79-Year-Old Woman With Jaundice and Anemia

Mazie Tsang, MD; Jayme L. Dahlin, MD, PhD; and Karna K. Sundsted, MD

A 79-year-old woman was referred by her primary care physician to the emergency department for evaluation and management of a 2-week history of jaundice, bilateral leg edema, and increased dyspnea. A complete blood count (CBC) obtained by her primary care physician was remarkable (reference ranges provided parenthetically) for a hemoglobin level of 5.8 g/dL (12.0-15.5 g/dL), which had decreased from 11.0 g/dL 4 months before presentation. She reported a 2-month history of intermittent right upper-quadrant pain, generalized weakness, fatigue, increased urinary frequency, decreased appetite, and subjective fevers and chills. She described dark-colored urine that began 2 days before her admission and progressive back and hip pain during the preceding year.

Review of systems was negative for hemoptysis, melena, and hematochezia. She reported that she had had no sick contacts, recent travel, or recent illnesses. She had no history of venous thromboembolism, personal or family history of hematologic or autoimmune diseases, Raynaud's phenomenon, or association of her symptoms with cold-temperature exposure.

Her medical history was notable for severe chronic obstructive pulmonary disease, chronic hypoxia on home oxygen, history of transient ischemic attack, coronary artery disease, myocardial infarction with coronary stent placed 15 years earlier, hypertension, and hypothyroidism. Medications at the time of presentation included albuterol, apixaban, levothyroxine, lisinopril, furosemide, metoprolol, and simvastatin. She was not on any known nonprescription medications or supplements.

On admission, her blood pressure was 143/48 mm Hg; heart rate, 78 beats per min; temperature, 36.8°C (oral); and respiratory rate, 21 breaths/min. Her SpO_2 fluctuated between 85% and 95% on 3 L/min via nasal cannula. Physical examination was notable for scleral icterus, jaundiced skin, and edema to the mid-tibial region bilaterally. No lymphadenopathy was found. Her abdomen was soft, minimally distended, and had no tenderness. Her liver was palpated 4 cm below the costal margin, but no splenomegaly was present.

Hemoccult stool test results were negative. Additional laboratory studies revealed the following: mean corpuscular volume 122.2 fL (81.6-98.3 fL); red blood cell (RBC) distribution width, 22.7% (11.9%-15.5%); platelet count, 487 × 10^9/L (150-450 × 10^9/L); and leukocytes 13.4 × 10^9/L (3.5-10.5 × 10^9/L). Initial chemistry study results were notable for the following: international normalized ratio, 1.2 (0.9-1.1); prothrombin time, 13.6 sec (9.4-12.5 sec); creatinine, 0.8 U/L (38-176 U/L); NT-pro-B typenatriuretic peptide, 1793 pg/mL (10-244 pg/mL for females 79 years of age; 1800 pg/mL suggested diagnostic cutoff for acute congestive heart failure in adults >75 years of age without renal failure). Electrolytes were within reference ranges. An initial chest radiograph revealed cardiac enlargement with pulmonary venous hypertension and mild interstitial edema.

1. **Which *one* of the following is the *most appropriate* next step in the management of this patient?**
   a. RBC transfusion
   b. Serum protein electrophoresis
   c. Echocardiogram
   d. Administration of intravenous immune globulin (IVIG)
   e. Administration of intravenous glucocorticoids

Stabilizing the patient takes immediate precedence, including correcting symptomatic anemia with RBC transfusions to provide adequate tissue oxygenation.[1] Our patient exhibited symptomatic anemia, as manifested

See end of article for correct answers to questions.

Resident in Internal Medicine, Mayo Clinic School of Graduate Medical Education, Rochester, MN (M.T.); Resident in Clinical Pathology, Brigham and Women's Hospital, Boston, MA (J.L.D.); Advisor to residents and Consultant in General Internal Medicine, Mayo Clinic, Rochester, MN (K.K.S.).

by weakness, dyspnea, fatigue, and a hemoglobin level of 5.8 g/dL (12.0-15.5 g/dL). Given her history of coronary artery disease, she was transfused with 2 units of packed RBCs while medical workup proceeded.

Further diagnostic studies, such as plasma electrophoresis and an echocardiogram, are reasonable elements of the workup, but they should be performed after patient stabilization. The use of IVIG or glucocorticoids would be premature until a working diagnosis is established, because these particular interventions are associated with several adverse effects, such as hemolysis and thromboembolic events in the case of IVIG, and hyperglycemia and increased susceptibility to infection with glucocorticoids.

Additional laboratory studies were ordered to investigate her profound anemia.

2. Which *one* of the following laboratory findings would be *most consistent* with an underlying hemolysis in this patient?

a. Myoglobinuria
b. Decreased absolute reticulocyte count
c. Elevated serum lactate dehydrogenase (LDH)
d. Elevated direct bilirubin
e. Elevated serum haptoglobin

Hemoglobinuria and hemosiderinuria can accompany hemolysis, whereas myoglobinuria is more associated with rhabdomyolysis. Laboratory findings that are associated with hemolysis include anemia, an increase in the absolute reticulocyte count, the presence of microspherocytes or schistocytes on peripheral blood smears, and elevated serum LDH levels. Serum indirect bilirubin is usually elevated, and the concentration of serum haptoglobin, which binds free hemoglobin in blood plasma, is usually substantially reduced in hemolysis.

Consistent with the preceding description, our patient had the following additional laboratory test results: serum haptoglobin <14 mg/dL (30-200 mg/dL); total serum bilirubin 4.9 mg/dL (0.1-1.2 mg/dL); indirect serum bilirubin, 4.4 mg/dL (0.0-1.2 mg/dL); serum LDH, 1266 U/L (122-222 U/L); and absolute reticulocyte count, 369.7 $\times$ 10^9/L (38.1-112.6 $\times$ 10^9/L). A peripheral blood smear was notable for marked anisopoikilocytosis with markedly increased spherocytes and polychromatic cells, whereas the white blood cells and platelets were morphologically unremarkable.

Additional laboratory workup was notable for positive direct antiglobulin test (DAT) results. In this test, patient RBCs are washed to eliminate weakly binding proteins and then are reacted with titers of antiserum or monoclonal antibodies to various immunoglobulins (the most common targets being IgG and C3d). RBCs with autoantibodies will bind the anti-immunoglobulin reagents and agglutinate to yield a positive test result.[2] In our patient, the monospecific DATs were weakly positive for anticomplement and strongly positive for anti-IgG. She had positive cold agglutinin screen results with positive titers at 1:64.

3. Based on the available data, which *one* of the following is the *most likely* diagnosis for this patient?

a. Cold agglutinin disease
b. Cryoglobulinemia
c. Idiopathic autoimmune hemolytic anemia (AIHA)
d. Severe aortic stenosis
e. Drug-related AIHA

A positive DAT can be observed in cold agglutinin disease, paroxysmal cold hemoglobinuria, idiopathic AIHA, and drug-related AIHA (with drug-related AIHA being less common than idiopathic AIHA). Cold agglutinin disease is associated with acrocyanosis or Raynaud's phenomena with cold exposure, as well as IgM autoantibodies that bind RBCs at temperatures below 37°C. In cold agglutinin disease, the DAT is typically C3-positive and IgG-negative, and cold agglutinin titers are often greater than 1:1000.[3] Our patient, however, had strongly positive anti-IgG titers, and only weakly positive cold agglutinin titers (<1:64). In general, a subset of patients (approximately 5%-10%) who have AIHA present with clinical and laboratory findings that are suggestive of warm AIHA but also positive cold agglutinin titers. In many of these "mixed" cases, the magnitude of cold agglutinin titers are generally low, as was the case with our patient. The DAT result is often negative in cases of cryoglobulinemia.

Hemolytic anemia can also be drug induced, and the list of offending agents is

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extensive.[4] However, the patient was not taking any of the most commonly associated medications, such as cephalosporins and penicillin derivatives. Therefore, her presentation (ie, positive DAT results and strongly positive anti-IgG test results) is most closely associated with idiopathic AIHA. Although malfunctioning heart valves can cause hemolysis, her aforementioned echocardiogram did not reveal evidence of abnormal heart valves or cardiac blood flow.

After primary stabilization of the patient and diagnosis, several treatment strategies are available for idiopathic AIHA.[5]

4. Which *one* of the following is *most appropriate* to use as a first-line agent for treatment of AIHA?

a. Glucocorticoids
b. Rituximab
c. Glucocorticoids plus rituximab
d. Azathioprine
e. Splenectomy

Historically, single-agent glucocorticoids are a reasonable first-line option because most AIHA patients treated with steroids exhibit clinical response in the form of rising hemoglobin levels within the first few weeks of treatment. Once the hemoglobin concentration is above a certain threshold (eg, 10 g/dL), the dose is usually tapered to attain the lowest dose capable of maintaining disease remission. Second-line agents can be used for AIHA that is refractory to glucocorticoids, as well as for relapsing cases. These options include rituximab, glucocorticoids plus rituximab, azathioprine, and splenectomy. Third-line treatment options include various cytotoxic agents and immunosuppressive agents.[5]

Our patient began taking daily oral prednisone (60 mg, or 1 mg/kg), iron, and folic acid because folate and iron deficiency can accompany chronic hemolysis. She was given sulfamethoxazole-trimethoprim and omeprazole for *Pneumocystis jirovecii* and gastrointestinal ulcer prophylaxis while on prednisone, respectively.

After initiating therapy for AIHA, we investigated an underlying etiology, including a possible malignancy, because the patient also reported constitutional symptoms, including fevers, night sweats, and anorexia. AIHA can be associated with certain types of lymphoproliferative disorders (LPDs). In one study, 18% of patients with idiopathic AIHA went on to develop an LPD, with a median presentation time of 24 months.[6] Some reported risk factors for a malignant LPD include advanced age, underlying autoimmune disease, and the presence of an underlying IgM monoclonal gammopathy.

Additional evaluation was unrevealing. Her total serum protein level was 6.5 g/dL (6.3-7.9 g/dL). Serum electrophoresis results revealed only a mild polyclonal hypergammaglobulinemia. Serum immunofixation test results were negative for the presence of monoclonal protein, and a urine electrophoresis test revealed only a small abnormality in the gamma globulin fraction. Test results for serum antinuclear, double-stranded deoxyribonucleic acid, and *Mycoplasma pneumoniae* antibodies were all negative. A computed tomography (CT) scan of the abdomen and pelvis revealed lytic bone lesions with a radiographic differential of multiple myeloma versus aggressive osteoporosis; otherwise, no acute processes were found within the abdomen or pelvis.

5. Given the patient's history and findings on evaluation, which *one* of the following additional tests is the *most appropriate*?

a. Magnetic resonance imaging of the spine
b. Cerebrospinal fluid oligoclonal bands
c. Serum anti-Xa levels
d. Kidney biopsy
e. Bone marrow biopsy

In our patient, we considered an accompanying LPD, including multiple myeloma, given her advanced age, the CT findings of bone lesions, and her increased back pain. Although our patient had lytic bones lesions on CT, magnetic resonance imaging would be unlikely to yield additional diagnostic information with respect to a potential malignancy or LPD. Although cerebrospinal fluid oligoclonal bands are immunoglobulins, they are associated with multiple sclerosis, and she did not have any remarkable neurologic findings on history or examination. Anti-Xa levels, which detect presence of circulating heparins and Xa inhibitors such as apixiban, would not rule out a potential LPD or yield additional

diagnostic clues. Multiple myeloma can involve the kidneys, but this patient had normal creatinine values. A bone marrow biopsy would therefore be the most useful test in diagnosing a potential LPD. Her bone marrow biopsy results revealed hypercellular bone marrow with moderate panhyperplasia but no definitive morphologic or immunophenotypic features of an LPD, plasma cell disorder, or other malignancy.

The patient was discharged on hospital day 7 and had subsequent outpatient follow-up for her presumed idiopathic AIHA. She eventually completed 4 weekly infusions of rituximab (an anti-CD20 monoclonal antibody), based on evidence, from a single-arm prospective trial, that low doses can induce sustained responses in patients who have AIHA.[7,8] Her response was monitored with weekly CBCs. At 12 weeks postdischarge, relevant laboratory findings were as follows: hemoglobin, 11.2 g/dL; absolute reticulocyte count, 121.1 $\times$ 10^9/L; indirect serum bilirubin, 0.0 mg/dL; and serum LDH, 604 U/L. Once her hemoglobin level reached 10 g/dL, her daily prednisone dose was tapered by 10 mg each week until the dose was 20 mg per day, after which her taper was adjusted to 5 mg each week.

DISCUSSION

Hemolysis can be broadly organized into intra- and extra-corpuscular etiologies. The former are usually hereditary, not acquired, and include defects in hemoglobin, membrane components (eg, congenital spherocytosis), and metabolism (eg, pyruvate kinase, glucose-6-phosphate dehydrogenase deficiencies), all of which can make the red cells susceptible to oxidant-mediated damage/hemolysis. The latter are usually an acquired process that destroys otherwise normal RBCs. Etiologies include antibodies directed against RBC components, splenic destruction, mechanical trauma, excessive oxidant exposure, and certain pathogens.

Autoimmune hemolytic anemia is an uncommon yet potentially fatal autoimmune phenomenon caused by autoantibodies that bind to the surface of RBCs, targeting them for destruction by the immune system. Many cases are idiopathic (approximately 50%), although some are linked to LPD, autoimmune diseases, drugs, or infection. Once AIHA is suspected clinically, the highest-yielding assay is generally considered to be the DAT, which detects the presence of RBC-binding autoantibodies.[2]

Given the seminal importance of the DAT in diagnosing AIHA, the possibility of DAT-negative AIHA is important to note. Such false-negative readings can result from the following: (1) IgG sensitization below the threshold of detection; (2) low-affinity IgG removed by preparatory washes that are not conducted at 4°C or are at low ionic strength; and (3) by red-cell sensitization by IgA or IgM alone, but without complement fixation.[9] If a DAT result is negative, yet clinical suspicion for AIHA is still high, reasonable next steps include ruling out plausible alternative diagnoses and conducting secondary tests for hemolysis (eg, augmented sensitivity tests, DATs with alternative washing steps, DATs with anti-IgA sera), which often need to be sent out to reference laboratories.[9]

Empiric treatment of suspected DAT-negative AIHA may be warranted, with treatment being the same as that for DAT-positive AIHA.[9] Patients who were treated for DAT-negative AIHA had outcomes similar to those of patients who had DAT-positive AIHA; in addition, they required a lower dose of steroid maintenance therapy and typically had a milder anemia and hemolysis compared with patients with DAT-positive AIHA.[10]

Polyspecific DAT reagents, which include antisera to both human IgG and C3d, are often used for screening purposes in transfusion medicine. Monospecific DATs, which contain antisera to either IgG or C3d (plus IgM and/or IgA in more specific cases), can be useful for narrowing the differential diagnosis in DAT-positive hemolytic anemias. Specifically, complement-positive DATs are more highly suggestive of paroxysmal cold hemoglobinuria or cold agglutinin disease, whereas IgG-positive DATs are more suggestive of warm antibody or drug-related hemolytic anemias.

Clinicians should search for an underlying cause in cases of idiopathic AIHA. A reasonable workup includes a detailed history for drug-induced AIHA, infection, autoimmune disease, and malignancy, as well as supportive laboratory diagnostics, such as autoimmune

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and infectious serology testing. A search for an underlying LPD or other malignancy by physical examination (eg, lymphadenopathy, splenomegaly), abdominal imaging, immunoglobulin testing (eg, serum protein electrophoresis, IFE), and a bone marrow biopsy, along with its ancillary studies, may be warranted.

The first-line treatment of warm AIHA is to administer glucocorticoids, which generate initial responses in most patients. In refractory cases, clinicians typically employ splenectomy or rituximab with or without glucocorticoids as second-line therapies that have been found to have short-term efficacy. Third-line agents, for which the evidence of efficacy is of considerably lower quality, include immunosuppressive agents (eg, azathioprine, cyclophosphamide), danazol, and IVIG.[11]

The prognosis of AIHA can depend on its underlying etiology, such as the presence of an LPD. In one study, after a mean follow-up of approximately 4 years, 47% of patients obtained complete remission and were no longer receiving treatment, 25% had complete or partial remission with <10 mg/day of glucocorticoids, and 31% still had active disease.[12]

CONCLUSION

This case reinforces the most appropriate approach to diagnosis and management of symptomatic AIHA, highlighting the importance of using laboratory medicine and hematopathology, choosing the proper diagnostic tests, and conducting timely red-cell transfusions. In our patient, diagnosis was achieved by integrating the following: CBC (hemoglobin, absolute reticulocytes, RBC distribution width); a peripheral smear (spherocytes); serum studies (bilirubin, LDH, haptoglobin); autoantibodies (DAT); bone marrow biopsy; and clinical immunology (immunoglobulin electrophoresis). Lastly, this case emphasizes that a complete workup should evaluate for causes of AIHA, such as LPDs.

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Correspondence: Address to Karna K. Sundsted, MD, Division of General Internal Medicine, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (sundsted.karna@mayo.edu).

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CORRECT ANSWERS: 1. a. 2. c. 3. c. 4. a. 5. e.