

Hemolytic anemia in a 26-year-old woman with vomiting and fatigue

Malik Elharram MD, Vladimir Sapon-Cousineau MD, Vicky Tagalakakis MD MSc

■ Cite as: *CMAJ* 2018 August 27;190:E1013-7. doi: 10.1503/cmaj.171147

A 26-year-old black woman underwent evaluation in the emergency department after two weeks of worsening nausea, vomiting and fatigue. Her medical history included having had malaria at eight years of age, for which she had received treatment in Eritrea. A complete review of systems was unremarkable aside from a two-day history of a mild bitemporal headache. The patient stated no recent changes in her dietary habits or bowel movements. She did not take any medication or over-the-counter products, and she had never used illicit drugs. She had no history of venous thromboembolism.

Our patient's vital signs were as follows: temperature 36.7°C, heart rate 87 beats/min, blood pressure 105/71 mm Hg, respiratory rate 16 breaths/min and oxygen saturation 100% on room air. She appeared fatigued and showed signs of pallor on her palms and nail beds. A complete neurologic examination that included cranial nerves, gait, reflexes, proprioception and sensation was unremarkable. The rest of the physical examination results were normal, including the absence of hepatosplenomegaly.

Laboratory investigations in the emergency department showed normocytic anemia (mean corpuscular volume [MCV] 89.9 [normal 80–96] fL), with hemoglobin 57 [normal 120–152] g/L, mild thrombocytopenia (platelets 119 [normal 150–400] × 10⁹/L) and normal leukocyte count and differential. A preliminary workup for anemia showed elevated lactate dehydrogenase (> 1800 [normal 110–220] U/L) and mildly elevated total bilirubin (23 [normal 3–17] µmol/L), with a conjugated bilirubin of 6 (normal 0–5) µmol/L. The reticulocyte count was normal (26 [normal 7–31] × 10³/L). These results, taken in totality, were consistent with hemolytic anemia. There were no previous laboratory results for comparison.

What further evaluation should this patient undergo?

- Fibrinogen level
- Coomb test
- Peripheral blood smear
- Malaria antigen test
- All of the above

The answer is (e). All of the above are appropriate diagnostic tests for further workup of a hemolytic anemia for this patient. We excluded disseminated intravascular coagulation on the basis of a

normal fibrinogen level and normal coagulation studies. To evaluate for autoimmune hemolytic anemia, we ordered a direct Coomb test, which was negative. This result, combined with a normal total complement test, allowed us to conclude that a diagnosis of autoimmune hemolytic anemia was unlikely. Direct Coomb tests have a sensitivity of 90%–95% and specificity of 99% in diagnosing autoimmune hemolytic anemia.¹ We chose to order a rapid malaria antigen test even in the absence of recent travel, given our patient's medical history. The test was negative. To assess for underlying microangiopathic hemolytic anemia, we requested a peripheral blood smear, which showed polychromatophilia, macro-ovalocytes, hypersegmented neutrophils and large platelets (Figures 1 and 2). In addition, schistocytes and fragmented cells were present (Figure 2).

Given the results of the peripheral blood smear, which of the following diagnoses should be considered?

- Microangiopathic anemia
- Sickle cell crisis
- Thalassemia
- Paroxysmal nocturnal hemoglobinuria
- Cobalamin deficiency

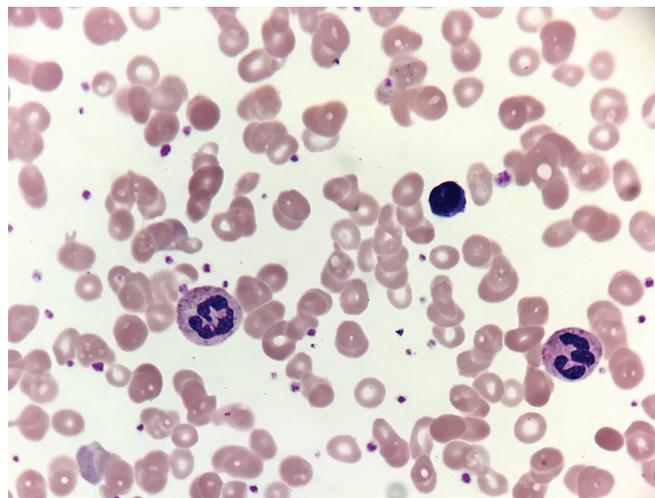


Figure 1: Peripheral blood smear from a 26-year-old black woman showing anisocytosis, poikilocytosis, macro-ovalocytes, hypersegmented neutrophils and large platelets.

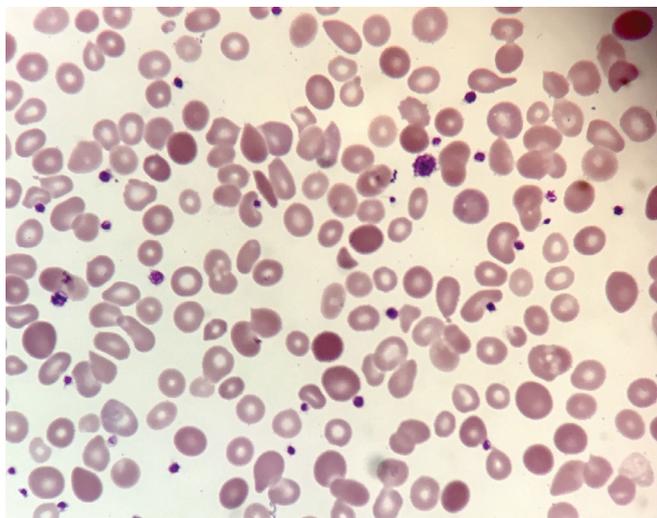


Figure 2: Peripheral blood smear from the same patient showing macroovalocytes and fragmented cells.

The answer is both (a) and (e). Both microangiopathic anemia and cobalamin (vitamin B₁₂) deficiency should be considered. Although our patient was originally from Sub-Saharan Africa, she had no history of sickle cell crisis and, with an absence of sickled red blood cells on peripheral smear, this diagnosis remained low on our differential. Paroxysmal nocturnal hemoglobinuria was possible given the presence of hemolysis with iron deficiency; however, it was unlikely, given our patient's negative history for

venous and arterial thromboembolism, and the absence of leukopenia. Thalassemia could present with hemolytic anemia; however, our patient's peripheral smear showed macrocytosis as opposed to microcytic hypochromic anemia, which is classically seen in thalassemia.

The presence of schistocytes and fragmented cells supported a diagnosis of microangiopathic hemolytic anemia. At this point, our differential diagnosis included thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (HUS) given the presence of thrombocytopenia and the findings consistent with microangiopathic hemolytic anemia on the peripheral blood smear. Thrombotic thrombocytopenic purpura was lower on our differential given the absence of other associated features, such as altered mental status, renal failure and fever. However, our patient presented with a bimodal headache and mild thrombocytopenia, which cautioned us to keep this life-threatening diagnosis in mind. Regarding HUS, the patient did not have any history of food-borne illness, exposure to a contaminated water supply or any preceding episode of diarrhea. Furthermore, she had relatively mild thrombocytopenia without the evidence of acute kidney injury commonly associated with HUS. Box 1 summarizes the differential diagnosis of thrombotic microangiopathy.²

We considered vitamin B₁₂ deficiency in our differential diagnosis given the presence of megaloblastic changes on the peripheral blood smear, including macro-ovalocytes, giant platelets and hypersegmented neutrophils. Furthermore, the inappropriately normal reticulocyte count in the context of

Box 1: Differential diagnosis of primary thrombotic microangiopathy²

Diagnosis	Cause	Clinical presentation
Acquired disorders		
Thrombotic thrombocytopenic purpura	Autoantibody inhibition of ADAMTS13 activity	Diverse, including weakness, gastrointestinal symptoms, purpura and neurologic symptoms; acute kidney injury is uncommon
Hemolytic uremic syndrome (Shiga toxin-mediated)	Enteric infection with Shiga toxin-secreting strain of <i>Escherichia coli</i> or <i>Shigella dysenteriae</i>	More common in children; presents with acute kidney injury after an episode of diarrhea
Complement-mediated	Autoantibody inhibition of complement factor H activity	Acute kidney injury
Drug-mediated (immune reaction)	Quinine and possibly other drugs with formation of drug-dependent antibodies	Sudden onset of severe systemic symptoms and anuric renal failure
Drug-mediated (non-immune reaction)	Multiple mechanisms	Gradual onset of renal failure over weeks or months
Hereditary disorders		
Thrombotic thrombocytopenic purpura	ADAMTS13 deficiency (homozygous or compound heterozygous for ADAMTS13 mutations)	Usually presents in children (also possible in adults); acute kidney injury is uncommon
Complement-mediated	Mutations in complement genes causing uncontrolled activation of alternative complement pathway	Usually presents in children (also possible in adults); acute kidney injury is common
Metabolism-mediated	Homozygous mutations in <i>MMACHC</i>	Usually presents in children younger than 1 year; 1 case reported in an adult with hypertension and acute kidney injury
Coagulation-mediated	Homozygous mutations in <i>DGKE</i>	Usually presents in children younger than 1 year with acute kidney injury

*Adapted from George JN, Nester CM. Syndromes of thrombotic microangiopathy. *N Engl J Med* 2014;371:654-66.

severe anemia pointed to underlying bone marrow failure. Vitamin B₁₂ deficiency was subsequently confirmed with a serum level of 62 (normal 140–700) pmol/L. In addition, the patient was found to have concomitant iron deficiency, with a ferritin level

of 38 (normal 50–300) µg/L, which could have been the reason for our patient's normal MCV.

Given the finding of severe vitamin B₁₂ deficiency, we gave our patient vitamin B₁₂ parenterally and folic acid supplementation.

Box 2 (part 1 of 2): Case series and reports of vitamin B₁₂ deficiency and hemolysis

Study	No. of patients	Mean or median age, yr	Mean cobalamin level, pg/mL*	Other laboratory values	Peripheral smear	Clinical presentation
Acharya et al. ⁴	3	Patient 1: 55 Patient 2: 58 Patient 3: 91	Patient 1: 167 Patient 2: 100 Patient 3: 162	Patient 1: Hg 5.0 g/dL; WBC 3100/mm ³ ; platelets 123 000/mm ³ ; reticulocytes 6.3%; MCV 134 fL; homocysteine 62.4 µmol/L; haptoglobin < 6 mg/dL; LDH 3152 U/L Patient 2: Hg 7.7 g/dL; WBC 3400/mm ³ ; platelets 99 000/mm ³ ; reticulocytes 6%; MCV 115 fL; homocysteine 88.8 µmol/L; LDH 788 U/L; serum haptoglobin < 7 mg/dL Patient 3: Hg 6.6 g/dL, MCV 146 fL; platelets 97 000/mm ³ ; homocysteine 129.7 µmol/L; haptoglobin < 7 mg/dL	Patient 1: Schistocytes and hypersegmented neutrophils Patient 2: Macrocytosis with anisopoikilocytosis, single hyperpigmented neutrophil/granulocyte with schistocyte Patient 3: NR	Patient 1: Lethargy, confusion, exertional dyspnea, difficulty walking, moderate lower extremity edema Patient 2: Progressively increasing fatigue, bilateral paresthesias, scleral icterus Patient 3: Increasing fatigue, exertional dyspnea, right upper quadrant pain
Andrès et al. ⁵	201	67 (SD ± 6)	Pseudothrombotic microangiopathy: 50	Mean hemoglobin 10.3 g/dL; no hematological abnormalities (56 [28%]); pancytopenia 12 (5%); pseudothrombotic microangiopathy 5 (3%); hemolytic anemia 3 (2.5%); positive anti-intrinsic factor antibodies 3 (2.5%)	Hypersegmented neutrophils and macrocytosis in two-thirds of patients	Asymptomatic: one-third of patients; mild sensory polyneuropathy or confusion: 92 patients (46%); physical weakness: 40 patients (20%); bilateral edema of the legs: 24 patients (12%); jaundice: 4 patients (2%)
Andrès et al. ⁷	6	77 (range 47–89)	52 (normal 12–74)	Hemoglobin 68 g/L; platelets 70 × 10 ⁹ /L; homocysteine 16.2 µmol/L; positive anti-intrinsic factor antibodies 4 patients (67%)	Megaloblastic anemia and schistocytes	Asthenia and dyspnea: 6 patients (100%); jaundice: 5 patients (83%); mild peripheral sensitive neuropathy: 3 patients (50%); impaired mental status: 1 patient (17%); peripheral infiltrated cutaneous purpura: 1 patient (17%)

Box 2 (part 2 of 2): Case series and reports of vitamin B₁₂ deficiency and hemolysis

Study	No. of patients	Mean or median age, yr	Mean cobalamin level, pg/mL*	Other laboratory values	Peripheral smear	Clinical presentation
Chhabra et al. ⁶	1	52	116	Hemoglobin 4.2 g/dL; platelets $76 \times 10^9/L$; MCV 136.8 fL; reticulocytes 2.48%; homocysteine > 50 $\mu\text{mol/L}$; LDH 4050 U/L; haptoglobin < 10 mg/dL; positive anti-intrinsic factor antibodies	Macrocytic dysmorphic red blood cells with slight basophilic stippling, occasional schistocytes with hypersegmented neutrophils	Fatigue and chest pain
Garderet et al. ⁸	1	38	Undetectable	Hemoglobin 4.5 g/dL; WBC $2.2 \times 10^3/L$; MCV 90 fL; platelets $5 \times 10^3/L$; haptoglobin 7 mg/dL; total bilirubin 12 mg/dL; positive anti-intrinsic factor antibodies	Hypersegmented neutrophils, anisocytosis, schizocytes, and poikilocytosis	Asthenia
Noël et al. ⁹	7	72 (range 43–78)	45 $\mu\text{mol/L}$	Hemoglobin 42 g/L; platelets $73 \times 10^9/L$; reticulocytes $13.1 \times 10^9/L$; MCV 110.6 fL; LDH 7310 IU/L; haptoglobin 0.1 g/L; reticulocytes $13.1 \times 10^9/L$	Schistocytes	Mild confusion: 2 patients (28.6%)
Kollipara et al. ¹⁰	1	42	59	Hemoglobin 6.8 g/dL; WBC $1.3 \times 10^9/L$; platelets $112 \times 10^9/L$; LDH 1155 U/L; haptoglobin < 10 mg/dL; positive antiparietal cell and anti-intrinsic factor antibodies	Normocytic, normochromic anemia with ovalocytes; tear-shaped red blood cells	Asymptomatic
Prueksaritanond et al. ¹¹	1	52	< 30	Hemoglobin 5.9 g/dL; MCV 87.1 fL; LDH 701 IU/L; platelets $161 \times 10^9/L$; WBC $4.7 \times 10^9/L$; serum haptoglobin undetectable; homocysteine 100 $\mu\text{mol/L}$; reticulocyte index 0.4%	Marked anisocytosis and poikilocytosis; tear drop cells, elliptocytes and multiple schistocytes	Increasing generalized fatigue and weakness; jaundice
Yeruva et al. ¹²	1	22	60	Hemoglobin 4.8 g/dL; platelets 91 000; haptoglobin 2 mg/dL; LDH 1868 IU/L; homocysteine 22.4 $\mu\text{mol/L}$; positive anti-intrinsic factor; positive antiparietal cell antibodies	Marked anisocytosis, hypochromasia with poikilocytosis, few schistocytes	Generalized fatigue and new onset dyspnea on exertion; pale conjunctiva with icteric sclera

Note: LDH = lactate dehydrogenase, MCV = mean corpuscular volume, NR = not recorded, SD = standard deviation, WBC = white blood cells.
*Unless otherwise specified.

We administered 1 unit of packed red blood cells, and her hemoglobin increased to 73 g/L with marked improvement in her symptoms. She was subsequently discharged home with vitamin B₁₂ (1000 μg subcutaneously daily for six weeks, then monthly), folic acid (5 mg orally daily) and iron sulfate (300 mg orally daily).

At six weeks' follow-up, her symptoms had completely resolved, with normalization of her blood counts (hemoglobin 137 g/L, platelets $279 \times 10^9/L$) and no signs of hemolysis (normal lactate dehydrogenase and total bilirubin). Of note, her serum vitamin B₁₂ level normalized, suggesting recovery of her tissue stores.

In the context of the patient's vitamin B₁₂ deficiency, what additional workup should be done?

- Bone marrow aspiration
- Hemoglobin electrophoresis
- Anti-intrinsic/antiparietal cell antibodies
- Genetic testing
- Methylmalonic acid and homocysteine levels

The answer is (c), anti-intrinsic/antiparietal cell antibodies. Homocysteine and methylmalonic acid are metabolic intermediates that accumulate in vitamin B₁₂ deficiency, and are typically reserved for cases in which the initial vitamin B₁₂ levels are borderline or inconclusive. Genetic testing should not be performed in this patient, as there is no suspicion for an underlying hereditary hemoglobinopathy. Furthermore, a bone marrow aspirate or biopsy is not appropriate to evaluate vitamin B₁₂ deficiency, as the findings typically do not distinguish these deficiencies from other hematologic disorders. Hemoglobin electrophoresis would be appropriate for a suspicion of sickle cell anemia (b) or thalassemia.

In the context of combined vitamin B₁₂ and iron deficiency, we strongly suspected a gastrointestinal malabsorption disorder. Our patient had no anti-intrinsic factor antibodies, but she did have antiparietal cell antibodies, which suggests but is not diagnostic of pernicious anemia. As part of the workup for her severe vitamin B₁₂ deficiency, she underwent a gastroscopy with biopsies that were consistent with autoimmune atrophic gastritis.

Discussion

Clinical manifestations of vitamin B₁₂ deficiency are often subtle; however, the consequences of this condition can be serious, with the potential of reversible bone marrow failure and demyelinating disease of the nervous system. Existing case reports and case series have reported substantial heterogeneity in the clinical presentation of patients with hemolysis secondary to vitamin B₁₂ deficiency.³⁻¹¹ In our review of the literature, these patients most commonly presented with fatigue, asthenia, sensory neuropathy or jaundice. A small proportion of patients, however, presented asymptotically, with evidence of hemolysis noticed incidentally on blood work (Box 2).

In the largest study involving patients with documented vitamin B₁₂ deficiency, Andrès and colleagues examined hematologic findings in 201 consecutive patients.⁴ The results showed that less than half of patients presenting with vitamin B₁₂ deficiency had the classical hematologic features of macrocytosis and

hypersegmented neutrophils on peripheral blood smear. Nearly one-third of patients presented without any hematologic findings. Life-threatening hematologic findings including symptomatic pancytopenia, pseudothrombotic microangiopathy and hemolytic anemia were found in about 10% of patients.⁴ Other studies in the literature have reported varying hematologic findings including pancytopenia⁹ or autoimmune hemolytic anemia with a positive direct Coomb test result.¹¹ Varying blood smear features have been reported, including hypersegmented neutrophils and macrocytosis, schistocytosis, normocytic and normochromic anemia, anisocytosis and poikilocytosis.³⁻¹¹ In our literature review, patients who presented with hemolysis usually had vitamin B₁₂ levels of less than 100 pg/mL. A few case reports, however, have described hemolysis in patients with vitamin B₁₂ levels between 100 and 200 pg/mL (Box 2).

The presentation of patients with vitamin B₁₂ deficiency can be quite diverse, with varying clinical and hematologic findings that can mimic other illnesses. The peripheral blood smear is an essential first step in investigating the cause of hemolysis, and evidence of megaloblastic changes and schistocytes should direct a physician to suspect vitamin B₁₂ deficiency as the cause of the anemia.

References

- Parker V, Tormey CA. The direct antiglobulin test: indications, interpretation, and pitfalls. *Arch Pathol Lab Med* 2017;141:305-10.
- George JN, Nester CM. Syndromes of thrombotic microangiopathy. *N Engl J Med* 2014;371:654-66.
- Stabler SP. Clinical practice. Vitamin B12 deficiency. *N Engl J Med* 2013;368:149-60.
- Acharya U, Gau JT, Horvath W, et al. Hemolysis and hyperhomocysteinemia caused by cobalamin deficiency: three case reports and review of the literature. *J Hematol Oncol* 2008;1:26.
- Andrès E, Affenberger S, Zimmer J, et al. Current hematological findings in cobalamin deficiency. A study of 201 consecutive patients with documented cobalamin deficiency. *Clin Lab Haematol* 2006;28:50-6.
- Chhabra N, Lee S, Sakalis EG. Cobalamin deficiency causing severe hemolytic anemia: a pernicious presentation. *Am J Med* 2015;128:e5-6.
- Andrès E, Affenberger S, Federici L, et al. Pseudo-thrombotic microangiopathy related to cobalamin deficiency. *Am J Med* 2006;119:e3.
- Garderet L, Maury E, Lagrange M, et al. Schizocytosis in pernicious anemia mimicking thrombotic thrombocytopenic purpura. *Am J Med* 2003;114:423-5.
- Noël N, Maigné G, Tertian G, et al. Hemolysis and schistocytosis in the emergency department: consider pseudothrombotic microangiopathy related to vitamin B12 deficiency. *QJM* 2013;106:1017-22.
- Kollipara VK, Brine PL, Gemmel D, et al. A case of asymptomatic pancytopenia with clinical features of hemolysis as a presentation of pernicious anemia. *J Community Hosp Intern Med Perspect* 2016;6:32493.
- Prueksaritanond S, Barbaryan A, Mirrakhimov AE, et al. A puzzle of hemolytic anemia, iron and vitamin B12 deficiencies in a 52-year-old male. *Case Rep Hematol* 2013;2013:708489.
- Yeruva SL, Manchandani RP, Oneal P. Pernicious anemia with autoimmune hemolytic anemia: a case report and literature review. *Case Rep Hematol* 2016;2016:7231503

Competing interests: Dr. Tagalakis reports personal fees from advisory boards (Sanofi, Pfizer, Bayer, Bristol-Myers Squibb and Servier), personal fees from speakers bureaus (Sanofi, Pfizer, Bristol-Myers Squibb), grants or honoraria from a commercial organization (Sanofi), and participation in a clinical trial within the past two years, outside the submitted work. No other competing interests were declared.

This article has been peer reviewed.

The authors have obtained patient consent.

Affiliations: Division of General Internal Medicine (Sapon-Cousineau, Tagalakis), Jewish General Hospital; Faculty of Medicine (Sapon-Cousineau, Elharram, Tagalakis), McGill University, Montréal, Que.

Contributors: Malik Elharram and Vladimir Sapon-Cousineau contributed equally to the production of the manuscript. Vicky Tagalakis contributed to drafting the manuscript and provided editorial review of the manuscript. All of the authors reviewed the final version to be published and agreed to act as guarantors of the work.

Correspondence to: Malik Elharram, malikelharram@gmail.com