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 B₉) deficiency and cobalamin (vitamin B₁₂) 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 B₁₂ deficiency, usually due to pernicious anemia.
- Both vitamin deficiencies cause hematologic signs and symptoms of anemia; vitamin B₁₂ deficiency also causes neurologic symptoms.
- Oral supplementation is available for both vitamin deficiencies; intramuscular vitamin B₁₂ supplementation should be used in cases involving severe neurologic symptoms or gastric or bowel resection.

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 B₉) and cobalamin (vitamin B₁₂) 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. Nonmegaloblastic causes of macrocytic anemia include ethanol abuse, myelodysplastic syndrome, aplastic anemia, hypothyroidism, liver disease, and drugs. 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 B₉ (folate) or vitamin B₁₂ (cobalamin) (Table 1). Less-frequent causes include congenital disorders (inborn errors of metabolism), drugs (particu-
MEGALOBLASTIC ANEMIA

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.

### TABLE 1

<table>
<thead>
<tr>
<th>Characteristics of vitamin B(_{12}) vs folate deficiency</th>
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<tbody>
<tr>
<td><strong>Vitamin B(_{12}) deficiency</strong></td>
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<tr>
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<tr>
<td><strong>Etiology</strong></td>
</tr>
<tr>
<td>Lack of intrinsic factor: pernicious anemia</td>
</tr>
<tr>
<td>Malabsorption: celiac disease, prior gastric or ileal surgery</td>
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<tr>
<td>Dietary deficiency less common</td>
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<tr>
<td><strong>Clinical presentation</strong></td>
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<tr>
<td>Hematologic findings: cytopenias</td>
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<td><strong>Evaluation</strong></td>
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<tr>
<td><strong>Differential diagnosis</strong></td>
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<tr>
<td>Other macrocytic anemias without megaloblastic features: liver disease, thyroid dysfunction, alcohol abuse</td>
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<tr>
<td>Myelodysplastic syndrome, acute myeloid leukemia</td>
</tr>
<tr>
<td>Nitrous oxide exposure</td>
</tr>
<tr>
<td>Medication effect</td>
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<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td></td>
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<tr>
<td>High-dose oral vitamin B(_{12}), daily</td>
</tr>
<tr>
<td><strong>Monitoring and follow-up</strong></td>
</tr>
<tr>
<td>Monitor hematologic response: complete blood cell count</td>
</tr>
<tr>
<td>Pernicious anemia: consider monitoring methylmalonic acid</td>
</tr>
</tbody>
</table>

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 B₁₂ DEFICIENCY

Vitamin B₁₂ is produced by microorganisms and is found almost exclusively in foods of animal origin. Normal body stores of vitamin B₁₂ are 3 to 5 mg, and the recommended adult daily intake is 2.4 μg.⁷,⁸

Causes of vitamin B₁₂ deficiency are listed in Table 2. Dietary deficiency of vitamin B₁₂ occurs less frequently than folate deficiency because body stores can last for years owing to efficient enterohepatic recycling mechanisms. Although uncommon, dietary B₁₂ deficiency can occur even in industrialized countries in strict vegans and vegetarians, or in breastfed infants of mothers with vitamin B₁₂ deficiency.

Complex absorption pathway
Dietary absorption of vitamin B₁₂ 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 B₁₂ is released and binds to haptocorrin.⁴,⁹

Simultaneously, gastric parietal cells secrete intrinsic factor, which cannot interact with the vitamin B₁₂-haptocorrin complex. Not until food moves into the duodenum, where trypsin and other pancreatic enzymes cleave haptocorrin, is vitamin B₁₂ free to bind to intrinsic factor.⁹ The resultant vitamin B₁₂-intrinsic factor complex binds to the cubam receptor on the mucosal surface of enterocytes in the ileum. From there, vitamin B₁₂ 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 B₁₂-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 B₁₂ 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 B₁₂ deficiency in most cases.

TABLE 2

Causes of vitamin B₁₂ deficiency

<table>
<thead>
<tr>
<th>Common causes (related to malabsorption)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune gastritis (pernicious anemia)</td>
</tr>
<tr>
<td>Celiac disease</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>Surgical gastrectomy, gastric bypass, ileal resection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Less common causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional (strict vegans, breastfed infants of mothers with vitamin B₁₂ deficiency)</td>
</tr>
<tr>
<td>Nitrous oxide abuse</td>
</tr>
<tr>
<td><em>Diplolebothrium latum</em> infection</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
</tr>
<tr>
<td>Drug effect (metformin, proton pump inhibitors)</td>
</tr>
<tr>
<td>Inherited disorders affecting intrinsic factor or the cubam receptor</td>
</tr>
<tr>
<td>Rare inherited disorder (e.g., methylmalonic acidemia, transcobalamin II deficiency)</td>
</tr>
</tbody>
</table>

Information from references 4, 5, and 7.

Pernicious anemia and autoimmune gastritis
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 B₁₂, 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 B₁₂ defi-
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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 × 10⁹/L (reference range 4.5–10)
- Hemoglobin 6.2 g/dL (14–18)
- Mean corpuscular volume 121.5 fl (80–95)
- Platelet count 41 × 10⁹/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 dysynchrony, 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 B₁₂ level < 150 pg/mL (232–1,245)
- Parietal cell antibody positive (1:40)
- Conventional cytogenetics: normal male karyotype

In conjunction with normal cytogenetic and next-generation-sequencing panel results, undetectable vitamin B₁₂ levels helped confirm severe vitamin B₁₂ 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.

FOLATE AND VITAMIN B₁₂ METABOLISM ARE INTERTWINED

Folate and vitamin B₁₂ 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. Insufficient folate or vitamin B₁₂ leads to decreased thymidine available for DNA synthesis, hampering cell division and replication.

In pyrimidine synthesis, 5,10-methylene-tetrahydrofolate serves as the methyl donor, 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-
ductase is targeted by multiple drugs,\textsuperscript{5,13} which have the effect of decreasing available deoxythymidylate for DNA synthesis, resulting in megaloblastic anemia.

\section*{Drug Effects}

Owing to vitamin fortification of common foods in developed countries, megaloblastic anemia related to vitamin deficiency is increasingly uncommon.\textsuperscript{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.\textsuperscript{2,4,13}

Drugs that affect purine synthesis include:\textsuperscript{2,13}:

\begin{itemize}
  \item Immunosuppressants, eg, azathioprine and mycophenolate mofetil
  \item Chemotherapeutics, eg, purine analogues (fludarabine, cladribine, and thioguanine)
  \item Allopurinol, a xanthine oxidase inhibitor used to treat gout
\end{itemize}

Drugs that affect pyrimidine synthesis include:\textsuperscript{13}:

\begin{itemize}
  \item Immunomodulatory drugs, eg, leflunomide and teriflunomide
  \item Chemotherapeutics, eg, cytarabine, gemcitabine, and fluorouracil
  \item Methotrexate, an immunosuppressant and chemotherapeutic
  \item Sulfa drugs and trimethoprim
\end{itemize}

Numerous drugs from multiple classes can reduce folate or vitamin B\textsubscript{12} absorption, although this rarely leads to clinically significant deficiency.
Vitamin B₁₂ deficiency causes hematologic and neuropsychiatric manifestations that may occur together or independently.⁵,¹⁶ Megaloblastic anemia due to folate deficiency and other causes shares the same hematologic manifestations as vitamin B₁₂ 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 dysynchrony.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 B₁₂ deficiency can cause subacute combined degeneration of the dorsal and lateral columns of the spinal cord. Patients may experience bilateral and symmetrical pares-
sensory and decreased vibratory and positional sense. Psychiatric manifestations include memory loss, delirium, dementia, depression, mania, and hallucinations.\textsuperscript{15,17,18}

Atypical presentations
Although neuropsychiatric symptoms often develop after hematologic abnormalities, more than 25\% of patients with neurologic manifestations of vitamin B\textsubscript{12} deficiency have either a normal hematocrit or a normal MCV.\textsuperscript{17}

Why certain patients are prone to hematologic complications of vitamin deficiency and other patients have neurologic sequelaes 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\textsubscript{12} and folate deficiency, possibly as a consequence of hyperhomocysteinemia.\textsuperscript{19} Atrophic glossitis (swollen, erythematous, smooth tongue) is a common, albeit nonspecific, finding in vitamin B\textsubscript{12} deficiency.

\section*{INITIAL EVALUATION}
While there is no gold standard for diagnosing megaloblastic anemia, appropriate clinical and laboratory evaluation can usually establish the correct diagnosis.

\subsection*{History and physical examination}
A complete history and physical examination are imperative. Targeted questions should cover the following areas\textsuperscript{20}:

\begin{itemize}
  \item Diet—vegan or vegetarian?
  \item Surgical history—gastric or ileal resection?
  \item Gastrointestinal symptoms—celiac disease or gastritis?
  \item Neurologic symptoms such as paresthesias, numbness, ataxia, or gait disturbances?
  \item Medications—folic acid antagonists, chemotherapeutics?
\end{itemize}

\subsection*{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),
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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 B12 deficiency.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.

Vitamin B12 laboratory evaluation

Specific laboratory evaluation for vitamin B12 deficiency begins with total serum cobalamin levels.21,22 Vitamin B12 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 B12.4 In addition, in pernicious anemia, anti-intrinsic factor antibodies can interfere with vitamin B12 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 B12 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 B12 metabolism and are increased in vitamin B12 deficiency. Homocysteine is also elevated in folate deficiency and renal disease but methylmalonic acid is not, making it a more specific marker of vitamin B12 deficiency.4

Vitamin B12 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

Lack of intrinsic factor secondary to pernicious anemia is the cause of vitamin B12 deficiency in most cases

Neuroimaging for atypical cases

Neuroimaging is unnecessary for patients with a classic clinical presentation of vitamin B12 deficiency. However, in suspected cases without hematologic manifestations, magnetic resonance imaging is indicated. The most consistent finding in vitamin B12 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 dysynchrony (ie, a large, immature-appearing nucleus with an open chromatin pattern accompanied by a mature-appearing cytoplasm). Findings are also apparent in the granulocytic lineage, as seen by giant metamyelocytes and bands. 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 B12 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. 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% erythroblasts and > 30% proerythroblasts) without increased myeloid blasts.

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 B12 and folate levels, conventional cytogenetics, and next-generation sequencing, can also help distinguish the 2 entities. Identifying an acquired clonal abnormality, such as a myelodysplastic syndrome-associated cytogenetic abnormality or mutation, would strongly support a neoplastic process.

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 B12 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...
Megaloblastic anemia 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 B12 deficiency generally resolve more slowly than hematologic symptoms and may not resolve completely.

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, folic acid.

Folate deficiency is typically treated with oral folic acid 1 to 5 mg per day. 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 B12 SUPPLEMENTATION

Prompt treatment is particularly important for patients with vitamin B12 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.

Formulations vary

Vitamin B12 can be supplemented in different forms; noted preferences vary worldwide: cyanocobalamin in the United States, hydroxycobalamin in Europe, and methylcobalamin in Asia. 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 B12 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 B12

About 1.2% of oral cobalamin is passively absorbed unbound, while the remainder requires intrinsic factor to be absorbed in the ileum. Eussen et al found that high-dose oral vitamin B12 (> 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 B12 even in pernicious anemia, a 2018 Cochrane review found a lack of data demonstrating equivalence to intramuscular administration, mainly due to a limited number of quality randomized studies.

### TABLE 3

Estimated cost of treatment per month for vitamin B12 and folate deficiency

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dose</th>
<th>Cost per month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 Intramuscular injection</td>
<td>1,000 μg/mL, single vial of 1 mL</td>
<td>$5–$15</td>
</tr>
<tr>
<td>Oral</td>
<td>1,000 μg/pill, 30 pills per month</td>
<td>$2–$5</td>
</tr>
<tr>
<td>Nasal spray</td>
<td>500 μg/spray, single spray per day, carton of 4</td>
<td>$500–$640</td>
</tr>
<tr>
<td>Sublingual lozenges</td>
<td>3,000 μg/lozenge, single lozenge per day, ~ 30 lozenges per month</td>
<td>$5</td>
</tr>
<tr>
<td>Folic acid</td>
<td>1 mg/pill, 30 pills per month</td>
<td>$3–$5</td>
</tr>
</tbody>
</table>

*The dose and cost are adapted from GoodRx.com.*
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. 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 B₁₂. 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).

In general, mild vitamin B₁₂ 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. Serum vitamin B₁₂ 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. We recommend checking a complete blood cell count and reticulocyte count 4 weeks after the initiation of vitamin B₁₂ 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. Delayed or incomplete response should prompt further evaluation for other causes of anemia, including iron deficiency. In their dose-finding study, Eussen et al 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 B₁₂ dosage, evidence nevertheless supports monitoring methylmalonic acid levels to assess response to B₁₂ supplementation, especially in patients with pernicious anemia. 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.

**REFERENCES**

8. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, SOCHA AND COLLEAGUES
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