REVIEW

CME MOC

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Severe megaloblastic anemia: Vitamin deficiency and other causes

ABSTRACT

Megaloblastic anemia causes macrocytic anemia from ineffective red blood cell production and intramedullary hemolysis. The most common causes are folate (vitamin B_9) deficiency and cobalamin (vitamin B_{12}) deficiency. Megaloblastic anemia can be diagnosed based on characteristic morphologic and laboratory findings. However, other benign and neoplastic diseases need to be considered, particularly in severe cases. Therapy involves treating the underlying cause—eg, with vitamin supplementation in cases of deficiency, or with discontinuation of a suspected medication.

KEY POINTS

The hallmark of megaloblastic anemia is macrocytic anemia (mean corpuscular volume > 100 fL), often associated with other cytopenias.

Dysplastic features may be present and can be difficult to differentiate from myelodysplastic syndrome.

Megaloblastic anemia is most commonly caused by folate deficiency from dietary deficiency, alcoholism, or malabsorption syndromes or by vitamin B_{12} deficiency, usually due to pernicious anemia.

Both vitamin deficiencies cause hematologic signs and symptoms of anemia; vitamin B_{12} deficiency also causes neurologic symptoms.

Oral supplementation is available for both vitamin deficiencies; intramuscular vitamin B_{12} supplementation should be used in cases involving severe neurologic symptoms or gastric or bowel resection.

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Not all megaloblastic anemias result from vitamin deficiency, but most do. Determining the underlying cause and initiating prompt treatment are critical, as prognosis and management differ among the various conditions.

This article describes the pathobiology, presentation, evaluation, and treatment of severe megaloblastic anemia and its 2 most common causes: folate (vitamin B_9) and cobalamin (vitamin B_{12}) deficiency, with 2 representative case studies.

MEGALOBLASTIC ANEMIA OVERVIEW

Megaloblastic anemia is caused by defective DNA synthesis involving hematopoietic precursors, resulting in ineffective red blood cell production (erythropoiesis) and intramedullary hemolysis. Macrocytic anemia with increased mean corpuscular volume (MCV), defined as more than 100 fL, is the hallmark of megaloblastic anemia, but leukopenia and thrombocytopenia are also frequently present.

The incidence of macrocytosis is as high as 4% in the general population, but megaloblastic anemia accounts for only a small fraction.[1] Nonmegaloblastic causes of macrocytic anemia include ethanol abuse, myelodysplastic syndrome, aplastic anemia, hypothyroidism, liver disease, and drugs.[2,3] Although these causes are associated with increased MCV, they do not lead to the other features of megaloblastic anemia.

The most frequent causes of megaloblastic anemia are deficiencies of vitamin B_9 (folate) or vitamin B_{12} (cobalamin) (**Table 1**). Less-frequent causes include congenital disorders (inborn errors of metabolism), drugs (particu-

TABLE 1

Characteristics of vitamin B_{12} vs folate deficiency

	Vitamin B_{12} deficiency	Folate deficiency
Etiology	Lack of intrinsic factor: pernicious anemia Malabsorption: celiac disease, prior gastric or ileal surgery Dietary deficiency less common	Dietary deficiency: alcoholism, countries without food fortification Malabsorption: developed countries Increased demand: pregnancy, hemolytic anemia, eczema
Clinical presentation	Hematologic findings: cytopenias Neuropsychiatric symptoms: paresthesias, decreased proprioception and vibratory sense, dementia, confusion	Hematologic findings: cytopenias
Evaluation	Clinical history and physical examination: symptoms secondary to anemia and hemolysis, neurologic symptoms Laboratory testing: serum vitamin B_{12}, methylmalonic acid, homocysteine, antiparietal cell and anti-intrinsic factor antibodies, serum gastrin Gastric biopsy for suspected pernicious anemia	Clinical history and physical examination: similar to vitamin B_{12} deficiency, except no neurologic symptoms Laboratory testing: serum folate, red blood cell folate, methylmalonic acid, homocysteine
Differential diagnosis	Other macrocytic anemias without megaloblastic features: liver disease, thyroid dysfunction, alcohol abuse Myelodysplastic syndrome, acute myeloid leukemia Nitrous oxide exposure Medication effect	Other macrocytic anemias without megaloblastic features: liver disease, thyroid dysfunction, alcohol abuse Myelodysplastic syndrome, acute myeloid leukemia Medication effect
Treatment	Parenteral vitamin B_{12} 1–2 times per week until symptoms improve, then monthly High-dose oral vitamin B_{12} daily	Oral folate daily
Monitoring and follow-up	Clinical follow-up for improvement of neurologic symptoms Monitor hematologic response: complete blood cell count Pernicious anemia: consider monitoring methylmalonic acid	Monitor hematologic response: complete blood cell count

larly chemotherapeutics and folate antagonists), micronutrient deficiencies, and nitrous oxide exposure.[4,5]

FOLATE DEFICIENCY

Folate is found in green leafy vegetables, fruits, nuts, eggs, and meats. Normal body stores of folate are 5 to 30 mg. The recommended daily allowance depends on age, sex, and pregnancy status, but is generally 400 µg in adults and 600 µg during pregnancy.[6]

Folate deficiency has 3 main causes[4,5]:

- **Reduced intake** from diets lacking folate (rare in countries with vitamin fortification) and alcoholism (see **Case 1**)
- **Decreased absorption** from disorders affecting nutrient absorption in the small bowel, eg, celiac disease, inflammatory bowel disease, and tropical sprue
- **Increased demand** from pregnancy, hemolytic anemia, puberty, and eczematous conditions.

VITAMIN B_{12} DEFICIENCY

Vitamin B_{12} is produced by microorganisms and is found almost exclusively in foods of animal origin. Normal body stores of vitamin B_{12} are 3 to 5 mg, and the recommended adult daily intake is 2.4 µg.[7,8]

Causes of vitamin B_{12} deficiency are listed in **Table 2**. Dietary deficiency of vitamin B_{12} occurs less frequently than folate deficiency because body stores can last for years owing to efficient enterohepatic recycling mechanisms. Although uncommon, dietary B_{12} deficiency can occur even in industrialized countries in strict vegans and vegetarians, or in breastfed infants of mothers with vitamin B_{12} deficiency.

Complex absorption pathway

Dietary absorption of vitamin B_{12} is a complex process that begins with haptocorrin (also known as transcobalamin I or R-binder) production by the salivary glands.

When food is digested in the stomach by gastric acid and pepsin, free vitamin B_{12} is released and binds to haptocorrin.[4,9]

Simultaneously, gastric parietal cells secrete intrinsic factor, which cannot interact with the vitamin B_{12}-haptocorrin complex. Not until food moves into the duodenum, where trypsin and other pancreatic enzymes cleave haptocorrin, is vitamin B_{12} free to bind to intrinsic factor.[9] The resultant vitamin B_{12}-intrinsic factor complex binds to the cubam receptor on the mucosal surface of enterocytes in the ileum. From there, vitamin B_{12} is transported into the circulation by multidrug resistance protein 1, where it is readily bound by its transport protein transcobalamin II.[7,9]

The vitamin B_{12}-transcobalamin complex then binds to the transcobalamin receptors on hematopoietic stem cells (and other cell types), allowing uptake of the complex, with subsequent lysosomal degradation of transcobalamin. Free vitamin B_{12} is then available for cellular metabolism.

Nearly every step of this pathway can be disrupted in various pathologic states, but lack of intrinsic factor secondary to pernicious anemia is the cause of vitamin B_{12} deficiency in most cases.

TABLE 2

Causes of vitamin B_{12} deficiency

Common causes (related to malabsorption)
Autoimmune gastritis (pernicious anemia)
Celiac disease
Inflammatory bowel disease
Surgical gastrectomy, gastric bypass, ileal resection
Less common causes
Nutritional (strict vegans, breastfed infants of mothers with vitamin B_{12} deficiency)
Nitrous oxide abuse
Diphyllobothrium latum infection
Pancreatic insufficiency
Drug effect (metformin, proton pump inhibitors)
Inherited disorders affecting intrinsic factor or the cubam receptor
Rare inherited disorder (eg, methylmalonic acidemia, transcobalamin II deficiency)

Information from references 4, 5, and 7.

Pernicious anemia and autoimmune gastritis

Macrocytic anemia with a mean corpuscular volume > 100 fL is the hallmark of megaloblastic anemia

Chronic atrophic autoimmune gastritis is an autoimmune process directed specifically at either gastric parietal cells or intrinsic factor, or both.[10–12] Parietal cell damage leads to reduced production of gastric acid and intrinsic factor, accompanied by a compensatory increase in serum gastrin levels. Decreased intrinsic factor leads to significantly reduced absorption of dietary vitamin B_{12}, resulting in pernicious anemia.

Chronic atrophic autoimmune gastritis affects the body and fundus of the stomach, replacing normal oxyntic mucosa with atrophic-appearing mucosa, often with associated intestinal metaplasia.[11]

The associated inflammatory infiltrate consists predominantly of lymphocytes and plasma cells. Enterochromaffin-like cell hyperplasia is also seen in biopsies of the fundus or stomach body (highlighted by staining for chromogranin A and synaptophysin) and is thought to be a precursor to neuroendocrine (carcinoid) tumors. In addition to having vitamin B_{12} defi-

CASE 1: An older man with suspected myelodysplastic syndrome

A 68-year-old man with no significant past medical history presented from prison to the emergency department with fatigue, occasional shortness of breath, weight loss, and numbness and tingling of both hands.

Initial complete blood cell count findings showed pancytopenia with macrocytic anemia, with the following values:

- White blood cell count 1.81 × 10^9/L (reference range 4.5–10)
- Hemoglobin 6.2 g/dL (14–18)
- Mean corpuscular volume 121.5 fL (80–95)
- Platelet count 41 × 10^9/L (150–450).

Because of his clinical symptoms and severe pancytopenia with macrocytosis, bone marrow biopsy was performed to evaluate for myelodysplastic syndrome and acute leukemia.

Bone marrow biopsy results

Findings from bone marrow aspirate smear and core biopsy included the following (**Figure 1**):

- Hypercellularity (70%–80%; reference range 30%–70%)
- Erythroid hyperplasia, indicated by a reduced ratio of myeloid to erythroid precursor cells (0.7; reference range 2–4:1) and 2% blasts
- Severe megaloblastic changes in the erythroid and granulocytic lineages; erythroid precursors showed significant nuclear-cytoplasmic dyssynchrony, multinucleation, nuclear budding, nuclear irregularities, and basophilic stippling; granulocytic precursors showed hypersegmentation of mature neutrophils and occasional giant metamyelocytes and band forms
- Mildly increased ring sideroblasts (10%) seen with iron stain
- Megakaryocyte dysplasia in the form of small hypolobated forms.

Bone marrow findings of multilineage dysplasia, in addition to megaloblastic changes, were strongly suggestive of myelodysplastic syndrome.

Further evaluation

Additional testing yielded the following results:

- Serum folate level 18.1 ng/mL (> 4.7)
- Serum vitamin B_{12} level < 150 pg/mL (232–1,245)
- Parietal cell antibody positive (1:40)
- Conventional cytogenetics: normal male karyotype
- Hematologic neoplasm next-generation-sequencing panel (62 genes): negative for disease-associated mutations.

In conjunction with normal cytogenetic and next-generation-sequencing panel results, undetectable vitamin B_{12} levels helped confirm severe vitamin B_{12} deficiency. This may be the underlying cause of the cytopenias and dysplasia. It was speculated that a restricted diet during incarceration was the source of the problem.

Treatment

Intramuscular cyanocobalamin (1,000 µg) was started, followed by high-dose oral cyanocobalamin (1,000 µg/day). Abnormal complete blood cell count findings improved, as did neurologic symptoms.

ciency, patients with chronic atrophic autoimmune gastritis are at increased risk of gastric adenocarcinomas and neuroendocrine tumors.

Hyperplasia of gastrin cells can be identified using gastrin immunohistochemistry on gastric antral biopsies. Serologic testing for antiparietal and anti-intrinsic factor antibodies, as well as increased serum levels of gastrin, help confirm the diagnosis.[10–12]

FOLATE AND VITAMIN B_{12} METABOLISM ARE INTERTWINED

Folate and vitamin B_{12} metabolism are intimately interconnected, so deficiency in either vitamin leads to many similar manifestations. Both vitamins are involved in single carbon transfer (methylation), which is necessary for the conversion of deoxyuridylate to deoxythymidylate.[7] Insufficient folate or vitamin B_{12} leads to decreased thymidine available for DNA synthesis, hampering cell division and replication.

In pyrimidine synthesis, 5,10-methylenetetrahydrofolate serves as the methyl donor,[7] after which it is converted to dihydrofolate, which must be reduced and then methylated to be used again. The reduction of dihydrofolate to tetrahydrofolate by dihydrofolate re-

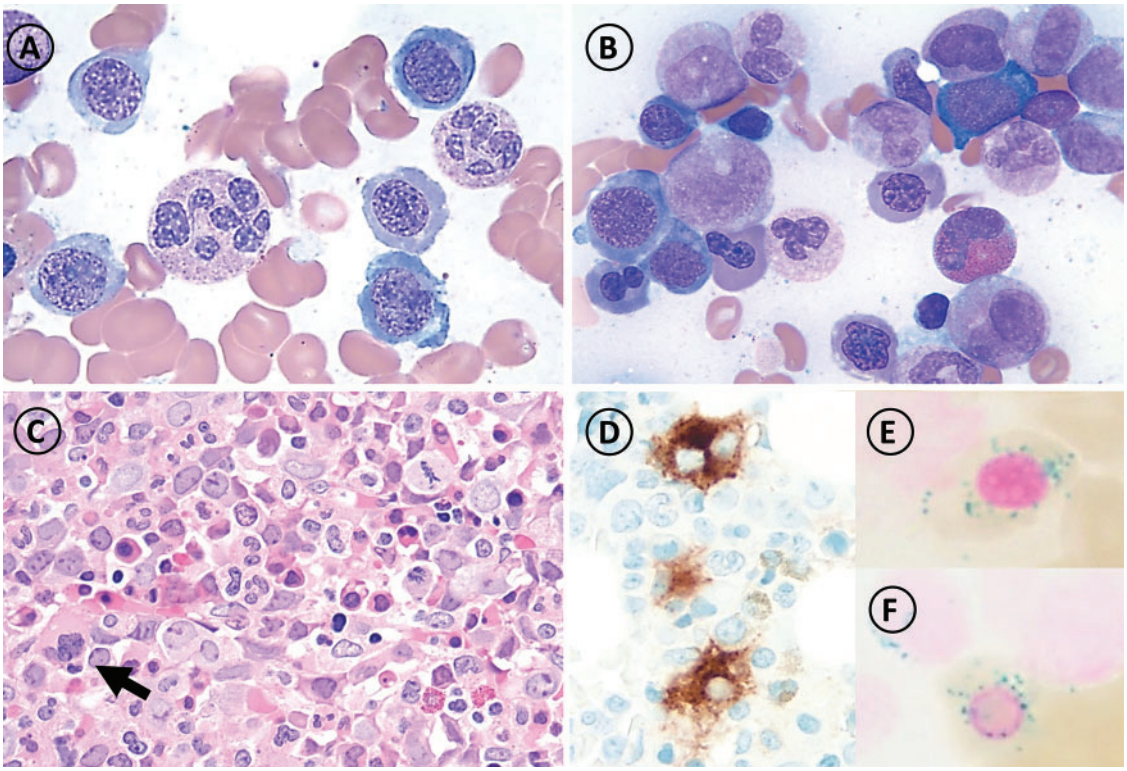

Figure 1. A,B: Bone marrow aspirate smears showing severe megaloblastic changes: nuclear-cytoplasmic dyssynchrony, binucleation, nuclear irregularity, and basophilic stippling in erythroid lineage cells, and also hypersegmentation, nuclear-cytoplasmic dyssynchrony, and giant metamyelocytes or band forms in granulocytes (Wright-Giemsa, × 1,000). **C:** Bone marrow core biopsy showing hypercellularity, erythroid hyperplasia, left shift in maturation, and small dysplastic megakaryocytes (arrow) (hematoxylin and eosin, × 400). **D:** Small dysplastic megakaryocytes highlighted by CD61 immunohistochemistry on the core biopsy. **E,F:** Increased ring sideroblasts in iron stain on the aspirate smears.

ductase is targeted by multiple drugs,[5,13] which have the effect of decreasing available deoxythymidylate for DNA synthesis, resulting in megaloblastic anemia.

DRUG EFFECTS

Owing to vitamin fortification of common foods in developed countries, megaloblastic anemia related to vitamin deficiency is increasingly uncommon.[2,14] However, this reduced incidence is offset by a growing list of drugs that can cause megaloblastic anemia by interfering with DNA synthesis in various ways.[2,4,13]

Drugs that affect purine synthesis include[2,13]:

- Immunosuppressants, eg, azathioprine and mycophenolate mofetil
- Chemotherapeutics, eg, purine analogues (fludarabine, cladribine, and thioguanine)
- Allopurinol, a xanthine oxidase inhibitor used to treat gout.

Drugs that affect pyrimidine synthesis include[13]:

- Immunomodulatory drugs, eg, leflunomide and teriflunomide
- Chemotherapeutics, eg, cytarabine, gemcitabine, and fluorouracil
- Methotrexate, an immunosuppressant and chemotherapeutic
- Sulfa drugs and trimethoprim.

Numerous drugs from multiple classes can reduce folate or vitamin B_{12} absorption, although this rarely leads to clinically significant deficiency.

CASE 2: A young woman with worsening anemia and family history of autoimmune disease

A young woman, age 17, presented to the emergency department with headache and abdominal pain that had worsened over the previous month. She had sought medical care several times over the past 6 months with similar symptoms, when moderate anemia was attributed to iron deficiency from heavy menses (the most common cause of anemia in women of reproductive age). Family history was notable for her sister having autoimmune thyroid disease and type 1 diabetes mellitus. On additional questioning, she reported paresthesias in the hands. Physical examination revealed decreased proprioception and vibratory sense and a wide-based gait.

Results of initial testing were as follows:

- Hemoglobin 6.8 g/dL (down from 8.5 g/dL at her last visit)
- Mean corpuscular volume 104.2 fL (elevated)
- White blood cell count 6.91 × 10^9/L (normal)
- Platelet count 300 × 10^9/L (normal)
- Peripheral blood smear: several hypersegmented neutrophils with no left-shift in maturation (**Figure 2**).

Further tests were performed:

- Direct antiglobulin test negative
- Serum iron, ferritin, and total iron-binding capacity normal
- Haptoglobin < 10 mg/dL (reference range 31–238)
- Lactate dehydrogenase 4,131 U/L (135–214)
- Relative reticulocytosis—reticulocyte count 48 × 10^9/L (18–100); 2.6% (0.4%–2.0%).
- Serum vitamin B_{12} < 150 pg/mL (232–1,245)
- Serum folate normal
- Serum methylmalonic acid 8,361 nmol/L (79–376)
- Antiparietal cell antibody negative
- Anti-intrinsic factor antibody positive.

The laboratory and clinical findings were consistent with vitamin B_{12} deficiency, and the presence of anti-intrinsic factor antibody confirmed the diagnosis of pernicious anemia. Although it tends to occur in older women, it is occasionally seen in young adults. A strong family history of autoimmune disease is common in patients with pernicious anemia.

She was also tested for the following:

- Serum thyroid-stimulating hormone level 6.72 µU/mL (0.40–2.80)
- Free thyroxine 1.3 ng/dL (0.8–1.5)
- Thyroid peroxidase antibody 1,224 IU/mL (< 5.6).

These findings indicate she is at risk for developing symptomatic thyroid disease.

Treatment

Treatment was started with parenteral cyanocobalamin, at first with daily intramuscular 1,000-µg cyanocobalamin injections. Treatments were then weekly, then monthly, with rapid improvement of hematologic symptoms and slower but complete resolution of her neurologic symptoms.

Future considerations

Given the personal and family history of autoimmune disease, a diagnosis of polyglandular autoimmune syndrome should be considered. Extensive clinical and laboratory evaluation for other signs of autoimmune disease is warranted. Antiadrenal and GAD65 antibody testing should be performed to assess risk for developing adrenal insufficiency.

CLINICAL FEATURES

Vitamin B_{12} deficiency causes hematologic and neuropsychiatric manifestations that may occur together or independently.[15,16] Megaloblastic anemia due to folate deficiency and other causes shares the same hematologic manifestations as vitamin B_{12} deficiency but lacks the neurologic features (see **Case 2**).[4,7]

Hematologic features

The most common hematologic manifestation is megaloblastic anemia, which includes macrocytic erythrocytes in the peripheral blood and megaloblastic precursor cells in the bone marrow that exhibit nuclear-to-cytoplasmic dyssynchrony.[7] Ineffective erythropoiesis leads to intramedullary hemolysis, classically with high lactate dehydrogenase and undetectable haptoglobin, but without schistocytes in the peripheral blood.

Symptoms secondary to anemia include fatigue, shortness of breath, and poor exercise tolerance.

Neuropsychiatric features

Vitamin B_{12} deficiency can cause subacute combined degeneration of the dorsal and lateral columns of the spinal cord. Patients may experience bilateral and symmetrical pares-

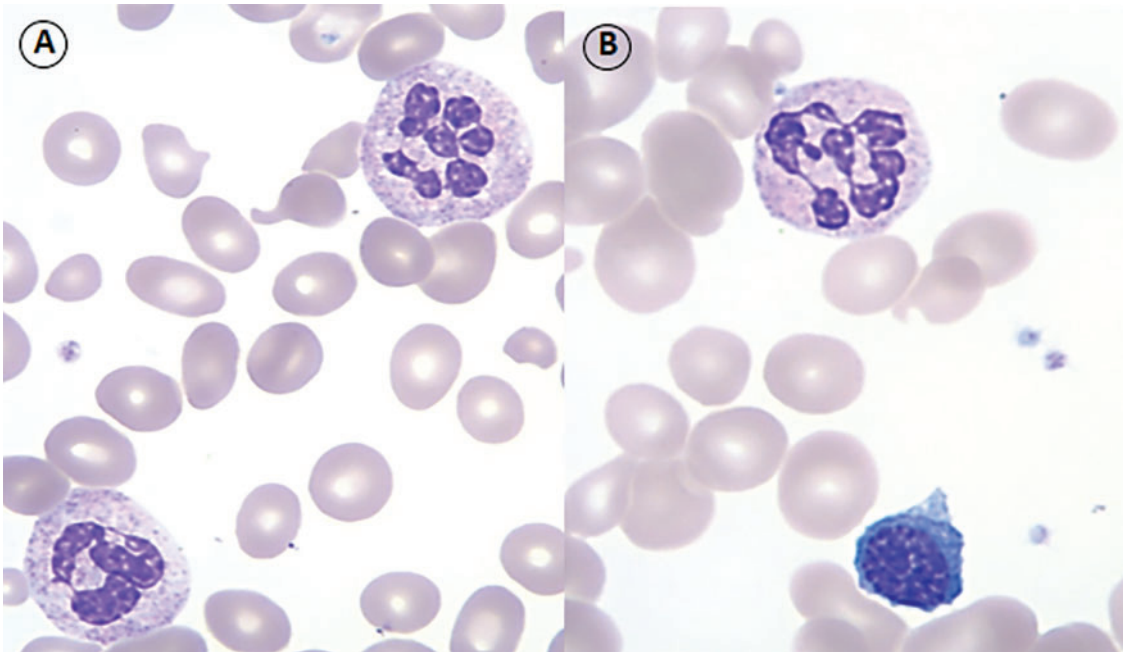

Figure 2. A,B: Two hypersegmented neutrophils (> 6 nuclear lobes) in a peripheral blood smear (Wright-Giemsa, × 1,000).

thesia and decreased vibratory and positional sense. Psychiatric manifestations include memory loss, delirium, dementia, depression, mania, and hallucinations.[15,17,18]

Atypical presentations

Although neuropsychiatric symptoms often develop after hematologic abnormalities, more than 25% of patients with neurologic manifestations of vitamin B_{12} deficiency have either a normal hematocrit or a normal MCV.[17]

Why certain patients are prone to hematologic complications of vitamin deficiency and other patients have neurologic sequelae remains unclear, but those with underlying abnormalities such as pre-existing neurologic comorbidities or bone marrow failure conditions may be more likely to develop side effects related to those conditions.

Other findings

An increased risk of thrombosis is seen in vitamin B_{12} and folate deficiency, possibly as a consequence of hyperhomocysteinemia.[19] Atrophic glossitis (swollen, erythematous, smooth tongue) is a common, albeit nonspecific, finding in vitamin B_{12} deficiency.

INITIAL EVALUATION

While there is no gold standard for diagnosing megaloblastic anemia, appropriate clinical and laboratory evaluation can usually establish the correct diagnosis.

Diets lacking folate are rare in countries with vitamin fortification

History and physical examination

A complete history and physical examination are imperative. Targeted questions should cover the following areas[20]:

- Diet—vegan or vegetarian?
- Surgical history—gastric or ileal resection?
- Gastrointestinal symptoms—celiac disease or gastritis?
- Neurologic symptoms such as paresthesias, numbness, ataxia, or gait disturbances?
- Medications—folate antagonists, chemotherapeutics?

Initial blood work

The complete blood cell count reveals anemia that is generally macrocytic (MCV > 100 fL). Anemia can be seen in isolation or with leukopenia or thrombocytopenia. Note that concurrent iron deficiency anemia can result in a normal MCV but increased red cell distribution width.

The peripheral blood smear shows morphologic changes in red blood cells (RBCs),

including marked size variation (anisocytosis) and abnormal morphology (poikilocytosis), including macro-ovalocytes, teardrop cells, microcytes, and in severe cases, schistocytes, basophilic stippling, Howell-Jolly bodies, and nucleated RBCs.

Polychromasia is not typically present. In the setting of cytopenias and neurologic symptoms, absence of schistocytes excludes thrombotic thrombocytopenic purpura.

Hypersegmented neutrophils (ie, ≥ 1% of neutrophils having 6 or more nuclear lobes, or ≥ 5% of neutrophils with 5 nuclear lobes) in the setting of macrocytic anemia are considered specific for megaloblastic anemia and are rarely seen in other diseases.[2,7]

Folate laboratory evaluation

Laboratory testing for suspected folate deficiency starts with evaluating serum or plasma folate. Fasting serum folate generally reflects tissue levels of folate; however, postprandial increases in folate occur and can cause falsely normal results in nonfasting samples.[6] After a meal, increased serum folate occurs within 2 hours, then quickly returns to baseline. Falsely elevated folate levels can also be seen with sample hemolysis and vitamin B_{12} deficiency. In the latter situation, inadequate vitamin B_{12} causes folate to be trapped in the 5-methyltetrahydrofolate state.[5]

An alternative method of evaluating folate stores is RBC folate, which reflects the folate status of the prior 3 months and has the advantage of not being affected by recent dietary intake. Disadvantages include slower turn-around time and higher cost. Also, recent transfusion of RBCs can lead to inaccurate results, as it will reflect the folate level of the donor.

Lack of intrinsic factor secondary to pernicious anemia is the cause of vitamin B_{12} deficiency in most cases

Vitamin B_{12} laboratory evaluation

Specific laboratory evaluation for vitamin B_{12} deficiency begins with total serum cobalamin levels.[21,22] Vitamin B_{12} levels lower than 200 pg/mL are highly suggestive of deficiency, although false-positive and false-negative results can happen. A normal cobalamin level makes deficiency unlikely, although it may occur in nitrous oxide exposure or abuse, which involves metabolically inactive vitamin B_{12}.[7] In addition, in pernicious anemia, anti-intrinsic factor antibodies can interfere with vitamin B_{12} assays, leading to falsely normal results.[5] On the other hand, pregnancy, drugs such as oral contraceptives and anticonvulsants, human immunodeficiency virus infection, and folate deficiency can falsely reduce vitamin B_{12} levels.

For borderline cobalamin levels (200–400 pg/mL), additional laboratory testing, including serum methylmalonic acid and serum homocysteine levels, should be performed.[5] Methylmalonic acid and homocysteine are intermediaries in vitamin B_{12} metabolism and are increased in vitamin B_{12} deficiency. Homocysteine is also elevated in folate deficiency and renal disease but methylmalonic acid is not, making it a more specific marker of vitamin B_{12} deficiency.[4]

Vitamin B_{12} deficiency secondary to increased intramedullary destruction of RBC precursors can cause undetectable haptoglobin levels and elevated lactate dehydrogenase and indirect bilirubin.

For suspected pernicious anemia, serologic testing for antiparietal cell and anti-intrinsic factor antibodies, as well as gastrin, are useful.[10] Antiparietal cell antibodies in patients with autoimmune pernicious anemia demonstrate high sensitivity (81%) and specificity (90%), while anti-intrinsic factor antibodies have high specificity (100%) but low sensitivity (27%–50%). The combination of these 2 tests significantly increases their diagnostic performance, with 73% sensitivity and 100% specificity in pernicious anemia.[23,24] Elevated gastrin is highly sensitive (85%) for pernicious anemia; however, it can also be elevated in Zollinger-Ellison syndrome, therapy with proton pump inhibitors or histamine 2 receptor blockers, *Helicobacter pylori* infection, or renal failure.[4,24]

SPECIAL TESTING

Neuroimaging for atypical cases

Neuroimaging is unnecessary for patients with a classic clinical presentation of vitamin B_{12} deficiency. However, in suspected cases without hematologic manifestations, magnetic resonance imaging is indicated. The most consistent finding in vitamin B_{12} deficiency is a symmetric, abnormally increased T2 signal intensity, involving the posterior or lateral columns (or both) in the cervical and thoracic spinal cord.[14]

Bone marrow aspiration and biopsy

If vitamin deficiency or drug effects cannot be determined clinically and by laboratory testing as the cause of anemia, bone marrow biopsy may provide useful information. In megaloblastic anemia, the bone marrow shows the following:

- Hypercellularity for age
- Erythroid predominance, with a decreased myeloid-to-erythroid ratio
- A left-shift in hematopoietic maturation.

Megaloblastic changes are best appreciated with bone marrow aspirate smears using Wright-Giemsa stain. The typical findings in the erythroid lineage include increased overall size and nuclear-cytoplasmic dyssynchrony (ie, a large, immature-appearing nucleus with an open chromatin pattern accompanied by a mature-appearing cytoplasm).[7] Findings are also apparent in the granulocytic lineage, as seen by giant metamyelocytes and bands.[7] Hypersegmented neutrophils can be seen in either peripheral blood or bone marrow smears. Occasionally, megakaryocytes are also affected, with large forms having hyperlobation and decreased cytoplasmic granularity.

In severe vitamin deficiency, dysplastic features can be observed, most often involving the erythroid lineage in the form of nuclear irregularities, eg, binucleation, multinucleation, nuclear fragmentation, and nuclear budding, which resemble features seen in myelodysplastic syndrome (see "Differential diagnosis" below).

Severe ineffective hematopoiesis can markedly increase iron stores (detectable with iron stain), although ring sideroblasts are rarely seen in megaloblastic anemia.

Gastric biopsy

Gastric biopsy can confirm chronic atrophic autoimmune gastritis.

DIFFERENTIAL DIAGNOSIS

Establishing the correct diagnosis of megaloblastic anemia is paramount, as the treatment and prognosis for different conditions can be vastly different. The differential diagnosis includes conditions that cause nonmegaloblastic macrocytic anemia, such as medication effects, ethanol abuse, hypothyroidism, liver disease, and post-splenectomy status. A detailed clinical and medication history and laboratory findings, including vitamin B_{12} and folate levels, can help determine the correct diagnosis.

Megaloblastic anemia can also mimic malignant conditions. Cytopenias, combined with severe megaloblastic findings in the bone marrow, overlap with the neoplastic processes of low-grade myelodysplastic syndrome or acute myeloid leukemia.[3,25,26] Diagnostic considerations include myelodysplastic syndrome with excess blasts and erythroid predominance, as well as pure erythroid leukemia (ie, a neoplastic proliferation of immature erythroid cells with > 80% erythroids and > 30% proerythroblasts) without increased myeloid blasts.[27]

Although myelodysplastic syndrome and severe megaloblastic anemia have overlapping features, careful morphologic evaluation of the bone marrow aspirate and biopsy can identify differentiating characteristics. Dysplastic features characteristic of myelodysplastic syndrome that are not typical of megaloblastic anemia include the following:

- Hyposegmentation or hypogranulation of granulocytes
- Hypolobation or small forms of megakaryocytes
- Hypogranular platelets
- Increased blasts.

Laboratory findings, including vitamin B_{12} and folate levels, conventional cytogenetics, and next-generation sequencing, can also help distinguish the 2 entities.[26] Identifying an acquired clonal abnormality, such as a myelodysplastic syndrome-associated cytogenetic abnormality or mutation, would strongly support a neoplastic process.

Vitamin B_{12} deficiency causes hematologic and neuropsychiatric manifestations

TREAT UNDERLYING PROBLEM

After establishing the diagnosis, treatment should be initiated promptly. Treatment is specific to the underlying condition and usually involves supplementing the deficient vitamin. With either vitamin B_{12} or folate supplementation, the rapid bone marrow response can push borderline iron stores into deficiency, so patients should be monitored for iron and provided with supplementation as needed. Megaloblastic anemia secondary

TABLE 3

Estimated cost of treatment per month for vitamin B_{12} and folate deficiency[a]

Formulation		Dose	Cost per month
Vitamin B_{12}	Intramuscular injection	1,000 µg/mL, single vial of 1 mL	$5–$15
	Oral	1,000 µg/pill, 30 pills per month	$2–$5
	Nasal spray	500 µg/spray, single spray per day, carton of 4	$500–$640
	Sublingual lozenges	3,000 µg/lozenge, single lozenge per day, ~ 30 lozenges per month	$5
Folic acid		1 mg/pill, 30 pills per month	$3–$5

[a]The dose and cost are adapted from GoodRx.com.

to drug effect is best treated by stopping the causative agent if feasible.

Generally, response to therapy is rapid, with hemoglobin levels improving within a week. Neurologic symptoms of vitamin B_{12} deficiency generally resolve more slowly than hematologic symptoms and may not resolve completely.

Hypersegmented neutrophils in the setting of macrocytic anemia are considered specific for megaloblastic anemia

FOLATE SUPPLEMENTATION

Megaloblastic anemia secondary to folate deficiency is generally treated with oral folate, as it is most often caused by dietary deficiency rather than malabsorption. For supplementation and treatment, it is available as either of the following:

- The synthetic form, known as folic acid or pteroylglutamic acid
- The naturally occurring form, folinic acid.

Folate deficiency is typically treated with oral folic acid 1 to 5 mg per day.[28] This dosage is more than the recommended dietary allowance of 400 µg per day, thereby allowing for adequate repletion even in the setting of malabsorption. Treatment is continued for the duration of hematologic recovery or until the cause of deficiency is addressed. In patients with malabsorption, treatment is continued indefinitely.

VITAMIN B_{12} SUPPLEMENTATION

Prompt treatment is particularly important for patients with vitamin B_{12} deficiency in order to prevent neurologic symptoms from becoming permanent.

Multiple supplementation options are available, with the choice depending on clinical and nonclinical factors. All forms are generally well tolerated, but adverse reactions such as hypersensitivity have been reported.[28,29]

Formulations vary

Vitamin B_{12} can be supplemented in different forms; noted preferences vary worldwide: cyanocobalamin in the United States, hydroxycobalamin in Europe, and methylcobalamin in Asia.[30] Although all forms are well absorbed, hydroxycobalamin may be best for those with inherited errors of cobalamin metabolism. Cyanocobalamin is more expensive but appears to be more stable for oral supplementation.

Vitamin B_{12} is available as a pill, sublingual lozenge, intranasal spray, and intramuscular injection. Oral and intramuscular administration are the most widely studied and used.

Oral vs intramuscular vitamin B_{12}

About 1.2% of oral cobalamin is passively absorbed unbound, while the remainder requires intrinsic factor to be absorbed in the ileum.[31] Eussen et al[32] found that high-dose oral vitamin B_{12} (> 200 × the recommended dietary allowance of 2.4 µg/day) produces adequate reductions in methylmalonic acid. However, despite multiple studies demonstrating the effectiveness of oral vitamin B_{12} even in pernicious anemia, a 2018 Cochrane review[33] found a lack of data demonstrating equivalence to intramuscular administration, mainly due to a limited number of quality randomized studies.

The most common oral dosage is 1,000 to 2,000 µg daily, compared with 1,000 µg intramuscularly daily for 7 days, then weekly for a month, then monthly thereafter.[34]

Advantages of intramuscular administration include improved adherence and less-frequent dosing during the monthly maintenance stage of treatment. As intramuscular administration avoids reliance on gastrointestinal tract absorption, it is particularly useful in patients who have undergone bowel surgeries or in patients with severe neurologic impairments who need optimal and quick repletion of vitamin B_{12}. Unless the patient self-administers it, the main disadvantages are the inconvenience and increased costs associated with receiving it at a medical facility. Actual monthly costs of oral and intramuscular formulations are otherwise similar (**Table 3**).[35]

In general, mild vitamin B_{12} deficiency should be treated with oral dosing, reserving intramuscular dosing for patients with significant neurologic symptoms, adherence issues, or extensive gastric or bowel resections. Patients with neurologic symptoms should have frequent injections until neurologic symptoms have disappeared and undergo more extended treatment if symptoms are severe.

Intranasal

Given the variable absorption of intranasal supplementation, closer clinical and serum methylmalonic acid monitoring is indicated to ensure therapeutic response. If the response is inadequate, switching to the intramuscular route should be considered.

Monitoring

There is no standard approach to monitoring response. Symptoms of anemia usually improve fairly quickly, but neurologic symptoms tend to resolve slowly or incompletely. The severity of neurologic symptoms at diagnosis may be predictive of outcome.[3,36]

Serum vitamin B_{12} levels fluctuate significantly with the timing of oral or intramuscular dosing, making testing of little value except in diagnosis. Serum methylmalonic acid levels do not necessarily correlate well with clinical improvement, as patients sometimes continue to report symptoms after levels have normalized. Therefore, a combination of clinical and laboratory testing is used to monitor therapy response.

Laboratory testing should include complete blood cell and reticulocyte counts. The reticulocyte count should increase after approximately 2 to 3 days, peaking at 5 to 7 days.[37] We recommend checking a complete blood cell count and reticulocyte count 4 weeks after the initiation of vitamin B_{12} therapy. The time point will also give an opportunity to reassess the symptoms and plan a transition to less-frequent dosing, if the response is adequate.

Hemoglobin typically starts increasing in a week, with expected complete normalization in 4 to 8 weeks.[37] Delayed or incomplete response should prompt further evaluation for other causes of anemia, including iron deficiency. In their dose-finding study, Eussen et al[32] reported absolute reductions of serum methylmalonic acid concentrations of at least 0.22 µmol/L at initial testing at 8 weeks and also at 16 weeks. Although the expected reduction of methylmalonic acid level is not standardized to vitamin B_{12} dosage, evidence nevertheless supports monitoring methylmalonic acid levels to assess response to B_{12} supplementation, especially in patients with pernicious anemia.[32,37] We recommend doing this at 4 weeks after initiation and on follow-up every 6 months to a year, as long as the complete blood cell count remains normal and there are no new symptoms. ■

Testing for suspected folate deficiency starts with serum or plasma folate

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