CLINICAL PROBLEM-SOLVING

The Essential Element

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In this Journal *feature, information about a real patient is presented in stages (boldface type) to an expert clinician, who responds to the information, sharing his or her reasoning with the reader (regular type). The authors' commentary follows.*

A 21-year-old female college student presented with a 10-day history of progressive fatigue, weakness, light-headedness, exertional dyspnea, and dark-colored urine, followed by an episode of syncope without injury. She reported no vomiting or diarrhea, and her oral intake had not decreased.

A cardiopulmonary disorder or anemia could explain the dyspnea, fatigue, weakness, and syncope. Dark urine could indicate concentrated urine due to volume depletion, hemoglobinuria arising from intravascular hemolysis, myoglobinuria arising from rhabdomyolysis, or bilirubinuria.

The patient had a history of irregular menstrual periods without menorrhagia. Her only medication was an oral contraceptive. She did not use tobacco, alcohol, herbal supplements, illicit substances, or intravenous drugs. The patient was not sexually active. She reported no recent travel. There was no known family history of disease. On physical examination, the temperature was 38.1°C (100.6°F), the heart rate 105 beats per minute and regular, the respiratory rate 18 breaths per minute, the blood pressure 127/59 mm Hg, and the oxygen saturation 99% while she was breathing ambient air. There was scleral icterus. Auscultation of the heart revealed regular tachycardia without extraneous sounds. The patient was alert and oriented, with normal strength, sensation, gait, and coordination. The remainder of the examination was normal.

Laboratory analysis revealed a white-cell count of 47,300 per cubic millimeter, with 67% polymorphonuclear cells and 7% band forms. The hemoglobin level was 3.9 g per deciliter, the mean corpuscular volume 136 fl, and the platelet count 249,000 per cubic millimeter. Reticulocytes accounted for 36% of erythrocytes. The serum creatinine level was 2.1 mg per deciliter (190 μmol per liter), the albumin level 2.6 g per deciliter, the aspartate aminotransferase level 50 U per liter (normal range, 19 to 45), the alanine aminotransferase level 9 U per liter (normal range, 8 to 37), the alkaline phosphatase level 8 U per liter, the total bilirubin level 6.8 mg per deciliter (120 μmol per liter), and the direct bilirubin level 2.7 mg per deciliter (46 μmol per liter). The international normalized ratio (INR) was 1.6, and the partial-thromboplastin time was 38.0 seconds (normal range, 22.0 to 32.0). Urinalysis showed 3+ blood and 2+ protein; there were 4 to 10 erythrocytes per high-power field.

Three units of packed erythrocytes were transfused, and the hemoglobin level increased to 7.8 g per deciliter. A peripheral-blood smear obtained after the transfusion revealed immature granulocytes and nucleated erythrocytes, marked anisopoikilocytosis, and numerous spherocytes and echinospherocytes (Fig. 1).

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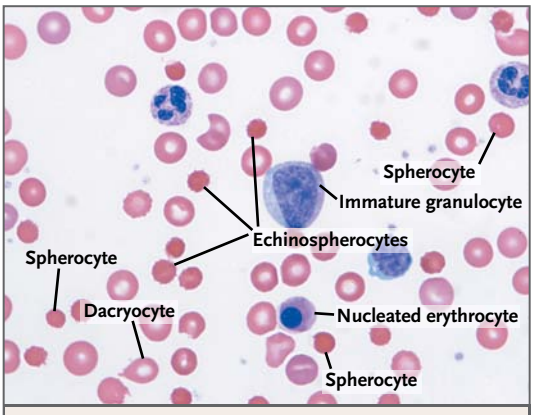

Figure 1. Peripheral-Blood Smear at the Time of Admission.

This smear, obtained after an erythrocyte transfusion, reveals immature granulocytes and nucleated erythrocytes, marked anisopoikilocytosis, and numerous spherocytes and echinospherocytes.

Profound anemia explains the presenting symptoms. The indirect hyperbilirubinemia and reticulocytosis suggest hemolysis (although direct hyperbilirubinemia would not be expected), and the fact that the elevated aspartate aminotransferase level is out of proportion to the alanine aminotransferase level corroborates that diagnosis. Hemolytic anemia may be acquired through immune, microangiopathic, or infectious processes or may be congenital owing to an abnormality in the erythrocyte membrane, enzymes, or hemoglobin. Spherocytes are seen in immune-mediated hemolysis and hereditary spherocytosis. Spherocytes, irregularly contracted erythrocytes, and bite cells can be identified in cases of oxidative hemolysis related to severe burns or glucose-6-phosphate dehydrogenase (G6PD) deficiency. Echinospherocytes may be seen after an erythrocyte transfusion, since erythrocytes may change shape during storage.

Given the fever, leukocytosis, and coagulopathy, the possibility of sepsis triggering disseminated intravascular coagulation should be addressed immediately. The elevated bilirubin and aminotransferase levels, coagulopathy, and hypoalbuminemia are also compatible with chronic liver disease. The patient's only hepatic risk factor is her use of an oral contraceptive, which can cause cholestasis. Clinicians must also be cognizant of the possibility of unreported hepatotoxic exposures.

The leukocytosis may also reflect a leukemoid reaction, which may occur in association with hemolysis, solid tumors, or infection. The marked reticulocytosis indicates a dynamic marrow response and most likely explains the macrocytosis. The smear does not contain blast cells suggestive of acute leukemia. The kidney injury may indicate pigment nephropathy due to intravascular hemolysis.

The serum iron level was 111 μg per deciliter (19.9 μmol per liter), the total iron-binding capacity 190 μg per deciliter (34 μmol per liter), and the transferrin saturation 58%. The vitamin B_{12} level was 1790 pg per milliliter (1321 pmol per liter), the folic acid level 12.7 ng per milliliter (28.8 nmol per liter), the serum lactate dehydrogenase level 735 U per liter (normal range, 120 to 240), and the serum haptoglobin level less than 8 mg per deciliter (normal range, 22 to 239). Results of the direct antiglobulin test were negative. Beta human chorionic gonadotropin was not detectable in the urine. The electrocardiogram and chest radiograph were unremarkable. Ultrasonography of the abdomen revealed thickening of the gallbladder wall; the liver, spleen, and kidneys were normal.

The elevated lactate dehydrogenase level and low haptoglobin level support a diagnosis of hemolysis; assessment for free hemoglobin in urine or plasma is a more specific way to distinguish between intravascular and extravascular hemolysis. The negative result on the direct antiglobulin test helps rule out autoimmune hemolytic anemia, although this test is negative in up to 10% of cases. Hereditary spherocytosis is unlikely given this patient's dramatic presentation in adulthood and the absence of splenomegaly. Gallbladder thickening makes acute cholecystitis possible. Given this finding and the abnormal liver-function tests, computed tomography (CT) or cholescintigraphy could be considered.

The patient's hemoglobin level rapidly declined to 4.8 g per deciliter. Bone marrow core biopsy and aspiration revealed 70% cellularity and trilineage hematopoietic maturation without evidence of dysplasia or neoplasm. Blood and urine cultures were sterile.

Intravenous methylprednisolone (1 mg per kilogram of body weight daily) was initiated for presumed autoimmune hemolytic anemia on the

second hospital day. However, the patient continued to require multiple erythrocyte transfusions for refractory anemia. The G6PD level was normal, as were the results on a blood smear to detect parasitic organisms, serologic testing for mycoplasma, an enzyme-linked immunosorbent assay for the human immunodeficiency virus, hemoglobin electrophoresis, flow cytometry for paroxysmal nocturnal hemoglobinuria, karyotyping, and a repeat direct antiglobulin test. The serum creatinine level peaked at 2.7 mg per deciliter (240 μmol per liter) on the fourth hospital day and subsequently normalized.

Given the concern for infection and the negative results of direct antiglobulin testing on two separate occasions, it would have been reasonable to withhold glucocorticoid therapy. The bone marrow examination shows no evidence of a hematologic cancer that might explain the leukocytosis and confirms that the marrow is not the source of the anemia. A normal G6PD level during hemolysis does not rule out this enzyme deficiency, since the younger, surviving erythrocytes are usually replete with G6PD; a repeat test in the weeks after the illness, when the entire spectrum of erythrocytes is present, is often required. Consideration of a possible underlying liver disease might illuminate a unifying diagnosis. In a young patient with intravascular hemolysis, liver dysfunction, and a very low alkaline phosphatase level, Wilson's disease should be considered.

The platelet count decreased to a nadir of 141,000 per cubic millimeter on the fifth hospital day. The serum fibrinogen level, results of serologic testing and a polymerase-chain-reaction assay for parvovirus, and the activity of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) were normal. A qualitative D-dimer test was positive, and the level of fibrin split products was elevated. The patient was given one dose of intravenous rituximab (375 mg per square meter of body-surface area) for presumed refractory hemolytic anemia on the seventh hospital day. The hemoglobin level stabilized at 8.4 g per deciliter thereafter, and on the ninth hospital day the patient was transitioned from methylprednisolone to prednisone with prolonged tapering. She was discharged on the 10th hospital day. She received three additional weekly doses of rituximab, and her symptoms resolved. Laboratory results 4 weeks after discharge revealed a hemoglobin level of 12.1 g per deciliter, a platelet count of 203,000 per cubic millimeter, a lactate dehydrogenase level of 244 U per liter, a haptoglobin level of less than 8 mg per deciliter, an aspartate aminotransferase level of 59 U per liter, an alanine aminotransferase level of 79 U per liter, and a total bilirubin level of 2.2 mg per deciliter (38 μmol per liter).

Disseminated intravascular coagulation appears to be unlikely given the normal fibrinogen level; elevations in the levels of D-dimer and fibrin split products are nonspecific findings and are common in hospitalized patients. ADAMTS13 activity was normal, whereas it is characteristically low in acquired thrombotic thrombocytopenic purpura.

Rituximab, a monoclonal antibody against CD20 on B cells, can be an effective second-line treatment for autoimmune hemolytic anemia in patients who have no response or an insufficient response to glucocorticoid therapy; the average response time is 4 to 6 weeks from the time of the first infusion. The rapid improvement in this patient after the administration of rituximab is atypical but may suggest that autoimmunity, independently of or in association with an underlying lymphoproliferative disorder, is playing a role. A partial response to rituximab raises the possibility of lymphoma, but results of basic imaging tests and bone marrow examination do not suggest an underlying hematologic cancer.

Although several test results appear to be consistent with hemolysis, the pattern of the aminotransferase levels — that is, the level of alanine aminotransferase being greater than that of aspartate aminotransferase — along with the elevated bilirubin level and coagulopathy continue to suggest an underlying hepatic disorder. Screening for Wilson's disease is warranted, with measurements of serum ceruloplasmin and urinary copper levels and an ophthalmologic examination for Kayser–Fleischer rings. Autoimmune hepatitis is possible, although the aminotransferase levels have not improved despite the use of potent immunosuppressive medications.

Three months after admission, the patient noted dark-colored urine, fatigue, and scleral icterus that progressed to jaundice. The white-cell count was 20,100 per cubic millimeter, the hemoglobin level 10.1 g per deciliter, and the platelet count

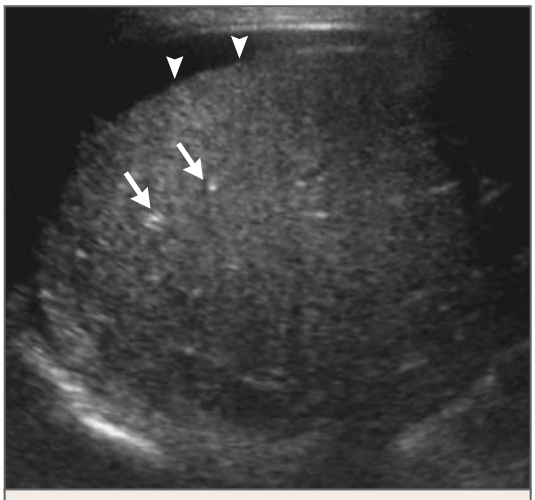

Figure 2. Ultrasonogram of the Liver.
The ultrasonogram shows multiple echogenic nodules (arrows), subtle micronodularity of the liver surface (arrowheads), and parenchymal heterogeneity.

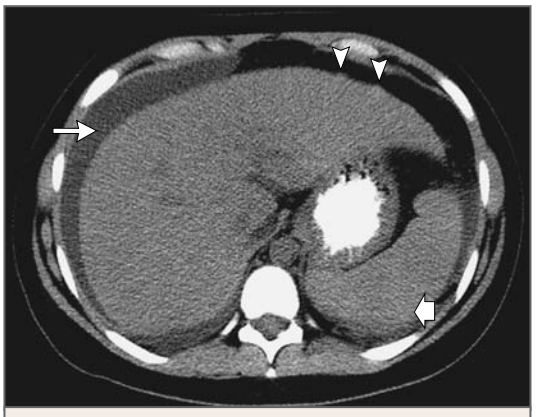

Figure 3. CT Scan of the Abdomen and Pelvis.
The scan shows moderate abdominal ascites (thin arrow), borderline splenic enlargement (thick arrow), and subtle micronodularity of the liver parenchyma (arrowheads).

189,000 per cubic millimeter. Reticulocytes accounted for 5.3% of erythrocytes. The serum creatinine level was 0.4 mg per deciliter (35 μmol per liter), the albumin level 2.6 g per deciliter, the aspartate aminotransferase level 123 U per liter, the alanine aminotransferase level 40 U per liter, the alkaline phosphatase level 57 U per liter, the total bilirubin level 28.3 mg per deciliter (484 μmol per liter), the direct bilirubin level 18.7 mg per deciliter (320 μmol per liter), the INR 2.2, and the partial-thromboplastin time 39.6 seconds.

The leukocytosis, anemia, and reticulocytosis are less pronounced than when this patient first presented but are still compatible with a leukemoid reaction and intravascular hemolysis; repeat examination of the peripheral-blood smear is warranted. Acute-on-chronic liver dysfunction can explain the coagulopathy. Given her immunosuppression, the search for a possible acute bacterial infection such as cholangitis should again be a priority. If infection is ruled out, Wilson's disease is the leading consideration in this young woman with severe liver disease and hemolysis. Relapse of a partially treated lymphoproliferative disorder or autoimmune disorder — both of which could have been stabilized with glucocorticoids and rituximab — are less likely possibilities.

Repeat ultrasonography of the abdomen revealed innumerable small echogenic liver nodules, a slightly nodular liver contour, a 12.7-cm spleen (borderline enlarged), and normal hepatic blood flow (Fig. 2). Reevaluation of the original ultrasonogram disclosed subtle heterogeneity of the liver parenchyma. CT of the abdomen and pelvis with the administration of contrast material revealed moderate ascites and hepatic micronodularity (Fig. 3). The serum ferritin level was 4304 ng per milliliter. Testing was negative for Epstein–Barr virus, hepatitis B and C viruses, anti–smooth-muscle antibody, antimitochondrial antibody, anti–liver–kidney microsomal antibody, and antinuclear antibody. The serum ceruloplasmin level was less than 4 mg per deciliter (normal range, 18 to 42).

The low serum ceruloplasmin level suggests but does not definitively establish the diagnosis of Wilson's disease. Detection of Kayser–Fleischer rings, a low serum copper level, or an elevated urinary copper level would corroborate the diagnosis. If Wilson's disease is confirmed, clinical and biochemical screening of first-degree relatives is advised, given the autosomal recessive pattern of transmission of this disease.

A 24-hour urinary copper level was 4703 μg (normal value, <55), and ophthalmologic slit-lamp examination revealed Kayser–Fleischer rings. The patient was given zinc acetate and trientine, but progressive renal insufficiency, liver dysfunction, thrombocytopenia, and coagulopathy developed.

She was treated with plasmapheresis and hemodialysis. On the sixth hospital day, she underwent orthotopic liver transplantation. Pathological examination of the explanted liver revealed cirrhosis with stainable copper in periseptal hepatocytes (Fig. 4). The hepatic copper concentration was 526 μg per gram of liver (dry weight) (normal range, 10 to 35). One year later, the patient reported no symptoms, and she had normal kidney and liver function.

Genetic testing revealed a known pathogenic heterozygous stop mutation in exon 2 (c.331C→T) and a novel mutation in intron 4 (IVS4-34G→A) of the ATP7B gene. The patient had one sibling, an 18-year-old sister, who was tested and found to have elevated aminotransferase levels, low serum ceruloplasmin and copper levels, and the same *ATP7B* mutations. With zinc acetate therapy, the sister's liver biochemical values have nearly normalized.

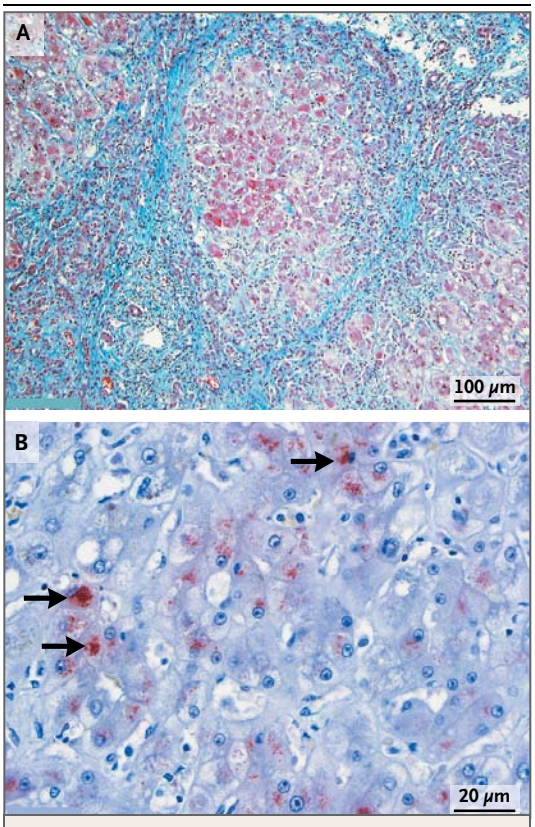

Figure 4. Histopathological Specimen of the Explanted Liver.

Panel A (trichrome stain) shows fibrosis that is consistent with cirrhosis. Panel B (rhodanine stain) shows a small amount of copper, identified as red–brown cytoplasmic granules (arrows), in periseptal hepatocytes.

COMMENTARY

Wilson's disease, an autosomal recessive disorder characterized by ineffective hepatic copper metabolism, affects approximately 1 in 40,000 people. It is caused by inactivating mutations in the ATP7B gene, which encodes a copper-transporting adenosine triphosphatase. Deficiency of this protein leads to impaired excretion of copper into bile and reduced incorporation of copper into precursors of ceruloplasmin, the major copper-carrying protein in the circulation. Copper accumulates in hepatocytes, is released into the plasma, and is deposited in other organs, most notably the brain, eyes, kidneys, and skin.[1]

Therapies for Wilson's disease include chelating agents that induce urinary copper excretion (e.g., trientine, penicillamine) and copper-absorption inhibitors (e.g., zinc acetate). Penicillamine should be avoided in patients with neuropsychiatric symptoms, since up to 50% of patients have worsening of these symptoms, which are frequently irreversible. Zinc is approved for maintenance therapy and is often used alone in presymptomatic patients or those with isolated aminotransferase elevations. In patients with mild-to-moderate hepatic dysfunction, copper-chelating agents with or without zinc are effective for rapid reduction of copper levels. Each of these agents has been reported to reduce morbidity and mortality among patients with Wilson's disease but must be administered indefinitely to prevent reaccumulation of copper. Tetrathiomolybdate is an experimental drug that forms stable complexes with copper and albumin and thus decreases copper absorption and deposition. If severe liver dysfunction has developed, medications are typically ineffective, and liver transplantation may be required, as it was in this patient.

Wilson's disease is fatal without treatment, but wide variations in its clinical presentation pose a challenge to rapid diagnosis.[2] Patients with Wilson's disease may present to the generalist, gastroenterologist, rheumatologist, neurologist, psychiatrist, ophthalmologist, or hematologist. More than half of patients have hepatic abnormalities, which can include elevations of liver enzyme levels, hepatomegaly, hepatitis, cirrhosis, and acute liver failure.[3] About one third of patients have neurologic manifestations —

specifically, movement disorders (e.g., dystonia, tremor, and ataxia), dysarthria, dysphagia, and memory loss. Psychiatric abnormalities, seen in approximately 10% of patients, include behavioral disturbances, depression, and psychosis. Copper deposition in the eye results in Kayser–Fleischer rings (golden-brown rings at the corneoscleral junction) or sunflower cataracts (radiating, multicolored central opacities); these ocular findings, which do not interfere with vision, are more common in patients with neurologic symptoms than in those without such symptoms. They are best evaluated on slit-lamp examination, but in some cases of advanced disease, they can be seen with the naked eye.

Parenchymal injury from the oxidative effect of copper leads to hepatocellular damage and elevated aminotransferase levels. The normal or low level of alkaline phosphatase is potentially explained by oxidative damage from free radicals or by competition at the active site of the alkaline phosphatase enzyme.[4] The combination of a ratio of alkaline phosphatase to total bilirubin of less than 4 and a ratio of aspartate aminotransferase to alanine aminotransferase of more than 2.2 was reported to have 100% sensitivity and specificity for the diagnosis of Wilson's disease in a small study involving patients with acute liver dysfunction, but these results were derived post hoc and require validation.[5]

The combination of unexplained intravascular hemolysis and liver dysfunction should prompt consideration of Wilson's disease. A negative result on the direct antiglobulin test in a patient with intravascular hemolysis should prompt an investigation for alternative causes of the hemolysis, although extravascular hemolytic anemia with a negative result on this test may occur in 5 to 10% of cases.[6] This patient had intravascular hemolytic anemia with a negative result on the direct antiglobulin test before the diagnosis of Wilson's disease was made — a presentation that has been reported in only 7 to 12% of patients.[7-10] Hepatic necrosis releases massive quantities of copper that inhibit erythrocyte enzymes and cause oxidative damage to erythrocyte membranes.[11] The resulting denatured hemoglobin forms intracellular inclusions that can be identified on the peripheral-blood smear by means of a specialized Heinz-body preparation.[12] Other findings consistent with oxidation-induced hemolysis include spherocytes, irregularly contracted erythrocytes, and bite cells.[13]

An elevated white-cell count, as observed in our patient, is not a component of current diagnostic guidelines for Wilson's disease[7] but has been reported to be an independent predictor of mortality among children with Wilson's disease and acute liver failure.[14] A leukemoid reaction can occur in cases of severe hemolytic anemia.[15] We suspect that the hemolytic episodes in our patient were driven by elevations in serum copper levels during times of hepatic necrosis and that the improvement was coincidentally rather than causally related to rituximab, which would not be expected to mitigate oxidative injury.

In hindsight, the combination of intravascular hemolysis and liver disease in a young patient would seem to point clearly to the diagnosis of Wilson's disease. However, the case was challenging to solve as it unfolded in real time. Hemolysis is an unusual initial manifestation of this uncommon disease, the biochemical manifestations of hemolysis overlapped with those of hepatic disease, extensive initial evaluations for both conditions were unrevealing, and there appeared to be a clinical response to immunosuppressive therapy (suggesting disorders other than Wilson's disease). Ultimately, the pattern of worsening liver function, hemolytic anemia, and corroborating test results established that excess copper was the essential element linking the multisystem abnormalities in this patient.

Dr. Saint reports receiving payment for board membership from and holding stock in Doximity. No other potential conflict of interest relevant to this article was reported.

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

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