A 61-year-old woman presents to the emergency department reporting fatigue, increasing dyspnea, and dark urine. The respiratory rate is 30 breaths per minute, the pulse 116 beats per minute, and the oxygen saturation 90% while she is breathing ambient air. She is pale, without splenomegaly or adenopathy. Her hemoglobin level is 5.4 g per deciliter, hematocrit 16.1%, and mean corpuscular volume 103 fl; the white-cell and platelet counts are normal. The percentage of reticulocytes is 15.7%, and the total bilirubin level is 9.7 mg per deciliter (166 μmol per liter). A peripheral-blood smear reveals numerous microspherocytes. A direct antiglobulin test is positive for IgG and weakly positive for C3d. Laboratory tests show a panagglutinin. She takes no regular medication. How would you further evaluate and treat this patient?

**The Clinical Problem**

Warm autoimmune hemolytic anemia (WAHA) leads to accelerated red-cell destruction due to the presence of warm agglutinins (almost always IgG antibodies) that bind to antigens on erythrocytes at a temperature of 37°C. The annual incidence of WAHA is 1 to 3 cases per 100,000 persons. The median age of onset is 52 years, but WAHA may occur at any age, and there is a slight female predominance in most series. Up to 30% of patients have a durable remission after initial therapy, but the rest have a chronic, relapsing course.

Most accelerated red-cell clearance is through the spleen and liver (extracellular hemolysis). IgG-coated red cells are recognized by splenic macrophages, which carry Fcγ receptors for the IgG heavy chain. This leads to either phagocytosis of the red cell or, more commonly, removal of a portion of the red-cell membrane, resulting in microspherocytes that are visualized on a peripheral-blood smear (Fig. 1A). Microspherocytes are less pliable than normal erythrocytes and become trapped in the splenic sinuses during their next passage through the spleen. Extracellular hemolysis in the spleen may also be due to antibody-dependent cell-mediated cytotoxicity from T cells that also possess Fc receptors. IgG subtypes can also activate complement, leading to deposition of C3 fragments on the red cell that are then removed by liver macrophages that carry receptors for C3 fragments. In severe cases, complement activation can lead to formation of the membrane attack complex (C5b-9) on the surface of red cells and result in intravascular hemolysis, which manifests as hemoglobinuria and markedly elevated lactate dehydrogenase levels.
all warm autoantibodies are polyclonal and react with all reagent red cells from the blood bank (panagglutinins), even when WAHA is associated with clonal B-cell lymphoproliferative diseases such as chronic lymphocytic leukemia (CLL).6,7

Approximately 50% of cases of WAHA are primary and idiopathic; the rest are secondary to other disorders (Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org) or medications.8 Immunodeficiency disorders that are associated with an increased risk of WAHA include common variable immunodeficiency9 and other primary immunodeficiency states, such as the autoimmune lymphoproliferative syndrome.10 Patients with this syndrome harbor germline mutations in genes such as FAS, FASL, or CASP10. These mutations lead to diminished Fas-mediated lymphocyte apoptosis and failure to delete autoreactive lymphocytes, and thus a predisposition to WAHA and other autoimmune diseases. Acquired lymphoproliferative diseases associated with WAHA include CLL, non-Hodgkin’s lymphoma, and Hodgkin’s lymphoma. Purine analogues such as fludarabine increase the risk and exacerbate the severity of WAHA among patients with CLL, as does a history of allogeneic bone marrow transplantation or solid-organ transplantation. Other risk factors for WAHA include rheumatologic conditions (e.g., systemic lupus erythematosus, scleroderma, and rheumatoid arthritis) and infections, including viral infections (especially in children, and in particular, human immunodeficiency virus [HIV] infection) and babesiosis in

Figure 1. Peripheral-Blood Specimens.
Panel A shows numerous microspherocytes (arrows) that are typically seen in warm autoimmune hemolytic anemia. Panel B shows red-cell agglutination (arrows) in a sample obtained from a patient with cold agglutinin disease.
patients without splenic function. In a single-institution study involving 86 patients with a diagnosis of babesiosis, WAHA developed in 6 patients, all of whom had undergone splenectomy, within 2 to 4 weeks after infection, whereas only 12 of the 80 patients in whom WAHA did not develop had undergone splenectomy.

Multiple medications are also associated with the development of WAHA. The most common drugs associated with WAHA are penicillins and cephalosporins. A recent search of the Food and Drug Administration database revealed 68 cases of WAHA associated with checkpoint inhibitors, which are known to be associated with immune-related complications. The reported incidence of WAHA associated with checkpoint inhibitors is 15 to 30% among adult patients with WAHA.

Among 109 consecutive patients with hemolytic anemia included in a retrospective study (of whom approximately 80% had WAHA), the median hematocrit was 24% and the median corrected percentage of reticulocytes was 5.0% (range, 0.1 to 45.0). In a smaller registry study in France, more than 90% of the patients had a low haptoglobin level, an elevated indirect bilirubin level, elevated lactate dehydrogenase levels, low haptoglobin levels, elevated indirect bilirubin levels, and a positive direct antiglobulin test.

Among 109 consecutive patients with hemolytic anemia included in a retrospective study (of whom approximately 80% had WAHA), the median hematocrit was 24% and the median corrected percentage of reticulocytes was 5.0% (range, 0.1 to 45.0). In a smaller registry study in France, more than 90% of the patients had a low haptoglobin level, an elevated indirect bilirubin level, and a low haptoglobin level. Bilirubin levels were elevated in more than 80% of the patients; one third had jaundice, more than 5% presented with chest pain, and more than 50% required blood transfusion. More than 20% of patients with WAHA have a concurrent monoclonal gammopathy, which is more than five times the expected rate for their age.

**STRATEGIES AND EVIDENCE**

**SIGNS AND SYMPTOMS**

Common symptoms of WAHA (fatigue, dyspnea, and palpitations) are proportional to the degree of anemia. Brisk intravascular hemolysis, which can be associated with chest pain, lethargy, and confusion, is a medical emergency. Physical examination reveals pallor and jaundice proportional to the degree of anemia and intravascular hemolysis. Cardiopulmonary signs such as tachycardia, peripheral edema, and elevated jugular venous pressure may occur. Splenomegaly may be present, especially in patients with an underlying lymphoproliferative disorder. Common laboratory features include a low hemoglobin level, reticulocytosis, elevated lactate dehydrogenase levels, low haptoglobin levels, elevated indirect bilirubin levels, and a positive direct antiglobulin test.

**DIAGNOSIS AND EVALUATION**

WAHA should be suspected in a patient who presents with anemia and laboratory evidence of hemolysis (i.e., an elevated lactate dehydrogenase level, an elevated indirect bilirubin level, and a low haptoglobin level). The diagnostic workup should include a complete blood count, a reticulocyte count, the markers of hemolysis listed above, a peripheral-blood smear, and a direct antiglobulin test (Fig. 2).

It is important to distinguish between WAHA and cold agglutinin disease, which is typically caused by IgM autoantibodies that react with polysaccharide antigens on red cells at tempera-
tures below the core body temperature and result in complement-mediated hemolysis. In cold agglutinin disease, the direct antiglobulin test is typically negative for IgG but positive for C3 fragments. Cold agglutinin disease is usually associated with antibody specificity to I or i red-cell antigens, a high titer of cold agglutinins (titer of >1:128), and a thermal amplitude (the temperature at which red cells agglutinate) higher than room temperature. A low titer of cold agglutinins or cold agglutinins with a thermal amplitude of 25°C or lower are not likely to be clinically significant; thus, communication with the blood bank is important to help distinguish between warm and cold autoimmune hemolytic anemia. Rarely, patients can present with mixed warm and cold autoimmune hemolytic anemia that is characterized by the presence of both a warm autoantibody and a cold-active IgM antibody.

A diagnosis of primary WAHA is defined by hemolytic anemia and a positive direct antiglobulin test in a patient who is not receiving a drug that can cause WAHA and does not have an underlying lymphoproliferative, infectious, autoimmune, or neoplastic disease (Table S1 in the Supplementary Appendix). WAHA in a patient with a negative direct antiglobulin test is rare (<5% of cases), but it should be suspected in patients with acquired hemolytic anemia and findings on a peripheral-blood smear that are consistent with WAHA. Paroxysmal nocturnal hemoglobinuria should be considered before making a diagnosis of direct antiglobulin test–negative WAHA. Direct antiglobulin test–negative WAHA may be due to pathogenic IgG autoantibodies below the sensitivity level of the direct antiglobulin test (approximately 500 IgG molecules per red cell), red cell–bound IgA or

Figure 2. Direct Antiglobulin Test (Direct Coombs’ Test).

The direct antiglobulin test is used to determine whether a patient’s red cells are coated with IgG, complement, or both. Historically, Coombs’ serum was made by immunizing rabbits with human immunoglobulin. The rabbits made antibodies against human immunoglobulin and complement that were subsequently purified to make Coombs’ reagent; thus, the antihuman globulin contained antibodies against IgG and complement. Newer reagents are made by injecting purified proteins. The test is performed by taking washed patient red cells (Panel A) and incubating them (Panel B) with antihuman IgG and antihuman C3d antibodies. In warm autoimmune hemolytic anemia, antihuman antibodies, anti-C3d antibodies, or both form links (agglutination) between red cells by binding the human antibodies on the patient’s red cells (Panel C). Agglutination is graded visually as negative to 4+, with values of 1 or higher indicating a positive test result.
monomeric IgM, or low-affinity autoantibodies. Specialized reference laboratories offer enhanced direct antiglobulin test assays to detect most cases of direct antiglobulin test–negative WAHA.²⁵,²⁶

Peripheral-blood flow cytometry may identify low-grade lymphoproliferative disorders and should be ordered for all adults with WAHA. Other tests warrant consideration in selected patients. These may include the d-dimer test, lower-extremity venous Doppler studies, and computed tomography (CT) of the chest if thromboembolic disease is suspected. Autoimmune panels should be performed in patients with signs and symptoms of rheumatologic disease. Bone marrow aspiration and biopsy and CT of the chest, abdomen, and pelvis may be indicated to rule out lymphoproliferative disorders, especially if the patient has lymphadenopathy, massive splenomegaly, weight loss, or unexplained fevers.

**IMMEDIATE MANAGEMENT**

WAHA with severe anemia (hemoglobin level <6 g per deciliter), hypoxia, confusion, or hemodynamic instability is a medical emergency, and urgent blood transfusion is indicated to reduce the likelihood of death from pulmonary edema, myocardial infarction, or arrhythmia. In virtually all cases, the cross-match will be “incompatible,” given that the autoantibodies recognize blood-group antigens and will react with virtually all donor red cells; in such cases, ABO- and RhD-matched blood should be administered.

The risk of a transfusion reaction with ABO- and RhD-matched blood is nearly zero among patients who have not been sensitized to foreign red-cell antigens, and it remains low (<10%), even in patients with a history of pregnancy or previous blood transfusion predisposing to sensitization. The benefits of red-cell transfusion outweigh the risks, even in patients with a predisposition to sensitization who have severe anemia due to WAHA.²⁷ However, in previously sensitized patients, blood should be infused slowly (over 2 to 3 hours), and patients should be monitored for fever, chills, and dyspnea. Extended phenotype matching for additional Rh subgroups (C, c, E, e, Kell, Kidd, S, and s) should be performed in patients with nonurgent cases of WAHA among whom there is a high risk of alloimmunization.

**DEFINITIVE THERAPY**

Since randomized trials are lacking, recommendations for drug treatment for WAHA are primarily based on case series and expert opinion. Treatment of WAHA that is attributable to other conditions or medications is generally the same as treatment for primary WAHA, but it may also include discontinuation of the use of offending drugs or treatment of underlying diseases such as poorly controlled HIV infection.

**First-Line Treatment**

Patients with a new diagnosis of symptomatic WAHA are treated with glucocorticoids (1–2 mg per kilogram of body weight per day of prednisone administered orally or an equivalent dose of methylprednisolone administered intravenously).²⁸,²⁹ It is recommended that this dose be continued until a hemoglobin level above 10 g per deciliter is achieved, a goal that is reached in up to 80% of patients within 2 to 3 weeks. A second agent is typically added if prednisone is not effective within 2 to 3 weeks after initiation. If effective, prednisone should be tapered over 4 to 6 months. The percentage of patients who remain in remission after discontinuing prednisone is unclear, but retrospective case studies suggest rates of 20 to 30%.³⁰ Most experts aim for a hemoglobin level of 10 mg per deciliter or higher with a dose of prednisone of less than 10 mg per day by 3 months after treatment; otherwise, a second agent is administered to avoid long-term complications of glucocorticoid use. Data from randomized trials to guide tapering strategies are lacking, but rapid tapers over 3 to 4 weeks are often associated with relapse.

Another option for first-line therapy in patients with WAHA is the use of rituximab with glucocorticoids; two randomized, controlled trials showed that combined therapy was superior to glucocorticoid monotherapy. One open-label, phase 3 trial in which 64 patients were randomly assigned to prednisolone with or without intravenous rituximab (at a dose of 375 mg per square meter of body-surface area weekly for 4 weeks) showed higher rates of relapse-free survival with combined therapy than with monotherapy at 36 months of follow-up (70% vs. 45%).³¹ There was no between-group difference in the rate of adverse effects. In another randomized, double-blind trial involving 32 patients with WAHA who had received prednisone for less than 6 weeks, patients assigned to intravenous rituximab (at a dose of 1000 mg, on day 1 and day 15) had higher rates of remission than those assigned to placebo at 1 year (75% vs. 31%) and at 2 years (62% vs. 19%).³²
<table>
<thead>
<tr>
<th>Treatment Option</th>
<th>Route</th>
<th>Dose</th>
<th>Serious Adverse Effects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-line treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucocorticoids (prednisone and methylprednisolone)</td>
<td>Oral or intravenous</td>
<td>Oral prednisone: 1–2 mg/kg of body weight/day; intravenous methylprednisolone: 500–1000 mg/day (begin slow taper to be completed over 4–6 mo) if hemoglobin level &gt;10 g per deciliter after 1–3 wk</td>
<td>Diabetes, osteoporosis, infections</td>
<td></td>
</tr>
<tr>
<td><strong>Second-line treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rituximab</td>
<td>Intravenous</td>
<td>375 mg/m² of body-surface area weekly in 4 doses</td>
<td>Reactivation of hepatitis B virus infection; progressive multifocal leukoencephalopathy</td>
<td>All patients should be screened for hepatitis B surface antigen before initiation of drug.</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>Oral</td>
<td>2 mg/m²/day; trough goal, 5–15 ng/ml</td>
<td>Pancytopenia, lymphoma, infections, opportunistic infections</td>
<td>Patients with the autoimmune lymphoproliferative syndrome have had a high response rate.</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>Oral</td>
<td>2 mg/m²/day</td>
<td>Lymphoma, lung disease, opportunistic infections</td>
<td></td>
</tr>
<tr>
<td>Immune globulin</td>
<td>Intravenous</td>
<td>500 mg/kg/day for 4 days or 1 g/kg/day for 2 days as an adjunct to glucocorticoids or mycophenolate mofetil</td>
<td>Aseptic meningitis, renal insufficiency, hemolytic anemia</td>
<td>Responses are often transient, so immune globulin is not often used as a stand-alone drug.</td>
</tr>
<tr>
<td><strong>Third-line treatment</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Azathioprine</td>
<td>Oral</td>
<td>1–2 mg/kg/day; maximum dose, 150 mg/day</td>
<td>Pancytopenia, infections, liver-function abnormalities</td>
<td></td>
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<tr>
<td>Cyclosporine</td>
<td>Oral</td>
<td>5 mg/kg/day divided every 12 hr; target trough levels of &gt;150 ng/ml and &lt;300 ng/ml</td>
<td>Renal and hepatic dysfunction, lymphoma, infections, hypertension</td>
<td></td>
</tr>
<tr>
<td>Pulse-dose cyclophosphamide</td>
<td>Intravenous</td>
<td>500–1000 mg/m²; 1–3 doses every 2–3 wk</td>
<td>Pancytopenia, infection, secondary malignancy, secondary cancer, infertility</td>
<td></td>
</tr>
<tr>
<td><strong>Fourth-line treatment</strong></td>
<td></td>
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<tr>
<td>High-dose cyclophosphamide or autologous bone marrow transplantation</td>
<td>Intravenous</td>
<td>50 mg/kg of ideal body weight/day for 4 consecutive days, followed by granulocyte colony-stimulating factor</td>
<td>Pancytopenia, severe infection, hematologic cysts, secondary cancer, secondary malignancy, disabling hypothyroidism, cardiac toxicity</td>
<td></td>
</tr>
</tbody>
</table>

* Splenectomy is also considered to be a second-line treatment. Associated adverse effects are thrombosis and bacterial infections (encapsulated organisms). Vaccination against Haemophilus influenzae, meningococcus, and pneumococcus 8 to 10 weeks before splenectomy is strongly recommended.
Second-Line and Other Treatments

Historically, splenectomy has been considered to be second-line therapy; however, owing to concerns regarding infection and thrombosis with splenectomy, rituximab is now preferred in patients with WAHA who are initially treated with glucocorticoid monotherapy and who do not have a response or who have disease that relapses after an initial response. Recent guidelines from the United Kingdom recommend rituximab over splenectomy. In a meta-analysis of 21 observational studies that included 154 patients with primary or secondary WAHA, the overall response rate among patients with relapsed WAHA or disease that was refractory to rituximab was 79%. Response can sometimes take several weeks, and the rate of relapse at 1 to 2 years ranges from 25 to 50%.

More than 50% of patients with relapsed or refractory WAHA have a response to splenectomy; however, of those who have a response, more than 25% have a relapse within a year; the longer-term durability of remission is unclear. Consequently, many hematologists prefer that patients try other relatively nontoxic therapies such as mycophenolate mofetil, azathioprine, intravenous immune globulin, or cyclosporine before undergoing splenectomy (Table 1). Data are lacking to guide the order in which to use these drugs. Case reports have described good outcomes in patients with severe refractory WAHA who receive intermittent intravenous (pulse-dose) cyclophosphamide or high-dose cyclophosphamide or who undergo allogeneic bone marrow transplantation. Patients with WAHA associated with the autoimmune lymphoproliferative syndrome have been reported to have a good response to sirolimus.

Areas of Uncertainty

Multicenter, randomized, controlled trials with long-term follow-up are needed to compare the benefits, risks, and costs of various treatments for WAHA and to guide how to sequence or combine them for the best outcomes. A number of targeted therapies are in development but are not approved for WAHA. Fostamatinib, a spleen tyrosine kinase inhibitor that prevents phagocytosis and immune activation in splenic macrophages, is approved for immune thrombocytopenia and is now being studied in WAHA (ClinicalTrials.gov number, NCT03764618). Other targeted strategies, including the use of proteasome inhibitors (e.g., ixazomib; NCT03965624), B-cell receptor inhibitors (e.g., ibrutinib; NCT03827603), and complement inhibitors (NCT03226678), are also being studied.

Guidelines

The British Committee for Standards in Haematology has published a guideline for the management of WAHA. This guideline is based largely on expert opinion, given the paucity of randomized trials. The present recommendations are largely consistent with this guideline, with the exception that it recommended glucocorticoid monotherapy as first-line treatment (it antedated one of the randomized trials that provided support for combined treatment with rituximab).

Conclusions and Recommendations

The woman described in the vignette has severe anemia, reticulocytosis, an elevated bilirubin level, and a positive direct antiglobulin test, consistent with WAHA. Given the hemoglobin level below 6 g per deciliter, I would immediately transfuse 1 to 2 units of ABO- and RhD-matched blood and monitor closely for evidence of a transfusion reaction (i.e., fever, chills, and worsening hemoglobinuria). I would promptly initiate treatment with glucocorticoids (starting with prednisone at a dose of 70 mg daily) and rituximab, on the basis of data from randomized trials providing support for the combination over glucocorticoid monotherapy. Given that the patient has dyspnea, tachycardia, and hypoxia, and recognizing the increased risk of venous thromboembolism associated with WAHA, testing is indicated to rule out this condition. I would monitor her hemoglobin levels every 8 hours until her symptoms improve and closely follow her lactate dehydrogenase levels and reticulocyte count. Additional blood transfusions may be necessary, with the goal of maintaining her hemoglobin level above 7 g per deciliter. Once her condition is stable, a workup for secondary causes of WAHA, including peripheral-blood flow cytometry and other testing as clinically indicated, is appropriate. She should be counseled that WAHA is often a chronic, relapsing condi-
tion, and additional therapies may be needed to achieve or maintain remission.

Dr. Brodsky reports receiving grant support and serving on an advisory board for Alexion Pharmaceuticals and receiving grant support and advisory board fees from Achillion Pharmaceuticals. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.


11. Conley CL, Lippman SM, Ness PM, Petz LD, Branch DR, Gallagher MT. Auto-


immune hemolytic anemia in adults: new insights based on a single-center experi-


matol 2017;92(8):E164-E166.


17. Shulman IA, Branch DR, Nelson JM, Thompson JC, Saxena S, Petz LD. Auto-

immune hemolytic anemia with both cold and warm autoantibodies. JAMA 1985; 253:1746-8.

18. Hill A, DeZern AE, Kinoshita T, Brod-

sky RA. Paroxysmal nocturnal haemoglob-


19. Kamesaki T, Oyamada T, Omine M, Ozawa K, Kaji E. Cut-off value of red-


22. Lechner K, Jäger U. How I treat auto-


23. Crowther M, Chan YL, Garbett IK, Lim W, Vickers MA, Crowther MA. Evi-

dence-based focused review of the treat-

ment of idiopathic warm immune hemo-


24. Birgins H, Frederiksen H, Hasselbalch HC, et al. Phase III randomized trial comparing glucocorticoid monotherapy versus glucocorticoid and rituximab in pa-

tients with autoimmune haemolytic anaem-


25. Michél M, Torriou L, Roudot-Thoraval F, et al. A randomized and double-blind controlled trial evaluating the safety and ef-
ficacy of rituximab for warm auto-immune hemolytic anemia in adults (the RAHI-


29. Maung SW, Leahy M, O'Leary HM, et al. A multi-centre retrospective study of ritux-


31. Miano M, Scalzoni M, Perri K, et al. Mycophenolate mofetil and sirolimus as second or further line treatment in chil-

dren with chronic refractory primitive or secondary autoimmune cytopenias: a sin-


32. Thabet AF, Faisal M. Pulse cyclophos-

phamide therapy in refractory warm auto-

immune hemolytic anemia: a new perspec-


33. Moyo VM, Smith D, Brodsky I, Crilley P, Jones RJ, Brodsky RA. High-dose cyclo-


34. Arenberg DC, Bussel JB, Hobbs MM, et al. Sirolimus is effective in relapsed/re-


36. Barbar S, Noventa F, Rossetto V, et al. A risk assessment model for the identifi-

cation of hospitalized medical patients at risk for venous thromboembolism: the