Immune Hemolytic Anemia

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INTRODUCTION

Hemolytic anemias are a diverse group of clinical disorders characterized by decreased survival of erythrocytes in the circulation. Because of their multiple causes, hemolytic anemias are often difficult for hematologists, as well as internists and primary care physicians, to diagnose. This clinical confusion can be lessened somewhat by following a logical, structured approach to diagnosis in patients suspected of having a hemolytic anemia.

First, it must be determined that hemolysis is actually present and that the anemia is not caused instead by bleeding or bone marrow failure. The laboratory and other tests most useful in determining whether or not hemolysis exists are listed in Table 1. Next, the most common causes of hemolysis (ie, acquired autoimmune hemolytic anemia, hypersplenism, congenital hemoglobinopathies) should be ruled in or out, with the specific cause identified, if possible. If a common cause is not easily ascertained, a “checklist” approach to the diagnosis of hemolytic anemia can be pursued. The numerous specific causes of hemolysis for the most part fall into 1 of 4 general categories: (1) processes outside the erythrocyte, (2) alterations of the erythrocytic membrane, (3) abnormalities in the hemoglobin molecule, or (4) decreased levels of an enzyme in erythrocytes (Table 2).1 When evidence of one of the disorders that commonly cause hemolytic anemia cannot be found, this checklist is useful as a guide to other problems to consider in making the diagnosis.

CLINICAL PRESENTATION

INITIAL EVALUATION OF CASE PATIENT

A 34-year-old man is seen in the emergency department reporting shortness of breath with exertion. He says that he has been unable to climb the stairs to his third-floor apartment during the past 2 weeks without stopping several times; previously, he could climb to the third floor very easily. Medical history is unremarkable except for arthroscopic knee surgery performed 2 years ago following a skiing injury. He takes no medicine other than an occasional nonsteroidal anti-inflammatory drug to treat headaches and knee pain. Family history reveals that his mother was treated for breast cancer 6 years ago; his father and 2 siblings are in good health. The patient does not smoke or use illicit drugs but does drink 2 to 3 beers each week.

Physical examination reveals a pale man in no distress at rest. Blood pressure is 140/76 mm Hg, pulse is 124 bpm, and respiratory rate is 22 breaths/min. He is afebrile. Nail beds and conjunctivae are pale; the sclerae
are icteric. Auscultation of the heart reveals a grade II/VI systolic ejection murmur at the apex; lungs are clear to auscultation. The liver and spleen are not palpable. Petechiae, bruises, adenopathy, and edema are not present, and there is no tenderness over the liver.

Results of laboratory testing show a hematocrit of 16%, a leukocyte count of $7.8 \times 10^3$/mm$^3$ (with a normal differential), and a platelet count of $417 \times 10^3$/mm$^3$. Reticulocyte count is 9.3% of erythrocytes. Total serum bilirubin level is 7.2 mg/dL. Fractionation of the bilirubin reveals a direct bilirubin level of 0.6 mg/dL, giving a calculated indirect bilirubin level of 6.6 mg/dL. Blood urea nitrogen level is within normal limits, as are serum levels of electrolytes, creatinine, aspartate aminotransferase (AST, SGOT), and alkaline phosphatase. Serum lactate dehydrogenase (LDH) level is 2900 U/L (normal, 200 to 600 U/L). Serum haptoglobin level is within normal limits. A peripheral blood smear reveals polychromatophilia and spherocytes.

**DETERMINING IF AN ANEMIA IS HEMOLYTIC**

- **What role do reticulocyte count, bilirubin level, and other laboratory measurements play in determining whether an anemia is hemolytic?**
- **How does a peripheral blood smear help in the diagnosis of hemolytic anemia?**

Diagnosis of any anemia involves examining a peripheral blood smear and obtaining a reticulocyte count. In general, when the reticulocyte count is elevated (ie, greater than 1.5%), hemolytic anemia should be suspected. However, an elevated reticulocyte count is not specific for diagnosing hemolytic anemia but can occur, for example, after an episode of acute bleeding or after initial treatment of iron, folate, or vitamin B$_12$ (cyanocobalamin) deficiency. Even the rebound erythrocyte production following a normal menstrual period can be associated with an elevated reticulocyte count in the range of 2% to 4%. Nevertheless, a reticulocyte count greater than 5% strongly suggests the presence of a hemolytic process.

An elevated reticulocyte count also is not totally sensitive for diagnosing hemolytic anemia. Approximately 10% of patients with hereditary spherocytosis and up to 50% of patients with thalassemia minor might have reticulocyte counts within the normal range. The more severe the hemolytic anemia, however, the more likely it is that the reticulocyte count will be elevated; patients with hemolytic anemia and reticulocyte counts within normal limits generally have minimal anemia.

Although the reticulocyte count is reported as a percentage of total erythrocytes, the reticulocyte number often is more informative. The normal reticulocyte number is 60–90 $\times 10^3$/mm$^3$. In a patient with a hematocrit of 20% and an erythrocyte count of $2 \times 10^6$/mm$^3$, an elevated reticulocyte count of 3% represents a reticulocyte number of only 60 $\times 10^3$/mm$^3$. In cases of clinically significant hemolytic anemia, a reticulocyte number greater than 150 $\times 10^3$/mm$^3$ is expected. The reticulocyte index (which adjusts for the fact that reticulocytes spend more time in the circulation when there is marked hemolysis and a very high reticulocyte count) adds little to the determination of whether anemia is or is not hemolytic.

The serum bilirubin level and bilirubin fractionation value are helpful in establishing that hemolytic anemia is present. Bilirubin is a breakdown product of erythrocytes, and its level will be elevated when these cells break down in the bone marrow (as in the ineffective erythropoiesis of myelodysplasia), in extravascular sites (as in reabsorbed hematomas), or in the circulation (as in hemolytic anemia). In all of these circumstances, the indirect or unconjugated bilirubin level is elevated. In contrast, an elevated direct or conjugated bilirubin level occurs in liver disease as a result of the regurgitation of bilirubin into the circulation from injured hepatocytes (in cases of hepatitis) or from the bile ducts (in cases of obstructive jaundice).

Unconjugated hyperbilirubinemia also can occur in Gilbert’s syndrome, a congenitally acquired nonlouse state in which the activity of an enzyme responsible for conjugating bilirubin (ie, hepatic uridine diphosphoglucuronosyltransferase) is decreased. Gilbert’s syndrome is present in 5% of the population but is rarely associated with bilirubin levels greater than 3.0 mg/dL. Because patients with Gilbert’s syndrome generally are not anemic, differentiating hemolytic anemia from Gilbert’s syndrome should not create a diagnostic problem. However, patients with Gilbert’s syndrome

### Table 1. Tests for Determining the Presence of a Hemolytic Process

<table>
<thead>
<tr>
<th>Laboratory measurements</th>
<th>Reticulocyte count</th>
<th>Serum bilirubin</th>
<th>Serum haptoglobin</th>
<th>Serum lactate dehydrogenase</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Indirect</td>
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<tr>
<td></td>
<td>Serum bilirubin</td>
<td>Serum haptoglobin</td>
<td>Serum lactate dehydrogenase</td>
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<tr>
<td>Other</td>
<td>Examination of the peripheral blood smear</td>
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</table>
sometimes develop anemia from other causes, and their anemia can mimic hemolytic anemia.

Although fractionation of the bilirubin is the standard means of documenting whether an elevated level involves either direct or indirect bilirubin, a helpful clue can come from examining the urine. Only direct bilirubin appears in the urine.

In chronic hemolytic anemia in which a steady state has been achieved, the total serum bilirubin level is rarely greater than 6 mg/dL, because erythrocyte production by the marrow cannot exceed 6 times the normal level. When a steady state has been achieved, destruction of erythrocytes also is no greater than 6 times the normal rate, so the indirect bilirubin is unlikely to be more than 6 times the normal rate. In the presence of chronic stable hemolysis combined with hepatic disease, the total serum bilirubin level can be markedly elevated, but the indirect serum bilirubin level is rarely greater than 6 mg/dL. If massive acute hemolysis occurs beyond the ability of the bone marrow

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**Table 2. Specific Causes of Hemolytic Anemia**

**Processes outside the erythrocyte**

- Antibody-mediated hemolysis
  - Hemolytic disease of the newborn
  - IgG warm antibody
  - IgM cold antibody
  - Paroxysmal cold hemoglobinuria
- Hypersplenism
- Microangiopathic hemolysis
  - Malfunctioning artificial heart valve
  - Renal vascular disorders
  - Thrombotic thrombocytopenic purpura
- Brown recluse spider bite†
- Burns†
- Clostridial sepsis†
- Heavy metal exposure†
  - Arsenic
  - Copper
  - Lead
- Leishmaniasis†
- Malaria†
- March hemoglobinuria†
- Snake bites†
- Toxoplasmosis†

**Alterations of the erythrocytic membrane**

- Hereditary elliptocytosis
- Hereditary spherocytosis
- Paroxysmal nocturnal hemoglobinuria
- Spur cell anemia

**Abnormalities in the hemoglobin molecule**

- Heat (oxidant)-sensitive hemoglobins
- Hemoglobin C disease
- Sickle cell anemia (hemoglobin S)
- Thalassemia
  - α-Thalassemia
  - β-Thalassemia
- Hemoglobin E and other disorders associated with uneven production of α- and β-hemoglobin chains

**Decreased levels of an erythrocytic enzyme**

- Glucose-6-phosphate dehydrogenase deficiency (90% or more of all cases of enzyme-deficient hemolysis)
- Pyruvate kinase deficiency (most other cases of enzyme-deficient hemolysis)
- Aldolase deficiency†
- Diphosphoglycerate mutase deficiency†
- Glutamylcysteine synthetase deficiency†
- Glutathione deficiency†
- Glutathione reductase deficiency†
- Hexokinase deficiency†
- Phosphofructokinase deficiency†
- Phosphoglucoisomerase deficiency†
- Phosphoglycerate kinase deficiency†
- Triose-phosphate isomerase deficiency†

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*Categorized according to the site of the pathophysiologic process.
†Rare cause of hemolytic anemia.

to compensate, significant elevations of indirect serum bilirubin levels (up to 15 mg/dL) can be seen. Patients with such massive hemolysis most likely would have an unstable and rapidly falling hematocrit.

Measurement of the serum LDH level is a sensitive but highly nonspecific screening test for hemolytic anemia. Erythrocytes contain the electrophoretically fast-moving fraction LDH-1, which is also present in myocardial muscle fibers and renal cortex cells. Consequently, the total serum LDH level and especially the LDH-1 fraction are not specific for hemolysis. However, a total serum LDH level can be helpful in ruling out hemolytic anemia. Given that the total serum LDH level might be elevated because of other LDH fractions (eg, hepatic LDH), LDH fractionation might be more helpful in confirming hemolysis; however, this procedure is rarely performed.

Serum haptoglobin is an $\alpha_2$-globulin that acts as a scavenger protein and binds any hemoglobin released into the blood from intravascular hemolysis. This binding is an important protective mechanism, because free hemoglobin can precipitate in the kidneys and cause renal failure, as can occur in mismatched transfusion reactions. Serum haptoglobin is less affected when hemolysis occurs at extravascular sites, specifically in the spleen.

Unfortunately, the serum haptoglobin test is of limited value for 2 reasons. First, serum haptoglobin is an acute phase reactant and thus will be elevated in patients with myocardial infarction, cancer, infection, and similar conditions. As such, the serum haptoglobin level in these patients, although potentially decreased by hemolysis, might still be within the normal range. Secondly, up to 30% of a transfused unit of blood can hemolyze intravascularly within 24 hours of transfusion and thus lower the serum haptoglobin level. Therefore, if the serum haptoglobin test is performed after a patient has received a transfusion, its results are of limited value.

Examination of a patient’s peripheral blood smear can be helpful in diagnosing hemolysis because several hemolytic disorders are associated with abnormal erythrocytic morphology. Although reticulocytes themselves can be detected only by special supravital stains, the presence of an elevated reticulocyte count is associated with polychromatophilia (ie, large dark cells) on routine peripheral blood smears. Spherocytes can be an indication of either hereditary spherocytosis or AIHA. Microangiopathic changes can occur in hemolytic anemias associated with thrombotic thrombocytopenic purpura, dysfunctioning artificial heart valves, or renal diseases (eg, transplant rejection, scleroderma). As their names suggest, elliptocytes can be seen in the peripheral blood smears of patients with hereditary elliptocytosis, and sickled cells can be seen in the peripheral blood smears of patients with sickle cell anemia. Whereas hemoglobin C usually is associated with crystals of abnormal hemoglobin, such crystals are generally not seen unless the patient has already had a splenectomy; however, target cells are usually present in hemoglobin C disease. Finally, marked microcytosis, anisocytosis, and poikilocytosis generally are seen in the peripheral blood smears of patients with thalassemia major (beyond what is seen in cases of iron deficiency); only minimal microcytosis usually is observed in cases of thalassemia minor, although anisocytosis and poikilocytosis might also be present.

When tests suggest that hemolysis, if present, is minimal, the patient should be carefully evaluated for occult bleeding. Occult gastrointestinal bleeding often is an intermittent problem and cannot be ruled out by a single pair of stool specimens that are negative for occult blood. Occult bleeding can indicate the presence of a life-threatening disorder; it would be disastrous, for example, to miss diagnosing a curable neoplasm by embarking on a search for a phantom hemolytic process.

**Autoimmune Hemolytic Anemia**

**Definition and Etiology**

AIHA occurs when patients produce autoantibodies that bind to erythrocytes, leading to their destruction and a resultant anemia. As such, AIHA represents a failure of self-tolerance. However, the specific mechanism by which self-tolerance fails in AIHA is not known. Although many cases of AIHA are idiopathic, some of the conditions associated with AIHA are autoimmune disorders (Table 3); the latter classification includes systemic lupus erythematosi and other disorders of the immune system (eg, chronic lymphocytic leukemia, Waldenström’s macroglobulinemia, and other lymphomas).

The syndromes of AIHA are generally classified on the basis of the relationship between antibody activity and temperature. Warm active antibodies are generally IgG molecules, which may or may not fix complement and have the greatest affinity for erythrocytes at body temperature (ie, 37°C [98.6°F]). Cold active antibodies are generally IgM molecules, which fix complement and have the greatest affinity for erythrocytes between 0°C (32°F) and 4°C (39.2°F). Because such low body temperatures are
incompatible with life, the biologic effect of cold antibodies depends on how much activity is present at colder temperatures (eg, at temperatures of 22°C [71.6°F] to 30°C [86°F], which actually can occur in the periphery on exposure to cold environments).

One reason to distinguish warm antibody–mediated from cold antibody–mediated hemolysis is that the mechanism of destruction of erythrocytes differs in these 2 types of hemolysis, thus necessitating different treatments. For patients with warm antibody–mediated hemolysis, the antigen-binding fragment (Fab) of the antibody attaches to antigens on the membrane of erythrocytes; cells are destroyed when the crystallizable fragment (Fc) of the IgG molecule attaches to cells in the monocyte-macrophage system. This scenario occurs most readily in the sinuses of the spleen, where erythrocytes are separated (relatively speaking) from plasma, leaving the antibody-coated erythrocyte more likely to bind to a splenic macrophage.

For patients with cold antibody–mediated hemolysis, complement on the cells leads to destruction anywhere in the circulation that complement is cleared. Quantitatively, this means that hepatic clearance is the dominant mode of clearance of erythrocytes in cold antibody–mediated hemolysis. Because warm antibodies also can fix complement, warm antibodies can be associated with hepatic destruction of erythrocytes as well.

**DIAGNOSTIC LABORATORY TESTING**

- What tests best establish the diagnosis of autoimmune hemolytic anemia?

AIHA should be suspected in any patient with acquired hemolytic anemia, especially when the peripheral blood smear shows spherocytosis (suggesting warm antibody–mediated hemolysis) or rouleaux formation (suggesting cold antibody–mediated hemolysis). However, even in the absence of those findings on peripheral blood smears, specific testing for possible AIHA is part of the evaluation of any patient with hemolytic anemia. The direct antiglobulin test (DAT), also known as the direct Coombs' test, is designed to detect immunoglobulin and/or complement on the surface of erythrocytes. In this test, a patient’s erythrocytes are first collected in EDTA to limit in vitro adherence of complement to the erythrocytes. Cells are washed to eliminate any nonspecifically bound proteins and then mixed with antiglobulin serum obtained from rabbits. Either polyspecific antiglobulin serum or monospecific anti-IgG or -C3 antiserum is used. The cell-antiserum mixture is then centrifuged to enhance agglutination.

Although positive results on this test can give further weight to the diagnosis of AIHA, the standard DAT is not extremely sensitive and requires 150 to 200 IgG molecules per cell to give positive results. Because autoimmune hemolysis can occur with fewer molecules per cell, more sensitive techniques might be necessary when AIHA is suspected and the standard DAT produces negative results. Unfortunately, these more sensitive tests also can produce negative results, in which case the diagnosis is established by exclusion of other causes and by a response to immunotherapy. The mechanism of DAT-negative immune hemolysis is not known, but such cases might be mediated by low-titer antibodies that are
lost in the wash phase of the DAT. When the DAT produces positive results, the antibody can be eluted off erythrocytes with acid or xylene, and the specificity of the antibody can be evaluated by means of the indirect antiglobulin test.

Whereas the DAT uses antiserum and patient cells, the indirect antiglobulin test uses the patient’s serum and a panel of erythrocytes to detect agglutination. This test is positive in approximately three fourths of patients who have AIHA, and its use has shown that autoantibodies can react against a number of erythrocytic antigens. Failure of autoantibodies to react against very rare Rh-null cells initially suggested that all autoantibodies react against a basic component of the Rh system. However, although the Rh antigen is the most common target antigen for warm antibody–mediated AIHA, other antigens (such as LW antigens, glycoporphins A, B, C, and D, and rarely Kidd or Kell group antigens) also can be the target antigen in AIHA.

CLINICAL FEATURES

The clinical picture in warm antibody–mediated AIHA, which represents the majority of cases of AIHA, can range from a minimal increase in hemolysis of no clinical significance to fatal fulminant hemolysis. Slow onset of weakness, fatigue, and exertional dyspnea is common. In elderly patients unable to tolerate anemia, angina or even bowel infarction can occur. Even if an underlying lymphoproliferative disorder is not present, splenomegaly commonly is observed, and lymphadenopathy occasionally can be seen. The presence of adenopathy and splenomegaly in association with AIHA must lead to a consideration of chronic lymphocytic leukemia or lymphoma. Also, because many cases of AIHA are drug related, a careful history of recent drug use (including prescription, over-the-counter, and illicit drugs) must be obtained.

In cases of cold antibody–mediated AIHA, the clinical picture is generally that of chronic hemolytic anemia but with exacerbations occurring during the winter. Fulminant hemolysis is rare, and the hemoglobin level is generally greater than 7 g/dL. Acrocyanosis can occur from agglutination of cells in the hands, nose, feet, or ears. A mottled appearance of the limbs, known as livedo reticularis, also may be present. As occurs in warm antibody–mediated AIHA, splenomegalgy also can be present. However, whenever more than minimal splenomegaly is present, lymphoma and infectious mononucleosis–related cold antibody–mediated hemolysis must be considered as possible causes. Whereas cold antibody–mediated AIHA is most commonly a disease of the elderly, young patients often experience postinfectious cold antibody–mediated AIHA after mycoplasmal infections or infectious mononucleosis.

MANAGEMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA

WARM ANTIBODY–MEDIATED HEMOLYSIS

Therapy of warm antibody–mediated hemolysis depends on the severity of the disease. Positive results on a DAT, if associated with a hematocrit that is within normal limits and a slightly elevated reticulocyte count, do not warrant corticosteroid therapy. Treatment may be limited to folate replacement and close observation to detect progressive anemia. Additionally, an evaluation seeking the underlying causes of the hemolysis is warranted.

Corticosteroid Therapy

When the degree of hemolysis is such that anemia is present, corticosteroids are the initial therapy of choice. The usual starting dose for prednisone is 1 mg/kg body weight per day, but higher doses can be used in the face of massive hemolysis. A response to corticosteroid therapy usually occurs within 4 to 7 days, and a slow increase in hemoglobin level of 2 to 3 g/dL per week is expected. Once the hemoglobin level reaches 10 g/dL, a slow tapering of the prednisone dose can begin. The generally recommended approach is to taper to a dose of 0.5 mg/kg over a period of 4 to 6 weeks. In a 70-kg (154-lb) patient, this tapering would represent a decrease from 70 mg/day to 35 mg/day. Once the latter level of daily prednisone is achieved, very slow tapering of the daily dose is recommended. The usual aim is to decrease the daily dose to 5 to 10 mg over a period of 3 to 4 months. Whether long-term maintenance at that level is necessary to prevent relapse has not been established by clinical trials. Many physicians simply stop administering prednisone after a slow taper to a daily dose of 5 mg and then follow patients for any signs of relapse.

The response rate to prednisone is excellent, with more than 80% of patients responding within a week and almost 90% of patients showing some improvement within 2 weeks of receiving the drug. Prednisone decreases hemolysis by 2 mechanisms. Its most immediate effect is to decrease clearance of erythrocytes by the monocyte-macrophage system. Animal studies have shown that prednisone is most effective in this regard when erythrocytes are covered with IgG alone. When
both IgG and complement are present, prednisone is less effective, and prednisone is least effective when only complement is present on the erythrocytes. This latter fact is consistent with the limited efficacy of prednisone in cold antibody–mediated hemolysis. A second documented mechanism of action of prednisone involves decreased production of autoantibody. As the response to prednisone occurs, results of the DAT generally remain positive, although the strength of the reaction decreases (eg, from a result reported as “+++” to one reported as “+”).

Although prednisone produces responses in the majority of patients with AIHA, clinical improvement is not equivalent to a complete recovery. Slightly more than half of the patients who respond to prednisone therapy will relapse, even if the corticosteroid is tapered gradually. It is estimated that prednisone alone will produce permanent responses in only a third of cases. For patients who are primarily refractory to prednisone or who require frequent retreatment with the drug, therapy with splenectomy or cytotoxic drugs is indicated.

**Splenectomy**

Splenectomy is indicated when patients require more than 20 mg/day of prednisone, when patients suffer from severe adverse effects at the dose required to maintain a response, or when frequent relapses require retreatment at a high dose (ie, near 1 mg/kg per day). Splenectomy theoretically would be effective because the spleen is both a major site of destruction of erythrocytes in warm antibody–mediated hemolysis and a major site of autoantibody production. As is the case with prednisone, splenectomy is of very limited value in patients who have cold antibody–mediated hemolysis.

Although the response rate to splenectomy varies considerably in published studies, a rate of approximately 75% seems consistent with the overall data. Despite the frequent relapses seen after splenectomy, it is estimated that a third to a half of patients have their disease permanently controlled by splenectomy. Additionally, many patients who require high doses of prednisone before splenectomy can be maintained on lower, safer doses after splenectomy.

**Immunosuppressive Therapy**

For patients not receiving or responding to corticosteroids who are not surgical candidates or for whom splenectomy fails as a treatment, immunosuppressive pharmacologic therapy is indicated. Azathioprine and cyclophosphamide are the most commonly used drugs. No data from controlled clinical trials are available, but reviews suggest a response rate of approximately 50% among patients with warm antibody–mediated AIHA who are refractory to corticosteroids and splenectomy.

No standard dose has been established, but it seems reasonable to initiate cyclophosphamide at a dose of 1.5 to 2.0 mg/kg per day or azathioprine at a dose of 2 mg/kg per day, with or without prednisone (1 mg/kg per day). When prednisone is included, its dose is usually tapered over 3 months; the immunosuppressive drug is continued for 6 months prior to dose reduction.

Immunoglobulin has been administered intravenously to patients with AIHA, but the results of this treatment have been far less encouraging in cases of AIHA than they are in cases of immune thrombocytopenia. Plasmapheresis has been attempted on occasion, but this approach has met with limited success, given the fact that most IgG is extravascular. Anecdotal success has also been reported with use of danazol and vinca-loaded platelets.

**Transfusions**

Transfusion therapy in patients with AIHA characterized by warm antibody–mediated hemolysis presents a major clinical problem because of difficulties in crossmatching and so should be avoided in most cases. However, in patients with critically low levels of hemoglobin, transfusions can be used as a life-saving treatment to temporize the patient until a response to corticosteroids or splenectomy occurs. In patients with warm autoantibodies, ABO and Rh typing generally presents little difficulty. If there is a question about the accuracy of typing, the antibody can be eluted from the cells and typing can be repeated.

The problem in arranging transfusions for patients with warm autoantibodies is that antibody screening is difficult to perform; crossmatching presents similar difficulties. Free autoantibody may react with all cells, rendering all crossmatches incompatible. When this circumstance occurs, the detection of alloantibodies induced by prior transfusions or pregnancies can be difficult. The best that a blood bank often can to do in this situation is to define the “least incompatible” units. If transfused, such units must be given slowly with close observation for signs of intravascular hemolysis (eg, development of back pain and/or hemoglobinuria). However, in most cases, transfused cells will be destroyed at a rate neither faster nor slower than that at which native erythrocytes are being destroyed.

**COLD ANTIBODY–MEDIATED HEMOLYSIS**

For many patients with cold antibody–mediated hemolysis, also known as cold agglutinin disease, the disease process is a chronic illness rather than a severe...
acute episode. As such, adequate therapy may require no more than having the patient avoid temperatures at which the antibody shows activity. In patients with more severe disease, more aggressive therapy is indicated.

As mentioned previously, prednisone therapy and splenectomy play no role in the usual management of cold antibody–mediated hemolysis. Instead, standard therapy is aimed at decreasing antibody production by means of immunosuppression. Cyclophosphamide and chlorambucil are the drugs most commonly used for this purpose. Chlorambucil is often administered at a dose of 2 to 4 mg/day and cyclophosphamide at a dose of 100 to 150 mg/day. Pulse therapy with higher levels of drugs given for 4 days every 2 to 3 weeks can also be employed.

Because the antibody in cold antibody–mediated hemolysis is IgM, which has an intravascular distribution, plasmapheresis is of greater theoretical value than in warm antibody–mediated hemolysis. Unfortunately, that theoretical value does not translate into significant clinical value, perhaps because plasmapheresis does nothing to decrease antibody production. Accordingly, plasmapheresis should not be used alone but instead should be combined with immunosuppressive therapy. Obviously, if plasmapheresis is employed, great care must be taken not to lower the temperature of the blood to the point where hemolysis will be increased.

Transfusion therapy also is not generally needed for patients with cold antibody–mediated hemolysis. However, when transfusions are needed, all testing must be performed at 37°C (98.6°F) to minimize the effects of the cold antibodies (or agglutinins) and allow for the detection of alloantibodies. Whereas most cold antibodies are directed against the I antigen, locating I\textsubscript{adult} cells is not practical because these cells are extremely rare. Instead the “least incompatible” units should be given through a blood warmer designed to keep the blood at 37°C (98.6°F).

**DRUG-RELATED AUTOIMMUNE HEMOLYTIC ANEMIA**

Although AIHA most often is an idiopathic process, drugs also can cause this type of anemia. Three main mechanisms of drug-induced immune hemolysis have been described: (1) drug adsorption, (2) neoantigen formation, and (3) true autoimmune disease. Moreover, there are other drugs that cause nonspecific binding of proteins (eg, immunoglobulins) to erythrocytes but do not cause hemolysis. Treatment of drug-related AIHA consists of recognizing the possible responsible drug (Table 4) and discontinuing its administration. In cases of α-methyldopa–related AIHA, therapy identical to that employed in drug-independent AIHA might be required.

**DRUG ADSORPTION**

In the drug adsorption–type of hemolysis, a drug binds to erythrocytic membranes, so the antibody is directed against the drug, not the erythrocytes. The prototype for this phenomenon is penicillin-induced hemolysis, which can occur when penicillin is given at doses greater than 10 million units per day. Because such doses are rarely used in clinical practice, the phenomenon is largely of academic interest. However, when such high doses are administered, approximately 3% of patients will develop antipenicillin antibodies. In contrast to true autoimmune disease, eluates from these patients do not react against normal cells because the antigen against which the antibody is directed is penicillin. It is worth noting that this phenomenon was first described in a transfusion recipient whose serum reacted with all erythrocytes that had been stored in penicillin. Because the antibody involved is an IgG antibody, other types of penicillin sensitivity (eg, urticaria, anaphylaxis) are usually not observed. The drug adsorption–type of hemolysis can also occur with cephalosporins, tetracycline, tolbutamide, and semisynthetic penicillins.

**NEOANTIGEN FORMATION**

A second mechanism of drug-induced hemolysis is neoantigen formation, previously known by the less accurate terms of “immune complex phenomenon” and “innocent-bystander mechanism.” Former theories held that, in this type of hemolysis, drugs formed a complex with an antidrug antibody, which then attached to an erythrocyte that was not a target of the antibody but was instead an innocent bystander. However, recent studies have shown that the antibodies react against a combined drug-erythrocyte complex. As in the penicillin mechanism of hemolysis, the drug or its metabolites are required for antibody binding to occur. Unlike penicillin, the drugs involved in this type of reaction bind only very loosely to erythrocytes. However, only a small amount of the drug is necessary for hemolysis to occur. This type of hemolysis is mediated by complement and can be quite massive, leading to hemoglobinuria and renal dysfunction.

**TRUE AUTOIMMUNE DISEASE**

A third type of drug-related autoimmune hemolysis is the α-methyldopa type of hemolysis. In this type of hemolysis, antibodies bind to erythrocytes in a
drug-independent manner. In essence, the process results from drug administration but is identical in every other respect to idiopathic autoimmune hemolytic anemia. Antibodies that are eluted off the erythrocyte in these cases will bind in vitro to erythrocytes of patients who have never received $\alpha$-methyldopa and are indistinguishable from antibodies seen in idiopathic AIHA. As many as a third of patients receiving $\alpha$-methyldopa will eventually have positive results on a DAT, but fewer than 1% of patients receiving the drug actually develop hemolysis.\(^1\) The specific mechanism by which $\alpha$-methyldopa alters the immune system and produces AIHA is unknown.

**NONHEMOLYTIC DRUGS**

Although not producing hemolysis, a final drug-related immune mechanism worth noting involves nonspecific attachment of proteins to erythrocytic membranes. These proteins lead to agglutination of erythrocytes and to positive results on a DAT but do not produce destruction of erythrocytes. Such a situation occurs in approximately 3% of all patients receiving cephalosporins.\(^7\) Cephalosporins, of course, also can produce hemolysis by means of either the drug absorption or the neoantigen method.

**PAROXYSMAL COLD HEMOGLOBINURIA**

A few words about paroxysmal cold hemoglobinuria (PCH) also are appropriate. PCH can be distinguished from cold antibody-mediated hemolysis by both clinical and immunologic characteristics. Originally described by Donath and Landsteiner in 1904,\(^1\) the condition is characterized by sudden onset of fever, back or leg pain, and hemoglobinuria after exposure to the cold. Cold exposure may be brief, and symptoms begin within minutes to hours. Urine is characteristically dark red to black but clears in color over the course of a few hours. The syndrome is more common in children but also can occur in adults.

In contrast to the IgM antibody in cold antibody-mediated hemolysis, the antibody in PCH is an IgG that binds to erythrocytes in the cold and fixes complement.
When the erythrocytes are warmed, hemolysis occurs. The Donath-Landsteiner test for this biphasic hemolysin involves incubating erythrocytes in the patient’s serum at 0°C (32°F) to 4°C (39.2°F) and then warming the cells to 37°C (98.6°F) to produce lysis. The antibody is specific for the P antigen. The generally accepted theory is that an antigen associated with a microorganism leads to production of the IgG antibody, which cross-reacts with the P blood-group system. Because IgG does not remain on the cell once complement is fixed, the DAT will have positive results if nonspecific antiglobulin antiserum or anti-complement antiserum is used, but not if specific anti-IgG antiserum is used.

In the past, PCH was associated with advanced or congenital syphilis. Patients with PCH still should be investigated for occult syphilis, but this association rarely is observed today.

The anemia associated with PCH can be severe because of massive intravascular hemolysis. A hemoglobin level of 5 g/dL is not uncommon. Although the reticulocyte count is elevated as recovery occurs, it may be misleadingly low if patients are seen at the onset of a hemolytic episode.

Treatment of PCH consists of keeping patients warm and giving them transfusions, as necessary. Although the antibody is directed against the P antigen, there is no need to search for rare pp units of blood; antibody will not fix to P antigen–positive cells unless the patient is exposed to cold temperatures.

**FOLLOW-UP OF CASE PATIENT**

- What type of anemia is most likely in the case patient?
- Does the presence of spherocytes in the case patient’s peripheral blood smear necessarily indicate a diagnosis of hereditary spherocytosis?
- Is the case patient’s normal serum haptoglobin level inconsistent with a diagnosis of autoimmune hemolytic anemia?
- Would the case patient’s urine be expected to contain increased levels of bilirubin?

Based on the clinical presentation and laboratory findings, a diagnosis of hemolytic anemia with spherocytosis is made in the case patient. AIHA is suspected. Spherocytes are not specific for hereditary spherocytosis but can indicate the presence of AIHA, especially in the absence of a positive family history. The serum haptoglobin level, although in the normal range, also is consistent with the diagnosis. Although the serum haptoglobin level generally is low in cases of intravascular hemolysis, the destruction of erythrocytes in AIHA (as well as in other hemolytic anemias) may be primarily splenic; as previously mentioned, the serum haptoglobin level is less affected when hemolysis occurs at such extravascular sites. Because the patient’s elevated serum bilirubin level is indirect, his urine would be straw colored (normal) rather than brown (indicating the presence of bilirubin).

To confirm the diagnosis of AIHA, a DAT is performed, with results reported as positive (“++++”). Specific tests reveal that IgG is present on the erythrocytes (results also are reported as “+++”) but that complement is absent. The patient is treated with prednisone (80 mg/day) and initially responds, with the hematocrit increasing to 42% as the reticulocyte count decreases to 3.4%. After the prednisone dose is slowly tapered to 20 mg/day, the hematocrit falls to 27%. The dose of prednisone is increased, leading to an increase in the hematocrit to 41%. However, despite slow tapering of the prednisone dose, the hematocrit again falls (to 26%) when the dose reaches 20 mg/day. At this point, the corticosteroid dose is again increased to raise the hematocrit above 30%, and a splenectomy is performed. This time, the patient is successfully tapered off prednisone without experiencing a relapse of the hemolytic anemia.

**SUMMARY**

The survival of erythrocytes in the circulation is decreased in patients with hemolytic anemia. Because hemolytic anemia is uncommon and because its causes are diverse, its presentation can cause confusion for clinicians. The first step in evaluating a patient suspected of having hemolytic anemia is to document that hemolysis is present by determining the reticulocyte count and the serum indirect bilirubin, LDH, and haptoglobin levels. Secondly, the specific cause of the hemolysis should be determined. If the hemolytic anemia is clearly acquired, one can start by evaluating the patient for the most common causes of hemolysis, namely acquired AIHA and hypersplenism.

AIHA comes in 2 varieties. In warm antibody–mediated AIHA, the antibody is usually an IgG, which may or may not fix complement. Destruction of erythrocytes occurs primarily in the spleen, and standard therapy is prednisone administration. Splenectomy is reserved for corticosteroid-refractory cases. In cold antibody–mediated AIHA, the antibody is an IgM that
fixes complement, leading to destruction of erythrocytes in the liver and elsewhere in the monocyte-macrophage system. Corticosteroids and splenectomy generally play no role in the management of cold antibody–mediated AIHA. In fact, this type of AIHA may be so mild as to not require therapy; treatment with chemotherapy or plasmapheresis is also possible. Appreciation of the underlying pathophysiology of AIHA may lead to a more rational approach to its diagnosis and treatment.

REFERENCES


