A 60-year-old woman presented with a 6-month history of progressive fatigue and frequent headache. The fatigue was worse in the afternoon and prevented the patient from performing her regular daily activities. She reported occasional blood-tinged urine and hematochezia, and no hematemesis or melena. Her medical history was notable for an unprovoked lower-extremity deep vein thrombosis (DVT) 1 year earlier, for which she had completed 6 months of warfarin therapy. Medications included omeprazole, zolpidem, and a daily calcium/vitamin D supplement.

On physical examination, she appeared to be in no distress. Her vital signs included the following: temperature 36.7°C; pulse rate 67 beats/minute; respiratory rate 18 breaths/minute; blood pressure 160/85 mm Hg; and oxygen saturation 97%, breathing room air. Lung auscultation revealed normal breath and heart sounds, without murmurs. Abdominal examination revealed marked tenderness in the left upper quadrant with no organomegaly. Tone, power, reflexes, and sensation were intact on neurologic examination, with no evidence of lateralizing. No evidence was found of petechiae, purpura, ecchymosis, or frank lymphadenopathy. The remainder of the physical examination was unremarkable.

Laboratory studies revealed the following (reference ranges provided parenthetically): leukopenia (white blood cell count 2.8 × 10^9/L [3.5-10.5 × 10^9/L]) with normal differential; macrocytic anemia (hemoglobin 10.9 g/dL [12.0-15.5 g/dL]); mean corpuscular volume 101.2 fL (81.6-98.3 fL); and a platelet count of 160 x 10^9/L (150-450 x 10^9/L). An electrolyte panel revealed a sodium level of 144 mmol/L (135-145 mmol/L); a potassium level of 4.0 mmol/L (3.6-5.2 mmol/L); a random glucose concentration of 81 mg/dL (70-140 mg/dL); a blood urea nitrogen level of 19 mg/dL (0.84-1.21 mg/dL); and a creatinine level of 0.9 mg/dL (0.8-1.3 mg/dL). Serum lactate dehydrogenase was 278 U/L (122-222 U/L), and serum haptoglobin was <14 mg/dL (30-200 mg/dL). Her percentage of reticulocytes was 2.2% (0.77%-2.36%) (absolute reticulocyte count was 70,400/cmm [19,500-125,750/cmm]). A blood smear showed moderate macrocytes and anisocytosis. Serum B12, folate, ferritin, and thyroid-stimulating hormone were within reference range. Total bilirubin level was 1.1 mg/dL (0.3-1.1 mg/dL) with direct bilirubin being 0.1 mg/dL (0.0-0.3 mg/dL). Serum alkaline phosphatase was 72 U/L (37-98 U/L), and aspartate aminotransferase was 18 U/L (8-43 U/L). A direct antiglobulin (Coombs) test result was negative. A splenic ultrasound was performed, given the left upper quadrant tenderness on abdominal examination, and showed a spleen measuring 11.7 x 10.4 x 4.8 cm (within normal limits), with no splenic lesions or other radiologic abnormalities.

1. Which one of the following diagnoses most likely fits the patient’s clinical presentation?
   a. Paroxysmal nocturnal hemoglobinuria
   b. Antiphospholipid syndrome
   c. Major depressive disorder
   d. Hyperviscosity syndrome
   e. Acute myeloid leukemia

This patient presents with evidence of intravascular hemolysis (low haptoglobin, elevated lactate dehydrogenase, macrocytosis), and a history of thrombosis. These features are common presenting manifestations of paroxysmal nocturnal hemoglobinuria (PNH), with hemoglobinuria seen in 62% of cases. The most common presenting symptoms of PNH include fatigue (80%) and headache (63%). Between 29% and 44% of diagnosed patients suffer at least 1 thromboembolic event during the course of the disease. Other complications include aplastic anemia and myelodysplastic...
syndrome. Although antiphospholipid syndrome could explain a history of unprovoked thrombosis, no other findings for this patient suggest the presence of the syndrome. Clinical depression could present with prolonged fatigue and vague symptoms of pain; however, it would not explain the presence of hemolytic anemia in this patient. Hyperviscosity should be considered, given the differential in patients with headache and anemia, but the absence of paraproteinemia makes this possibility less likely. Fundoscopic evaluation and serum viscosity in the appropriate setting would be helpful in making this diagnosis. Acute myeloid leukemia is a known complication of PNH, but the absence of blasts in peripheral blood suggests that this may not be present. Acute myeloid leukemia by itself does not explain the presence of intravascular hemolysis.

The constellation of fatigue, hemolytic anemia, and history of DVT in this patient was suggestive of the presence of PNH. Nevertheless, because PNH is so rare, other causes for hemolytic anemia should be investigated (Supplemental Table, available online at http://www.mayoclinicproceedings.org). Urinalysis was obtained and demonstrated free hemoglobin in the urine.

2. Which one of the following tests would be most appropriate to confirm the suspected diagnosis in this patient?
   a. Clinical diagnosis alone; no further laboratory testing necessary
   b. Flow cytometry with monoclonal antibodies to CD55 (decay accelerating factor) or CD59
   c. Sucrose lysis test
   d. Bone marrow biopsy
   e. Urinary protein electrophoresis

A diagnosis of PNH should be considered in patients with intravascular hemolysis and a negative Coombs test result. A diagnosis of PNH requires confirmation by additional laboratory testing. In PNH, the patient’s red blood cells are hemolysed by the complement pathway, owing to partial or complete absence of glycosyl phosphatidyl inositol (GPI)—linked proteins on the surface of the cells. These missing proteins are the result of a somatic mutation in the phosphatidylinositol N-acetylglicosaminyltransferase subunit A (PIG-A) gene along the X chromosome. At least 12 GPI-linked proteins have been identified that are lacking in patients with PNH, with CD55 and CD59 being involved in the complement pathway. The identification of deficiency in GPI-linked proteins in PNH has resulted in the development of flow cytometric methods for diagnosis. Before detection of these missing GPI-linked proteins, PNH was diagnosed using either the sucrose lysis test or the Ham acid hemolysis test, which measure hemolysis in a patient’s serum after agents that activate complement have been added. The use of flow cytometry has now made these less-quantifiable tests obsolete.

Bone marrow biopsy may be performed in patients with PNH if aplastic anemia is suspected. Marrow-derived cells are at various stages of hematopoietic maturation and therefore have variable expression of GPI anchor proteins compared with the mature cells from the peripheral blood. Because of this variability, peripheral blood cells are actually preferred for flow cytometric diagnosis. Urine protein electrophoresis has no role in the diagnosis of PNH.

In this patient, flow cytometry was performed on red blood cells, granulocytes, and monocytes and confirmed the presence of a PNH clone of 3.25% in red blood cells, 15% in granulocytes, and 13% in monocytes.

3. Which one of the following is the most appropriate treatment modality for this patient’s condition?
   a. Scheduled, recurrent packed red cell transfusions
   b. Anticoagulation with warfarin indefinitely
   c. Immediate bone marrow transplant
   d. Eculizumab
   e. Corticosteroids

Patients with PNH may receive blood transfusions if they develop clinically relevant anemia. However, transfusions alone will not improve quality of life or lower the risk of thromboembolic events in these patients. In addition, blood transfusions should be used with caution, as they may result in transient hemoglobinuria due to hemolysis of the patient’s complement-sensitive red cells. The use of long-term anticoagulation in patients...
with PNH who have recurrent thromboembolic events may be recommended; however, this approach should always be combined with definitive therapy for the underlying condition. In the absence of simultaneous treatment for PNH, anticoagulation would not be the best treatment option for this patient. Bone marrow transplantation may be considered for patients with severe complications from PNH; however, it is associated with substantial morbidity and mortality and should be considered only for severe, refractory disease.

Eculizumab is a monoclonal antibody that selectively targets and inhibits the complement cascade by binding to the terminal complement component, C5. In doing so, eculizumab prevents red cell hemolysis as well as platelet activation. Since its introduction, eculizumab has drastically reduced the risk for thrombosis, decreased transfusion dependence, and substantially improved quality of life for patients with PNH. Corticosteroids can improve hemoglobin levels and reduce hemolysis in some patients with PNH, but their long-term toxicity and limited efficacy make them less favorable.

Owing to this patient’s history of prior DVT and disabling symptoms of fatigue, eculizumab was initiated soon after diagnosis. She was additionally restarted on warfarin for secondary prevention of thromboembolic events.

4. Which one of the following adverse effects should this patient have been counseled about before initiation of eculizumab?
   a. Headaches
   b. Persistent pyrexia
   c. Risk of seizure
   d. Osteoporosis
   e. QT prolongation

   Headaches are commonly noted after the first 2 doses of eculizumab, but they resolve later. Other common adverse effects experienced by patients include abdominal pain and diarrhea. Research reviewing adverse events in patients using eculizumab has found that patients are more likely to experience adverse effects early, with no evidence for cumulative toxicity with long-term administration of eculizumab. Disclosing the potential risk of headache in this patient would be especially important, given that she was already experiencing frequent migraines.

Although pyrexia can occur with eculizumab, it is usually mild and thought to be related to viral infection. Eculizumab has never been shown to be associated with seizure activity, osteoporosis, or QT prolongation.

The potential benefits and adverse effects of eculizumab were thoroughly discussed with this patient before starting therapy. She developed an increase in the frequency of headaches during the first several weeks of treatment; this increase later resolved.

5. Which one of the following infections may this patient be at risk after starting eculizumab?
   a. Neisseria meningitidis
   b. Human immunodeficiency virus
   c. Fungal infections
   d. Mycobacterium tuberculosis
   e. Clostridium difficile

   By inhibiting the complement cascade, eculizumab is thought to increase risk for infection with encapsulated organisms, including Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis. This theory stems from a known increased frequency of Neisserial infection in individuals who have inherited deficiency of terminal complement proteins. Although not a common adverse effect of treatment, Neisserial sepsis is the most serious potential complication of eculizumab therapy. Patients should therefore be vaccinated against N meningitidis 2 weeks before therapy begins and should be revaccinated every 3 to 5 years after starting treatment. They should also be warned that the risk of Neisserial sepsis is 0.5% per year, even after vaccination, and that they should seek immediate medical attention if they develop symptoms of infection. Certain areas of the world, including the United Kingdom, have a high prevalence of Neisseria serogroup B, which is not susceptible to the current tetra- valent vaccine. In these areas, prophylactic antibiotic treatment with penicillin may be advised.

Although eculizumab is believed to increase risk for encapsulated organisms, no evidence has indicated that patients are at increased risk for human immunodeficiency
virus, fungal infections, mycobacterium, or other bacterial infections. Our patient was counseled on the potential risk of infection and was vaccinated against *N meningitidis* before starting eculizumab. She was seen again in the hematology clinic 6 months after initiating therapy and reported improvement in her fatigue and migraines. Her hemoglobin at that time had increased to 13.7 g/dL.

**DISCUSSION**

A high level of clinical suspicion is required to diagnose PNH, given that presenting symptoms may be indolent and nonspecific. Further, PNH is a rare disorder, with an estimated incidence of 1 to 10 cases per million per year, so it may remain undiagnosed. The average length of time from symptom onset to diagnosis is 1 to 2 years. In a survey of patients with PNH, 79% reported seeing more than 1 provider before PNH diagnosis. The median age of onset is in the thirties, with nearly equal distribution in men and women.

Paroxysmal nocturnal hemoglobinuria is an acquired clonal disorder that stems from a somatic mutation in the *PIGA* gene on the X chromosome. The gene encodes an enzyme required for the biosynthesis of the GPI anchor. The GPI anchor is a glycolipid that anchors cell surface proteins, including CD55 and CD59. Paroxysmal nocturnal hemoglobinuria occurs when *PIGA* gene mutations occur in self-renewing progenitor stem cells in the bone marrow, producing erythroid, myeloid, and lymphoid cell lineages with missing cell surface proteins. Both CD55 and CD59 are glycoproteins involved in complement inactivation; in their absence, PNH cell clones are greatly susceptible to lysis mediated by the complement cascade. CD55 accelerates destruction of C3 convertase, thereby reducing the amount of cleaved C3, which physiologically slows the alternative pathway of complement activation. CD59 interacts directly with the membrane-attacking complex, blocking C9 aggregation and preventing lytic pore formation.

Complement-mediated lysis is more likely at a lower pH. This explains the morning hematuria reported by many patients, as hemolysis is prevalent at night, with a transient rise of carbon dioxide levels in the setting of physiologic hypoventilation. As the PNH clone establishes dominance, signs of bone marrow failure may develop.

The major clinical manifestation of PNH is anemia. This anemia is generally secondary to a combination of intravascular hemolysis and bone marrow failure. The patient did report hematochezia; however, this was a confounding symptom given that she had a history of hemorrhoids and that the hematochezia resolved with stool softeners. Thrombosis is another common manifestation, with venous thrombosis being more common than arterial thrombosis. Thrombosis is a major cause of morbidity and mortality in PNH and may occur in uncommon sites, including cerebral venous sinuses, hepatic vein, splenic vein, and portal vein. The etiology for thrombosis is thought to be multifactorial. Primarily, prothrombotic microparticles result from the absence of CD55 and CD59. Further, the nitric oxide level increases in response to free hemoglobin. This increase promotes platelet activation and aggregation. Acute thrombotic episodes should be treated with anticoagulation and eculizumab. Nitric oxide is consumed by free hemoglobin in PNH, leading to decreased nitric oxide levels, which in turn may alter smooth muscle tone, leading to smooth muscle dystonia. Common symptoms of smooth muscle dystonia in PNH include abdominal pain, esophageal spasm, dysphagia, and erectile dysfunction.

Paroxysmal nocturnal hemoglobinuria is generally suspected in the presence of a Coombs-negative hemolytic anemia, aplastic anemia, or spontaneous thrombosis in the presence of concomitant anemia or cytopenia. Patients should undergo a standard workup of hemolytic anemia, including a complete blood count, a reticulocyte count, a peripheral blood smear, measures of serum haptoglobin, serum lactate dehydrogenase, and direct and indirect bilirubin, a direct Coombs test, and a urine test for hemoglobin. However, the confirmatory diagnosis requires peripheral blood flow cytometry with fluorescently labeled monoclonal antibodies that bind to GPI-anchored proteins. These proteins include CD55 and CD59; they are typically reduced in PNH. To establish a diagnosis, the result has to be confirmed with 2 independent flow cytometry reagents on 2 or more cell lineages.
The mainstay of treatment for PNH includes eculizumab, a complement-inhibiting humanized monoclonal antibody that binds to C5. It is administered intravenously every 7 days for the first 5 weeks and biweekly thereafter. By inhibiting C5, it compensates for CD59 deficiency as it prevents formation of the membrane attack complex. Patients on eculizumab should be vaccinated against Neisseria and advised that the infection occurs at a rate of 0.5% per year, or 5% after 10 years, on eculizumab treatment, despite vaccination. This risk stems from terminal complement blockade predisposing to secondary immunodeficiency. Bone marrow transplant is the only curative therapy; however, it should be offered to patients who fail eculizumab therapy, given the high risk of transplant-related morbidity and mortality in classic PNH.

SUPPLEMENTAL ONLINE MATERIAL
Supplemental material can be found online at http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

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REFERENCES

CORRECT ANSWERS: 1. a. 2. b. 3. d. 4. a. 5. a.