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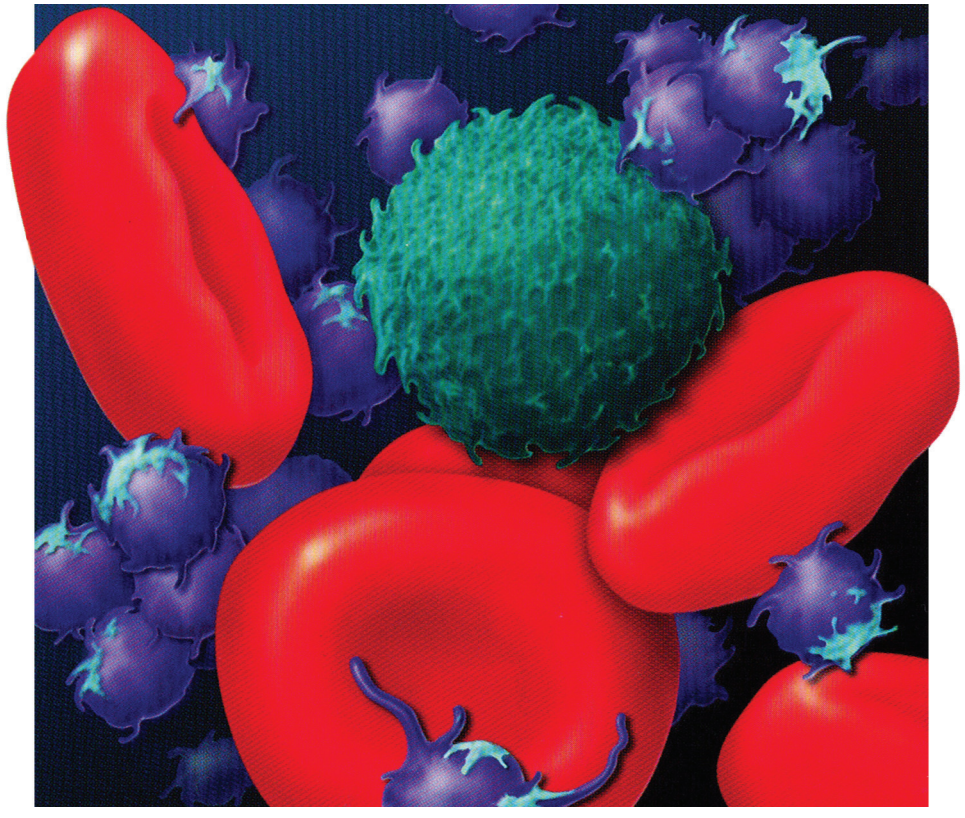

Autoimmune Hemolytic Anemia

HOSPITAL PHYSICIAN®

HEMATOLOGY BOARD REVIEW MANUAL

STATEMENT OF EDITORIAL PURPOSE

The *Hospital Physician Hematology Board Review Manual* is a study guide for fellows and practicing physicians preparing for board examinations in hematology. Each manual reviews a topic essential to the current practice of hematology.

NOTE FROM THE PUBLISHER:
This publication has been developed without involvement of or review by the American Board of Internal Medicine.

Autoimmune Hemolytic Anemia

Contributor:
Thomas G. DeLoughery, MD
Professor of Medicine, Pathology, and Pediatrics, Division of Hematology/Medical Oncology, Departments of Medicine and Pediatrics, and Division of Laboratory Medicine, Department of Pathology, Oregon Health Sciences University, Portland, OR

Table of Contents

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Thomas G. DeLoughery, MD

INTRODUCTION

The autoimmune hemolytic anemias (AIHA) are rare but important hematologic diseases. They can range in severity from mildly symptomatic illness to a rapidly fatal syndrome. The incidence of AIHA is estimated to be between 0.6 and 3 cases per 100,000 persons.[1,2] AIHA is mediated by antibodies, and in the majority of cases immunglobulin (Ig) G is the mediating antibody. This type of AIHA is referred to as "warm" AIHA because IgG antibodies bind best at body temperature. "Cold" AIHA is mediated by IgM antibodies, which bind maximally at temperatures below 37°C. This manual reviews the most common types of AIHA, with emphasis on diagnosis and treatment (**Table 1**).

PATHOGENESIS

In most cases, the ultimate etiology of AIHA is unknown. In warm AIHA, the target epitopes in most cases are Rh proteins.[2] What leads the immune system to target these proteins is unknown, but one theory is that an initial immune response to a foreign antigen starts to cross-react with the Rh proteins and the immune system fails to suppress this autoreactive response, leading to hemolysis. In IgG-mediated (warm) hemolysis, the red cells become coated with IgG molecules, which mark the cells for uptake and destruction by splenic macrophages.[3] In "cold" AIHA, IgM molecules fix complement to the surface of red blood cells. Rarely, this can lead to activation of the full complement cascade, resulting in red cell lysis, but more often it is stopped at the C3 stage, leading to C3-coated red cells which are then taken up by hepatic macrophages.[4]

DIAGNOSIS

SUSPECTING THE DIAGNOSIS

In many patients, it is the symptoms and signs of anemia that lead to suspicion of hemolysis. Older patients often present earlier in the course of the disease due to lack of tolerance of anemia, especially if there is a sudden drop in the red blood cell count. Dark, cola-colored urine resulting from the presence of free hemoglobin may be noted by some patients. Patients with rapid-onset hemolysis may note lumbar back pain, and those with cold agglutinins often note symptoms related to agglutination of red cells in the peripheral circulation, such as the development of acrocyanosis in cold weather.[5] In rare cases, patients will have abdominal pain when eating cold food due to ischemia related to agglutination of red cells in the viscera. Some patients with cold agglutinins can have an exacerbation of their hemolysis with cold exposure.

Unlike patients with immune thrombocytopenia (ITP), those with AIHA may have mild splenomegaly on exam. The presence of enlarged lymph nodes or massive splenomegaly should raise concern about concomitant lymphoma or chronic lymphocytic leukemia (CLL).

MAKING THE DIAGNOSIS

The 2 key steps in diagnosis are (1) demonstrating hemolysis and (2) demonstrating the autoimmune component.

Laboratory Evaluation for Hemolysis

Hemolysis is proven by finding evidence of both red cell breakdown and the compensatory increase in red cell production this stimulates (**Table 2**). The following sections discuss the laboratory tests that are performed to investigate hemolysis.

Lactate dehydrogenase. When red cells undergo hemolysis, they release their contents, which are mostly comprised of hemoglobin but also include lactate dehydrogenase (LDH), an enzyme found in high concentration in red cells. Most patients with hemolysis will have an elevated LDH level, making this a sensitive test. However, because many other processes, including liver disease and pneumonia, also raise the serum LDH level, this finding is not specific for hemolysis.

Serum bilirubin. Hemoglobin is salvaged by haptoglobin, and the heme moiety is broken down first to bilirubin and then to urobilinogen, which is excreted in the urine.[2] Bilirubin produced from the breakdown

Table 1. Types of Autoimmune Hemolytic Anemia

Warm antibody
Primary
Secondary
Systemic lupus erythematosus
Chronic lymphocytic leukemia
Cold antibody
Post-infectious
Mycoplasma, Epstein-Barr virus, cytomegalovirus
Associated with lymphoproliferative disease
Paroxysmal cold hemoglobinuria
Drug-induced

Table 2. Tests for Autoimmune Hemolytic Anemia (AIHA) and Expected Findings in Hemolysis

Test	Expected Result in AIHA
Direct antiglobulin test	Positive
	IgG ± C3 in warm-antibody–mediated AIHA
	C3 in cold-antibody–mediated AIHA
Haptoglobin	Decreased
Indirect bilirubin	Increased
Lactate dehydrogenase	Increased
Reticulocyte count	Increased
Urine hemosiderin	Present

of heme is not conjugated, but rather is delivered to the liver, where it is conjugated and excreted into the bile. In hemolysis, the concentration of *unconjugated* bilirubin (indirect bilirubin) is increased, while in liver disease the level of *conjugated* bilirubin (direct bilirubin) is increased. However, if the patient has concomitant liver disease with an increased direct bilirubin level, the serum bilirubin test is not reliable.

Serum haptoglobin. Haptoglobin binds free serum hemoglobin and is taken up by the liver. Haptoglobin usually falls to very low levels in hemolysis. A confounder is that haptoglobin is an acute phase reactant and can rise with systemic disease or inflammation. However, patients with advanced liver disease will have low haptoglobin levels due to lack of synthesis, and up to 2% of the population may congenitally lack haptoglobin.[1]

Serum hemoglobin. If the hemolysis is very rapid, the amount of free hemoglobin released will overwhelm the binding capacity of haptoglobin and lead to free hemoglobin in the plasma. This can be crudely quantified by examining the plasma color. Even minute amounts of free hemoglobin will turn the plasma pink. In fulminant hemolysis, the plasma will be cola-colored.

Reticulocyte count. In most patients with hemolysis, the destruction of red cells is accompanied by an increase in the reticulocyte count. Reticulocytes are red cells that still contain RNA and are a marker of red cells that are approximately 24 hours old or less. Traditionally, reticulocytes were measured manually by staining the blood smear with vital blue and counting the percentage of cells that absorb the stain; this percentage needs to be adjusted for the hematocrit. Usually a percentage above 1.5% is considered indicative of an elevated reticulocyte count. Recently, automated complete blood count machines have taken advantage of the fact that reticulocytes will absorb certain stains; these machines can directly measure the reticulocyte count via flow cytometry, which results in an "absolute" reticulocyte count. The reticulocyte count obtained using this method does not have to be corrected for hematocrit, and levels of approximately 90,000/μL are considered raised. However, the reticulocyte count can also be raised in blood loss or in patients who have other causes of anemia (eg, iron deficiency) under treatment. In addition, as many as 25% of patients with AIHA will never have raised counts for various reasons, such as nutritional deficiency, autoimmune destruction of red cell precursors, or lack of erythropoietin.

Blood smear. The blood smear provides vital information. The hallmark laboratory parameter of AIHA is spherocytes seen on the blood smear. In AIHA, antibodies and/or complement attach to the red cells, and when the antibodies or complement are taken up by macrophages in the spleen some of the red blood cell membrane is removed as well, decreasing the surface area of the cell. As the surface area of the red cell decreases with each pass through the spleen, the cell's shape changes from a bioconcave disk to a sphere before the cell is destroyed, reflecting the fact that a sphere has the smallest surface area for a given volume. The vast majority of patients with AIHA will have spherocytes on the blood smear. However, spherocytes are not specific to AIHA, as they can be seen in hereditary spherocytosis, Wilson's disease, clostridial sepsis, and severe burns.

Patients with cold agglutinins will often have red cell agglutination on the blood smear. In addition, patients with AIHA will often have a raised mean corpuscular volume (MCV) for 2 reasons. In patients with brisk reticulocytosis, the MCV will be raised due to the large size of the reticulocyte. In patients with cold agglutinin disease, the MCV may be falsely raised due to clumping of the red blood cells.

Urinary hemosiderin. When hemoglobin is excreted by the kidney, the iron is deposited in the tubules. When the tubule cells are sloughed off, they appear in the urine. The urine can be stained for iron, and a positive result is another sign of hemolysis. Hemosiderinuria is a later sign of hemolysis, as it takes 1 week for iron-laden tubule cells to be excreted in sufficient quantities to be detected in the urine.

Urinary hemoglobin. One other sign of hemolysis is the presence of hemoglobin in the urine. A quick way to demonstrate hemoglobinuria is to check the urine with a dipstick followed by a microscopic exam. In hemolysis, the dipstick will detect "blood," while the microscopic exam will be negative for red cells.

Laboratory Evaluation for Autoimmune Component

The autoimmune component is shown by demonstrating the presence of either IgG molecules or complement on the surface of red blood cells.[4,6] This can be done by performing the direct antiglobulin test (DAT) or Coombs test. IgG bound to red cells will not agglutinate them, but if IgM that is directed against IgG or C3 is added, the red cells will agglutinate, proving that there is IgG and/or C3 on the red cell membrane. The finding of a positive DAT in the setting of a hemolytic anemia is diagnostic of AIHA. Beware of individuals with concomitant weak positive DAT and other causes of hemolysis. The strength of the DAT result and the degree of hemolysis must match in order to conclude that the hemolysis is immune-mediated.

There are several pitfalls to the DAT. One is that a positive DAT is found in 1:1000 patients in the normal population and in up to several percent of ill patients, especially those with elevated gamma globulin, such as patients with liver disease or HIV infection.[6] Administration of intravenous immunoglobulin (IVIG) can also create a positive DAT. Conversely, patients can have AIHA with a negative DAT.[7–9] For some patients, the number of IgG molecules bound to the red cell is below the detection limit of the DAT reagents. Other patients can have IgA or "warm" IgM as the cause of the AIHA.[10] Specialty laboratories can test for these possibilities. The diagnosis of DAT-negative AIHA should be made with caution, and other causes of hemolysis, such as hereditary spherocytosis or paroxysmal hematuria, should be excluded.

TRANSFUSION THERAPY

Performing transfusions can be very difficult in patients with AIHA.[2] The presence of the autoantibody can interfere with typing of the blood and almost always interferes with the crossmatch since this final step consists of mixing the patient's serum or plasma with donor red cells. In most patients with AIHA, the autoantibodies will react with any donor cells, rendering a negative crossmatch impossible. Without the crossmatch, the concern is that underlying alloantibodies can be missed. Studies indicate that 15% to 32% of patients will have underlying alloantibodies, which can lead to transfusion reactions.[2] However, there are 2 considerations that may mitigate these concerns.[11,12] First, patients who have never been transfused or pregnant will rarely have alloantibodies. Second, a patient who has been transfused in the remote past may have an anamnestic antibody response but not an immediate hemolytic reaction.

The transfusion service can take several steps to identify alloantibodies. Occasionally, if the autoantibody is weakly reacting when the patient's serum is tested against a panel of reagent red cells, the alloantibodies can be identified by their stronger reactions as compared with the weakly reactive autoantibody. The most common technique for identifying alloantibodies is the autoadsorption technique.[4,13] This involves incubating the patient's red cells with the patient's serum to adsorb the autoantibody. After a period of incubation, the cells are pelleted and the serum is collected as the supernatant. The adsorbed serum may be incubated with another sample of the patient's cells for a second adsorption if the initial agglutination reactions of the patient's serum with the reagent cells were strong. After 1 to 3 adsorptions, the adsorbed serum is tested with a red cell panel in order to check for "leftover" alloantibodies.

When a patient is first suspected of having AIHA, a generous sample of blood should be given to the transfusion service to allow for adequate testing. Many centers will test the blood not only for blood groups ABO and D but also perform full Rh typing plus check for Kidd, Duffy and Kell status.[14] This can allow transfusion of phenotypically matched red blood cells to lessen the risk of alloantibody formation.

One difficult issue is timing of transfusion. Clinicians are often hesitant to transfuse patients with AIHA due to fear of reactions, but in patients with severe anemia, especially elderly patients or those with heart disease, transfusion can be lifesaving. Since in some cases it may take hours to screen for alloantibodies, it is often preferable to transfuse patients with severe anemia and observe carefully for reaction.

WARM AUTOIMMUNE HEMOLYTIC ANEMIA

In AIHA, hemolysis is mediated by antibodies that bind to the surface of red blood cells. AIHA in which IgG antibodies are the offending antibodies is referred to as *warm AIHA*. "Warm" refers to the fact that the antibody binds best at body temperature (37°C). In warm AIHA, testing will show IgG molecules attached to the surface of the red cells, with 50% of patients also showing C3. Between 50% and 90% of AIHA cases are due to warm antibodies.[14,15] The incidence of warm AIHA varies by series but is approximately 1 case per 100,000 patients per year; this form of hemolysis affects women more frequently than men.[1,2]

The goal of therapy in warm AIHA can be hard to define. However, most would agree that a hematocrit above 30% (or higher to prevent symptoms) with a minimal increase in the reticulocyte count—reflective of a significantly slowed hemolytic process—is a reasonable goal. Initial management of warm AIHA is prednisone at a standard dose of 1 mg/kg daily (**Table 3**).[16] Patients should be also started on proton-pump inhibitors to prevent ulcers. It can take up to 3 weeks for patients to respond to prednisone therapy. Once the patient's hematocrit is above 30%, the prednisone is slowly tapered. Although approximately 80% of patients will respond to steroids, only 30% can be fully tapered off steroids. For patients who can be maintained on a daily steroid dose of 10 mg or less, steroids may be the most reasonable long-term therapy. In addition, because active hemolysis leads to an increased demand for folic acid, patients with warm AIHA are often prescribed folic acid 1 mg daily to prevent megaloblastic anemia due to folic acid deficiency.

For patients who cannot be weaned from steroids or in whom steroid therapy fails, there is no standard therapy. Currently, the 2 main choices are splenectomy or rituximab (anti-CD20) therapy. Splenectomy is the classic therapy for warm AIHA. Reported response rates in the literature range from 50% to 80%, with 50% to 60% remaining in remission.[17–20] Timing of the procedure is a balance between allowing time for the steroids to work and the risk of toxicity of steroids. In a patient who has low presurgical risk and has either refractory disease or cannot be weaned from high doses of steroids, splenectomy should be done sooner. Splenectomy can be delayed or other therapy tried first in patients who require lower doses of steroids or have medical risk factors for surgery. Most splenectomies are performed via laparoscopy. The small incisions allow for quicker healing, and the laparoscopic approach provides better visualization of the abdomen to find and remove accessory spleens. When splenectomy is performed by experienced surgeons, the mortality rate is low (<0.5%).[21]

Table 3. Treatment of Warm Autoimmune Hemolytic Anemia

First line
Prednisone 1 mg/kg/day
Folic acid 1 mg/day
Second line
Rituximab 375 mg/m^2 weekly for 4 weeks
or
Splenectomy
Third line
Azathioprine 125 mg/day
Cyclophosphamide 1 g/m^2 IV every 28 days
Mycophenolate 500–100 mg twice daily
Cyclosporine
Danazol 200 mg 4 times daily
Alemtuzumab

The most concerning complication of splenectomy is overwhelming post-splenectomy infection (OPSI).[22] In adults, the spleen appears to play a minimal role in immunity except for protecting against certain encapsulated organisms. Splenectomized patients infected with an encapsulated organism (eg, *Pneumococcus*) will develop overwhelming sepsis within hours. These patients will often have disseminated intravascular coagulation and will rapidly progress to purpura fulminans. Approximately 40% to 50% of patients will die of sepsis even when the infection is detected early. The overall lifetime risk of sepsis may be as high as 1:500. The organism that most commonly causes sepsis in splenectomized patients is *Streptococcus pneumoniae*, reported in over 50% of cases. *Neisseria meningitidis* and *Haemophilus influenzae* have also been implicated in many cases.[23] Overwhelming sepsis after dog bites has been reported due to *Capnocytophaga canimorsus* infections. Patients are also at increased risk of developing severe malaria and severe babesiosis.[22]

Patients who have undergone splenectomy need to be warned about the risk of OPSI and instructed to report to the emergency department readily if they develop a fever greater than 101°F (38.3°C) or shaking chills. Once in the emergency department, blood cultures should be obtained rapidly and the patient

Table 4. Recommendations to Prevent Post-Splenectomy Sepsis

Pneumococcal vaccination
Administer 7- or 13-valent vaccine at least 2 weeks before splenectomy, and then administer 23-valent vaccine 2 months later
Administer 23-valent vaccine in 5 years, and then every 5 to 10 years depending on titers
Meningococcal vaccination
Administer vaccine at least 2 weeks before splenectomy and then boosters every 5 years
Haemophilus influenzae
Administer vaccine 2 weeks before splenectomy

Note: If rituximab therapy is being considered before splenectomy, need to vaccinate either before rituximab or wait at least 6 months after therapy.

Table is based on information from Rodeghiero and Ruggeri.[22]

started on antibiotic coverage with vancomycin and ceftriaxone (or levofloxacin if allergic to beta-lactams).[24] For patients in remote areas, some physicians will prescribe prophylactic antibiotics to take while they are traveling to a health care provider or even recommend a "standby" antibiotic dose to take while traveling to health care.[2] This usually consists of amoxicillin or a macrolide for penicillin-allergic patients.

Patients in whom splenectomy is being planned or considered should be vaccinated for pneumococcal, meningococcal, and influenza infections (**Table 4**).[22] If there is a plan to treat with rituximab, patients should first be vaccinated since they will not be able to mount an immune response after being treated with rituximab.

Rituximab therapy is the other option for patients who do not achieve remission with steroid therapy.[2,25] Most data for rituximab are case reports and case series, but there appears to be a response rate in the 50% to 80% range, with 50% of these being complete responses. These responses appear to be durable, but as in ITP, repeat treatment is effective. An important consideration is that most patients respond gradually to rituximab over months, so a rapid response should not be expected. Most studies have used the traditional dosing of 375 mg/m^2 weekly for 4 weeks. A study that evaluated a smaller rituximab dose of 100 mg weekly for 4 weeks reported initial response rates of 100% and 2-year response rates of 80%, but further study is needed for this novel dosing.[26]

The major side effects of rituximab are infusion reactions, which are often worse with the first dose. These reactions can be controlled with antihistamines, steroids, and, for severe rigors, meperidine. Rarely, patients can develop neutropenia (approximately 1:500) that appears to be autoimmune in nature. Infections appear to be only minimally increased with the use of rituximab.[27] One group at risk is chronic carriers of hepatitis B virus, who may experience a reactivation of the virus that can be fatal. Thus, patients being considered for rituximab need to be screened for hepatitis B virus carrier state. Patients receiving rituximab are at very slight risk for progressive multifocal leukoencephalopathy, which is more common in cancer patients and heavily immunosuppressed patients. The overall risk is unknown but is less than 1:50,000.

The therapeutic options for patients who do not respond to either splenectomy or rituximab are much less certain.[2,16] Although intravenous immune globulin is a standard therapy for ITP, response rates are low in warm AIHA.[21] Numerous therapies have been reported in small series, but no clear approach has emerged. Options include azathioprine, cyclophosphamide, mycophenolate, cyclosporine, danazol, and alemtuzumab. Our approach has been to use mycophenolate for patients requiring high doses of steroids or transfusions. Patients who respond to lower doses of steroids may be good candidates for danazol to help wean them off steroids.

Warm AIHA can complicate several diseases. Patients with systemic lupus erythematosus (SLE) can develop warm AIHA as part of their disease complex. The initial treatment approach is the same, but data suggest that splenectomy may not be as effective.[18,21] Also, many SLE patients have complex medical conditions, making surgeries riskier. For SLE patients who are refractory or cannot be weaned from steroids, rituximab may be the better choice.

Of the malignances associated with AIHA, CLL has the strongest association.[1,28] Series show that 5% to 10% of patients with CLL will have warm AIHA. AIHA can appear concurrent with CLL or develop during the course of the disease. The introduction of purine analogs such as fludarabine led to a dramatic increase

in the incidence of warm AIHA in treated patients.[29] It is speculated that these powerful agents reduce the number and effectiveness of T cells that hold in check the autoantibody response, leading to warm AIHA.[30] However, when these purine analogs are used in combination with agents such as cyclophosphamide or rituximab (with their immunosuppressive effects), the rates of warm AIHA have been lower. [28]

The approach to patients with CLL and warm AIHA depends on the state of their CLL.[28] For patients who have low-stage CLL that does not need treatment, the standard approach to warm AIHA should be steroids, splenectomy, and rituximab.[29] For patients with higher-stage CLL, the treatment for the leukemia will often provide therapy for the warm AIHA. The combination of rituximab-cyclophosphamide-dexamethasone has been reported to be effective for both the AIHA and CLL components.[31]

A rare but important variant of warm AIHA is Evans syndrome.[32] This is the combination of AIHA and ITP. Approximately 1% to 3% of AIHA cases are the Evans variant. The ITP can precede, be concurrent with, or develop after the AIHA. The diagnosis of Evans syndrome should raise concern for underlying disorders. In young adults, immunodeficiency disorders such as autoimmune lymphoproliferative disease (ALPS) need to be considered. In older patients, Evans syndrome is often associated with T cell lymphomas. The sparse literature on Evans syndrome suggests that it can be more refractory to standard therapy, with response rates to splenectomy in the 50% range.[32,33] In patients with lymphoma, antineoplastic therapy is crucial. There is increasing data showing that mycophenolate may be effective for patients with ALPS in whom splenectomy or rituximab therapy is unsuccessful.

In rare patients with warm AIHA, IgA or IgM is the implicated antibody. The literature suggests that patients with IgA AIHA may have more severe hemolysis.[10] Patients with IgM AIHA often have a severe course with a fatal outcome.[34] In such cases, the patient's plasma may show spontaneous hemolysis and agglutination. The DAT may not be strongly positive or may show C3 reactivity only. The clinical clues are C3 reactivity with no cold agglutinins and severe hemolysis, sometimes with an intravascular component. Treatment is the same as for warm AIHA, including the use of rituximab.[35]

Table 5. Treatment of Cold Autoimmune Hemolytic Anemia

First line
Rituximab 375 mg/m^2 weekly for 4 weeks
Folic acid 1 mg/day
Keep patients and infused products warm
Second line
Rituximab 375 mg/m^2 days 1, 29, 57, 85 *with*
Fludarabine 40 mg/m^2 orally days 1–5, 29–34, 57–61, and 85–89
Third line
Bortezomib
Eculizumab

COLD AUTOIMMUNE HEMOLYTIC ANEMIA

In cold AIHA, the hemolysis is mediated by IgM antibodies directed against red cells.[4] As discussed earlier, the term "cold" refers to the fact that the antibody binds maximally at temperatures below 37°C. The most efficient temperature for binding is called the "thermal amplitude," and, in theory, the higher the thermal amplitude, the more virulent the antibody. An antibody titer can be calculated at each reaction temperature from 4°C to 37°C by serial dilutions of the patient's serum prior to incubating with reagent red cells. Rarely, the IgM can fix complement rapidly, leading to intravascular hemolysis. In most patients, complement is fixed through C3, and the C3-coated red cells are taken up by macrophages in the mononuclear phagocyte system, primarily in the liver.[15]

The DAT in patients with cold AIHA will show cells coated with C3. The blood smear will often show agglutination of the blood, and if the blood cools before being analyzed, the agglutination will interfere with the analysis. Titers of cold agglutinin can range from 1:1000 to over 1 million. The IgM autoantibodies are most often directed against the I/i antigens on the red blood cell membrane, with 90% against I antigen.[4] The I antigen specificity is typical with primary cold agglutinin disease and after *Mycoplasma* infection. The i antigen specificity is most typical of Epstein-Barr virus and cytomegalovirus infections in children. In young patients, cold AIHA often occurs following an infection, including viral and *Mycoplasma* infections, and the course is self-limited.[4,36] The hemolysis usually starts 2 to 3 weeks after the illness and will last for 4 to 6 weeks. In older patients, the etiology in over 90% of cases is a B-cell lymphoproliferative disorder, usually with monoclonal kappa B-cells.[5] The most common disorders are marginal zone lymphoma, small lymphocytic lymphoma, and lymphoplasmacytic lymphoma.[15]

It is important to diagnose cold AIHA because the standard therapy for warm AIHA (steroids) is ineffective in cold AIHA. Because C3-coated red cells are taken up primarily in the liver, removing the spleen is also an ineffective therapy. Simple measures to help with cold AIHA should be employed.[5] Patients should be kept in a warm environment and should try to avoid the cold. If transfusions are needed, they should be infused via blood warmers to prevent hemolysis. In rare patients with severe hemolysis, therapeutic plasma exchange (TPE) can be considered.[37] Given that the culprit antibody is IgM—mostly intravascular—use of TPE may slow the hemolysis to give time for other therapies to take effect.

Treatment of cold AIHA remains difficult (**Table 5**). Because most patients with primary AIHA have underlying lymphoproliferative diseases, chlorambucil has been used in the past to treat cold AIHA. However, responses were rare and the drug could worsen the anemia.[37] Currently, the drug of choice is rituximab. Response is seen in 45% to 75% of patients, but is almost always a partial response and retreatment is often necessary.[5,21,26] As with other autoimmune hematologic diseases, there can be a delay in response that ranges from 2 weeks to 4 months (median time, 1.5 months).[5] Given the lack of robust response (complete and durable) with rituximab, the Berentsen group has explored adding fludarabine to rituximab.[38] In a prospective trial, the overall response rate was 76%, with a 21% complete-response rate, but there was also a 41% incidence of grade 3/4 hematologic toxicity. Although more toxic, this combination can be considered in patients with aggressive disease. A single case report of the use of bortezomib showed a good response.[39] A novel approach presented in 1 case report was the use of the C5 complement inhibitor eculizumab to halt the hemolysis, but further study of this agent is also required.[36,40]

Since most patients with cold AIHA are older, a frequent issue that must be considered is cardiac surgery. The concern is that the hypothermia involved with most heart bypass procedures will lead to agglutination and hemolysis. The development of normothermic bypass has expanded treatment options. A recommended approach in patients who have known cold agglutinins is to measure the thermal amplitude of the antibody preoperatively. If the thermal amplitude is above 18°C, normothermic bypass should be done, if feasible.[41] If not feasible, preoperative TPE should be considered.

A unique cold AIHA is paroxysmal cold hemoglobinuria (PCH).[15] This cold hemolytic syndrome most often occurs in children following a viral infection, but in the past it complicated any stage of syphilis.[42] The mediating antibody in PCH is an IgG antibody directed against the P antigen on the red blood cell. This antibody binds best at temperatures below 37°C, fixing complement at cold temperatures, but then activates the complement cascade at body temperature.[6] Because this antibody can fix complement, hemolysis can be rapid and severe, leading to extreme anemia. The DAT is often weakly positive but can be negative. The diagnostic test for PCH is the Donath–Landsteiner test. This complex test is performed by incubating 3 blood samples, one at 0° to 4°C, another at 37°C, and a third at 0° to 4°C and then at 37°C. The diagnosis of PCH is made if only the third tube shows hemolysis.[4] PCH can persist for 1 to 3 months but is almost always self-limiting. In severe case, steroids may be of benefit.

DRUG-INDUCED HEMOLYTIC ANEMIA

AIHA caused by a drug reaction is rare, with a lower incidence than drug-related ITP. The rate of severe drug-related AIHA is estimated at 1:1,000,000, but less severe cases may be missed.[43] Most patients will have a positive DAT without signs of hemolysis, but in rare cases patients will have relentless hemolysis resulting in death.

Multiple mechanisms for drug-induced immune hemolysis have been proposed, including drug-absorption (hapten-induced) and immune complex mechanisms.[43,44] The hapten mechanism is most often associated with the use of high-dose penicillin.[45] High doses of penicillin lead to incorporation of the drug into the red cell membrane by binding to proteins. Patients will manifest a positive DAT with IgG antibody but not complement.[46] The patient's plasma will be reactive only with penicillin-coated red cells and not with normal cells. As mentioned, very few patients will have hemolysis, and if they have hemolysis, it will resolve in a few days after discontinuation of the offending drug.[47]

Binding of a drug-antibody complex to the red cell membrane may cause hemolysis via the immune complex mechanism.[47] Again, most often the patient will have just a positive DAT, but rarely patients will have life-threatening hemolysis upon exposure or reexposure to the drug. The onset of hemolysis is rapid, with signs of acute illness and intravascular hemolysis. The paradigm drug is quinine, but many other drugs have been implicated. Testing shows a positive Coombs test

with anti-complement but not anti-IgG.[46] This pattern is due to the effectiveness of the tertiary complex at fixing complement. The patient's plasma reacts with red cells only when the drug is added.

A form of immune complex hemolysis associated with both disseminated intravascular coagulation (DIC) and brisk hemolysis has been recognized. Patients who receive certain second- and third-generation cephalosporins (especially cefotetan and ceftriaxone[48]) have developed this syndrome.[45,49–53] The clinical symptoms start 7 to 10 days after the drug is administered; often the patient has only received the antibiotic for surgical prophylaxis. Immune hemolysis with acute hematocrit drop, hypotension, and DIC ensues. The patients are often believed to have sepsis and are often reexposed to the cephalosporin, resulting in worsening of the clinical status. The outcome is often fatal due to massive hemolysis and thrombosis.[50,54–56]

Finally, 8% to 36% of patients taking methyldopa will develop a positive DAT after 6 months of therapy, with less than 1% showing hemolysis.[47,57] The hemolysis in these patients is indistinguishable from warm AIHA, consistent with the notion that methyldopa induces an autoimmune hemolytic anemia. The hemolysis often resolves rapidly after stopping the methyldopa, but the Coombs test may remain positive for months.[57] This type of drug-induced hemolytic anemia has been reported with levodopa, procainamide, and chlorpromazine, but fludarabine is the most common cause currently.

In many patients, the first clue to the presence of drug AIHA is the finding of a positive DAT. Rarely, patients will have severe hemolysis, but in many patients the hemolytic process is mild and may be wrongly assumed to be part of the underlying illness. Finding the offending drug can be a challenge, unless a patient has recently started one new drug; in a hospitalized patient on multiple agents, identifying the problem drug is difficult. Patients recently started on "suspect drugs," especially the most common agents cefotetan, ceftriaxone, and piperacillin, should have these agents stopped (**Table 6**).[43,44,58] Specialty laboratories such as the Blood Center of Wisconsin or the Los Angeles Red Cross can perform in vitro studies of drug interactions that can confirm the clinical diagnosis of drug-induced AIHA.

Therapy for patients with positive DAT without signs of hemolysis is uncertain. If the drug is essential, then the patient can be observed. If the patient has hemolysis, the drug needs to be stopped and the patient observed for signs of end-organ damage. It is doubtful that steroids or other autoimmune-directed therapy is effective. For patients with the DIC-hemolysis sydrome, there are anecdotal reports that TPE may be helpful.[43]

Table 6. Drugs Implicated in Autoimmune Hemolytic Anemia

Most Common
Cefotetan
Ceftriaxone
Piperacillin
Fludarabine
Other Implicated Drugs
Acetaminophen
Amphotericin B
Beta-lactams (any)
Carboplatin
Cephalosporins (any)
Chlorpromazine
Chlorpropamide
Cimetidine
Erythromycin
Isoniazid
Levodopa
Melphalan
Methyldopa
Nonsteroidal anti-inflammatory agents
Omeprazole
Oxaliplatin
Probenecid
Procainamide
Quinidine
Quinine
Rifampin
Sulfa drugs
Teniposide
Tetracycline

Table is based on information from Garratty,[43] Pierce and Nester,[44] and DeLoughery.[58]

SUMMARY

AIHA can range from an abnormal laboratory test (positive DAT and signs of hemolysis) to an acute, life-threatening illness. Treatment is guided by the laboratory work-up and evaluation of the patient's clinical status. While rituximab is promising for many patients, the lack of robust clinical trials hinders the treatment of patients who fail standard therapies.

BOARD REVIEW QUESTIONS

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