantibodies with an underlying disorder, a new-onset RBC autoantibody may prompt a clinician to investigate, for example, for the presence of a lymphoproliferative disorder. For cold RBC autoantibodies that cause white, painful ischemia in the digits, the differential diagnosis includes Reynaud phenomenon.

The laboratory differential diagnosis associated with a positive DAT can be challenging, especially given that up to 15% of hospitalized patients may have a positive DAT.⁷ Other causes of a positive DAT include drug effect, IgG sensitization, or a nonspecific binding of the patient's immunoglobulins onto their own cells, and RBC alloantibodies in patients with a history of recent transfusion. An eluate performed on the DAT⁺ RBCs is invaluable for classification because eluted antibodies that do not react with panel RBCs are not likely classic RBC autoantibodies, and newly formed RBC alloantibodies will react in very specific patterns with panel RBCs that contain the implicated antigen.

CURRENT TREATMENT

Definitive therapy for secondary AIHA and RBC autoantibodies is to treat the underlying disorder, such as chemotherapy for the lymphoproliferative disorder, antibiotics for *Mycoplasma pneumoniae*, and corticosteroids for systemic lupus erythematosus, for example. For idiopathic cases of AIHA, corticosteroids are the first line of treatment for most patients. Intravenous immune globulin may also be of some benefit. Splenectomy can also serve as a therapeutic option by removing the major reticuloendothelial reservoir. However, splenectomy incurs possible surgical complications and also a risk of overwhelming sepsis by encapsulated organisms, such as pneumococcus and meningococcus. Finally, immunosuppressive therapy, such as cyclophosphamide and chlorambucil, and monoclonal antibody therapy with rituximab can also be of some benefit.

Simple recommendations for patients with AIHA and cold autoantibodies can include using a blood warmer for transfusions and for the patient to avoid cold environments, where the cold activates the autoantibody and can cause precipitation of the antibody, thus leading to intravascular thrombosis and infarction, most commonly of the digits, ears, and nose. Furthermore, hypothermic procedures, such as cardiopulmonary bypass or neurosurgery, should be avoided until the underlying cause has been treated, and the antibody is no longer present.

Another consideration is to associate the patient's medical record with an alert, signaling an autoantibody has been present in the past. This may alert future technicians and physicians that workup for transfusion will likely take significantly longer than with most patients and, thus, finding compatible units may be more difficult, promoting discussion about the urgency and necessity of transfusion and being proactive, rather than reactive in urgent situations.

In cases of life-threatening anemia in patients with AIHA and/or RBC autoantibodies, emergency transfusion with uncrossmatched blood should be recommended because complete testing to rule out underlying alloantibodies can take hours.⁸ Clinicians should be aware of the increased risk of incompatible blood and the possibility that the RBC autoantibody may also lyse the transfused cells. However, those complications are generally outweighed by the need of the patient for the oxygen-carrying capacity of the transfused cells, and no transfusion should be withheld if a patient's life is dependent on transfusion.

Treatment for acute paroxysmal cold hemoglobinuria is typically supportive, and the anemia often resolves on its own in several weeks.⁹ When acute or chronic PCH results in severely symptomatic anemia, transfusion may be necessary. Although blood products should be compatible, they do not necessarily need to be P-negative (a rare phenotype, which is difficult to acquire in the blood bank). Blood products should be warmed to prevent further hemolysis. Glucocorticoids have also been used to decrease the antibody production and complement activation of red blood cell destruction; however, data supporting their efficacy is sparse in PCH.¹⁰ For both acute and chronic PCH, avoidance of cold temperatures is of great importance.

PROGNOSIS

The prognosis for autoantibodies is related to the ability to treat the underlying condition. For those that respond well to treatment of the precipitating event, the autoantibody should no longer be clinically or pathologically significant. For those autoantibodies that are idiopathic and when primary treatment is unsuccessful, second-line treatments are available with some success, as discussed above.

Ultimately, autoantibodies serve as a source of both clinical and laboratory diagnostic challenge but can also be an opportunity to identify an underlying, undiagnosed condition, such as malignancy. Pathologists must be able to recognize these antibodies, direct transfusion protocols around this complicating factor, and communicate with the treating physician on the implications surrounding the autoantibodies. Such cases afford the pathologist an opportunity not only to directly assist with clinical management but also to have valuable insight into a diagnosis.

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