Vitamin B\textsubscript{12} Deficiency

Sally P. Stabler, M.D.

A 57-year-old woman reports increasing symptoms of painful paresthesias in both legs for the past 18 months. Physical examination reveals impaired position sense and vibration sense. The serum vitamin B\textsubscript{12} level is 205 pg per milliliter (151.2 pmol per liter), which is above the lower end of the laboratory reference range. The hematocrit is 42\%, with a mean corpuscular volume of 96 fl. The serum methylmalonic acid level is 3600 nmol per liter (normal level, <400), and the serum homocysteine level 49.1 μmol per liter (normal level, <14). How should this patient be further evaluated and treated?

**THE CLINICAL PROBLEM**

The recognition and treatment of vitamin B\textsubscript{12} deficiency is critical since it is a reversible cause of bone marrow failure and demyelinating nervous system disease. Vitamin B\textsubscript{12} (cobalamin) is synthesized by microorganisms and detected in trace amounts mostly in foods of animal origin.\textsuperscript{1} Uptake in the gastrointestinal tract depends on intrinsic factor, which is synthesized by the gastric parietal cells, and on the “cubam receptor” in the distal ileum.\textsuperscript{2} The most frequent cause of severe vitamin B\textsubscript{12} deficiency is a loss of intrinsic factor due to autoimmune atrophic gastritis,\textsuperscript{3} historically called “pernicious anemia,” even though many patients present with mainly neurologic manifestations.\textsuperscript{4,5}

**PATHOPHYSIOLOGY OF VITAMIN B\textsubscript{12} DEFICIENCY**

Vitamin B\textsubscript{12} is a cofactor for only two enzymes: methionine synthase and l-methylmalonyl–coenzyme A mutase\textsuperscript{6,7} (see Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The interaction between folate and B\textsubscript{12} is responsible for the megaloblastic anemia seen in both vitamin deficiencies. Dyssynchrony between the maturation of cytoplasm and that of nuclei leads to macrocytosis, immature nuclei, and hypersegmentation in granulocytes\textsuperscript{6} in the peripheral blood (Fig. 1A). The hypercellular and dysplastic bone marrow can be mistaken for signs of acute leukemia (Fig. 1B).\textsuperscript{10} The ineffective erythropoiesis results in intramedullary hemolysis and release of lactate dehydrogenase, features that are similar to those of microangiopathic hemolytic anemia.\textsuperscript{8} Clinical and laboratory findings of megaloblastic anemia in the peripheral blood and bone marrow are shown in Figure 2.

Vitamin B\textsubscript{12} is necessary for the development and initial myelination of the central nervous system as well as for the maintenance of its normal function. Demyelination of the cervical and thoracic dorsal and lateral columns of the spinal cord, occasional demyelination of cranial and peripheral nerves, and demyelination of white matter in the brain\textsuperscript{5} (i.e., “combined-systems disease” or “subacute combined degeneration”) can occur with vitamin B\textsubscript{12} deficiency (Fig. 2). Pathologi-
VITAMIN B\textsubscript{12} DEFICIENCY

- Vitamin B\textsubscript{12} deficiency causes reversible megaloblastic anemia, demyelinating neurologic disease, or both.
- Autoimmune gastritis (pernicious anemia) is the most common cause of severe deficiency.
- Methodologic problems may compromise the sensitivity and specificity of current vitamin B\textsubscript{12} assays.
- Measurement of methylmalonic acid, homocysteine, or both is used to confirm vitamin B\textsubscript{12} deficiency in untreated patients; an elevated level of methylmalonic acid is more sensitive and specific for the diagnosis.
- For patients with pernicious anemia or malabsorption, lifelong vitamin B\textsubscript{12} therapy is indicated.
- High-dose oral vitamin B\textsubscript{12} tablets (1000 to 2000 µg) taken daily are as effective as intramuscular monthly injections in correcting blood and neurologic abnormalities.

Dietary Deficiency in Infancy and Childhood

The infant of a mother with vitamin B\textsubscript{12} deficiency may be born with the deficiency or it may occur if he or she is exclusively breast-fed, usually between 4 and 6 months of age. Typical manifestations of vitamin B\textsubscript{12} deficiency in children include failure of brain development and overall growth and development, developmental regression, hypotonia, feeding difficulties, lethargy, tremors, hyperirritability, and coma (Fig. 2). Brain imaging may reveal atrophy and delayed myelination. Anemia may be present. Vitamin B\textsubscript{12} replacement results in rapid improvement in responsiveness, and many infants recover fully. However, the longer the period of deficiency, the more likely that there will be permanent disabilities. Mothers of infants with

KA \begin{align*}
\text{KEY CLINICAL POINTS} \\
\text{VITAMIN B}_{12} \text{ DEFICIENCY} \\
\text{• Vitamin B}_{12} \text{ deficiency causes reversible megaloblastic anemia, demyelinating neurologic disease, or both.} \\
\text{• Autoimmune gastritis (pernicious anemia) is the most common cause of severe deficiency.} \\
\text{• Methodologic problems may compromise the sensitivity and specificity of current vitamin B}_{12} \text{ assays.} \\
\text{• Measurement of methylmalonic acid, homocysteine, or both is used to confirm vitamin B}_{12} \text{ deficiency in untreated patients; an elevated level of methylmalonic acid is more sensitive and specific for the diagnosis.} \\
\text{• For patients with pernicious anemia or malabsorption, lifelong vitamin B}_{12} \text{ therapy is indicated.} \\
\text{• High-dose oral vitamin B}_{12} \text{ tablets (1000 to 2000 µg) taken daily are as effective as intramuscular monthly injections in correcting blood and neurologic abnormalities.}
\end{align*}
vitamin B₁₂ deficiency often have unrecognized pernicious anemia, but alternatively, they may have a history of gastric bypass surgery, the short-gut syndrome, or a long-term vegetarian or vegan diet. Tandem mass spectrometry, used in neonatal screening programs in all 50 states, may detect nutritional B₁₂ deficiency owing to an increase in propionyl carnitine, but direct measurement of methylmalonic acid has higher sensitivity. Other causes of B₁₂ deficiency in children, such as ileal resections, the Imerslund–Gräsbeck syndrome, inflammatory bowel disease, and pernicious anemia, are listed in Table 1.

**Strategies and Evidence**

**Evaluation**
Both the clinical recognition of vitamin B₁₂ deficiency and confirmation of the diagnosis by means of testing can be difficult. An approach to testing is shown in Table 2.

The patient’s history may include symptoms of anemia, underlying disorders causing malabsorption, and neurologic symptoms. The most common neurologic symptoms are symmetric paresthesias or numbness and gait problems. The physical examination may reveal pallor, edema, pigmented changes in the skin, jaundice, or neurologic defects such as impaired vibration sense, impaired position and cutaneous sensation, ataxia, and weakness (Fig. 2).

Bone marrow biopsy and aspiration are not necessary for the diagnosis of megaloblastic anemia and may be misleading in cases of severe pancytopenia with hypercellularity, increased erythroblasts, and even cytogenetic abnormalities, confusing the diagnosis with acute leukemia. Imaging of the spinal cord is not indicated in patients with recognized vitamin B₁₂ deficiency, but in cases of severe myelopathy that are not initially recognized as the result of vitamin B₁₂ deficiency, there is characteristic hyperintensity on T₂-weighted imaging, described as an inverted V-shaped pattern in the cervical and thoracic spinal cord.

**Vitamin B₁₂ Assay**
The first test performed to confirm the diagnosis of vitamin B₁₂ deficiency is generally measurement of the serum vitamin B₁₂ level. Although an extremely low level (<100 pg per milliliter [<73.8 pmol per liter]) is usually associated with clinical deficiency, such low levels are infrequently observed. Both false negative and false positive values are common (occurring in up to 50% of tests) with the use of the laboratory-reported lower limit of the normal range as a cutoff point for deficiency. The high rate of false negative and false positive results may be
Brain
- Altered mental status
- Cognitive defects
- "Megaloblastic madness": depression, mania, irritability, paranoia, delusions, lability

Spinal cord
- Myelopathy
- Spongy degeneration
- Paresthesias
- Loss of proprioception: vibration, position, ataxic gait, limb weakness; spasticity (hyperreflexia); positive Romberg sign; Lhermitte's sign; segmental cutaneous sensory level

Autonomic nervous system
- Postural hypotension
- Incontinence
- Impotence

Peripheral nervous system
- Cutaneous sensory loss
- Hyporeflexia
- Symmetric weakness
- Paresthesias

Bone marrow
- Hypercellular, increased erythroid precursors
- Open, immature nuclear chromatins
- Dysynchrony between maturation of cytoplasm and nuclei
- Giant bands, metamyelocytes
- Karyorrhexis, dysplasia
- Abnormal results on flow cytometry and cytogenetic analysis

Abnormalities in infants and children
- Developmental delay or regression, permanent disability
- Does not smile
- Feeding difficulties
- Hypotonia, lethargy, coma
- Hyperirritability, convulsions, tremors, myoclonus
- Microcephaly
- Choreoathetoid movements

Infertility

Peripheral blood
- Macrocytic red cells, macroovalocytes
- Anisocytosis, fragmented forms
- Hypersegmented neutrophils, 1% with six lobes or 5% with 5 lobes
- Leukopenia, possible immature white cells
- Thrombocytopenia
- Pancytopenia
- Elevated lactate dehydrogenase level (extremes possible)
- Elevated indirect bilirubin and aspartate aminotransferase levels
- Decreased haptoglobin level
- Elevated levels of methylmalonic acid, homocysteine, or both

Optic atrophy, anosmia, loss of taste, glossitis

Bone/marrow
- Hypercellular, increased erythroid precursors
- Open, immature nuclear chromatin
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Optic atrophy, anosmia, loss of taste, glossitis
due to the fact that only 20% of the total measured vitamin B₁₂ is on the cellular delivery protein, transcobalamin; the remainder is bound to haptocorrin, a protein of unknown function.²⁷ Most laboratories now perform automated assays of vitamin B₁₂ on platforms used for many other analytes. There is often poor agreement when samples are assayed by different laboratories or with the use of different methods.³¹-³⁴ Because intrinsic factor is used as the assay-binding protein, anti–intrinsic factor antibodies (which are common in pernicious anemia) must be removed chemically from the sample, which has proved to be problematic in the automated assays.³⁵,³⁴ Recent studies show normal values³⁴ or falsely high values³³ of vitamin B₁₂ in many patients with pernicious anemia. New assays of holotranscobalamin (to measure the vitamin B₁₂ saturation of transcobalamin) provide a modest improvement in specificity over that provided by assays of total serum vitamin B₁₂, but they have not been clinically validated²⁷-²⁹ and are not yet available commercially in the United States.

Given the limitations of available assays, clinicians should not use a laboratory’s reported lower limit of the normal range to rule out the diagnosis of vitamin B₁₂ deficiency in patients with compatible clinical abnormalities. Clinicians should also recognize that vitamin B₁₂ values are frequently low in patients without other metabolic or clinical evidence of vitamin B₁₂ deficiency (i.e., megaloblastic anemia or myelopathy).

**Measurement of Serum Methylmalonic Acid and Total Homocysteine**

Measurement of methylmalonic acid, total homocysteine, or both in patients with suspected vitamin B₁₂ deficiency, then malabsorption must be present. Testing for pernicious anemia is described in Table 2. A positive test for anti–intrinsic factor or anti–parietal-cell antibodies is indicative of pernicious anemia; surveillance for autoimmune thyroid disease is reasonable in patients with positive antibody tests. Chronic atrophic gastritis can be diagnosed on the basis of an elevated fasting serum gastrin level and a low level of serum pepsinogen I.³,¹⁰ Some experts recommend endoscopy to confirm gastritis and rule out gastric carcinoid and other gastric cancers, since patients with pernicious anemia are at increased risk for such cancers.³

The Schilling test of radioactive vitamin B₁₂
### Table 1. Causes and Treatment of Vitamin B<sub>12</sub> Deficiency.

<table>
<thead>
<tr>
<th>Cause</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe malabsorption</td>
<td>Intramuscular cyanocobalamin at a dose of 1000 µg administered intramuscularly daily or every other day for 1 week, then weekly for 4 to 8 weeks, and then monthly for life, or oral cyanocobalamin at a daily dose of 1000 to 2000 µg for life.</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Pernicious anemia (autoimmune gastritis)</td>
<td>Administer iron and folic acid replacement as needed for full hemoglobin response, especially in patients with intestinal disease; perform serological tests for other autoimmune conditions, especially rheumatoid arthritis in patients with pernicious anemia; perform upper gastrointestinal endoscopy in patients with symptoms of gastric cancer or iron deficiency.</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Total or partial gastrectomy</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Gastric bypass or other bariatric surgery</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Gastrectomy or partial gastrectomy</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Inflammatory bowel disease, tropical sprue</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Imerslund–Gräsbeck and other syndromes†</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Mild malabsorption</td>
<td>Oral cyanocobalamin at a dose of 500 to 1000 µg daily or intramuscular cyanocobalamin at a daily dose of 1000 µg or every other day for 4 to 8 weeks, then weekly for 4 to 8 weeks, and then monthly for life.</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Protein-bound vitamin B&lt;sub&gt;12&lt;/sub&gt; malabsorption</td>
<td>Oral cyanocobalamin at a dose of 500 to 1000 µg daily or intramuscular cyanocobalamin at a daily dose of 1000 µg or every other day for 4 to 8 weeks, then weekly for 4 to 8 weeks, and then monthly for life.</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Mild atrophic gastritis</td>
<td>Oral cyanocobalamin at a dose of 500 to 1000 µg daily or intramuscular cyanocobalamin at a daily dose of 1000 µg or every other day for 4 to 8 weeks, then weekly for 4 to 8 weeks, and then monthly for life.</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Use of metformin&lt;sup&gt;14&lt;/sup&gt;</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Use of drugs that block stomach acid</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Dietary deficiency</td>
<td>Same as for pernicious anemia</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Adults</td>
<td>Oral supplements containing &gt;2 µg of vitamin B&lt;sub&gt;12&lt;/sub&gt; as folic acid or B&lt;sub&gt;12&lt;/sub&gt;-enriched formula or food.</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Infants</td>
<td>Intramuscular cyanocobalamin at a dose of 1000 µg daily or intramuscular cyanocobalamin at a daily dose of 1000 µg or every other day for 4 to 8 weeks, then weekly for 4 to 8 weeks, then monthly for life.</td>
<td>Same as for pernicious anemia</td>
</tr>
<tr>
<td>Breastfeeding in infants with vitamin B&lt;sub&gt;12&lt;/sub&gt;–deficient mothers&lt;sup&gt;13,16&lt;/sup&gt;</td>
<td>Oral supplementation with 112 µg of vitamin B&lt;sub&gt;12&lt;/sub&gt; daily for the first 4 weeks, then vitamin B&lt;sub&gt;12&lt;/sub&gt;–enriched formula or food.</td>
<td>Same as for pernicious anemia</td>
</tr>
</tbody>
</table>

<sup>1</sup> Perform tests for iron deficiency, anemia of chronic kidney disease, and anemia of chronic inflammation; these conditions coexist frequently in older adults, may limit the response to treatment, and may require further treatment.

<sup>2</sup> Genetic counseling to detect vitamin B<sub>12</sub> deficiency in family members.

<sup>3</sup> Confirm metabolic response in infants or refer parents to genetics specialist for evaluation; provide nutritional counseling for mothers.

<sup>4</sup> Dietary supplements containing >2 µg of vitamin B<sub>12</sub> are recommended for older adults with pernicious anemia.

<sup>5</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>6</sup> Refer parents to genetics specialist for evaluation; provide nutritional counseling for mothers.

<sup>7</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>8</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>9</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>10</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>11</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>12</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>13</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>14</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>15</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.

<sup>16</sup> Consult metabolic bone disease, tropical sprue, and other syndromes.
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Children

Diseases similar to those causing malabsorption in adults

100 μg of intramuscular vitamin B₁₂ monthly or high-dose oral vitamin B₁₂ daily in younger children; treatment as adults in older children

Confirm pernicious anemia or congenital malabsorption

Recreational or occupational abuse of nitrous oxide†

Intramuscular cyanocobalamin at a dose of 1000 μg administered on the same schedule as that for pernicious anemia above and for life if underlying pernicious anemia is present

Evaluate for vitamin B₁₂ malabsorption; provide addiction counseling

Nitrous oxide anesthesia in occult pernicious anemia

* Intramuscular hydroxocobalamin can be substituted for intramuscular cyanocobalamin, but document the long-term response if it is administered at 3-month intervals.
† Experts are not in agreement about the necessity or frequency of routine upper endoscopy in patients with pernicious anemia. However, symptoms suggestive of gastric atrophy, unexplained iron deficiency, and proven gastrointestinal blood loss should prompt a full investigation.
‡ Congenital malabsorption of vitamin B₁₂ results from mutations of the ileal cubam receptor, cubilin, or amnionless (as in the Imerslund–Gräsbeck syndrome) and from mutations in gastric intrinsic factor. These syndromes are usually manifested in infancy and early childhood, although studies have shown a delay in onset even into adolescence.†
§ Nitrous oxide inactivates the vitamin B₁₂-dependent enzyme methionine synthase and causes formation of vitamin B₁₂ analogues and gradual tissue depletion of vitamin B₁₂.

© CLINICAL PRACTICE

DIRECTIONS 

High-Dose Oral Treatment

1. Start with 2.4 μg per day, but higher amounts — 4 to 7 μg per day — which are common in persons who eat meat or take a daily multivitamin, are associated with lower methylmalonic acid values.
2. Healthy older adults should consider taking supplemental crystalline vitamin B₁₂ as recommended by the Food and Nutrition Board. However, most patients with clinical vitamin B₁₂ deficiency have malabsorption and will require parenteral or high-dose oral replacement. Adequate supplementation results in resolution of megaloblastic anemia and resolution of or improvement in myelopathy.
3. Injected vitamin B₁₂, however, is no longer available. A potential replacement, absorption test is under development.

The daily requirement of vitamin B₁₂ has been set at 2.4 μg per day, but higher amounts — 4 to 7 μg per day — which are common in persons who eat meat or take a daily multivitamin, are associated with lower methylmalonic acid values. Healthy older adults should consider taking supplemental crystalline vitamin B₁₂ as recommended by the Food and Nutrition Board. However, most patients with clinical vitamin B₁₂ deficiency have malabsorption and will require parenteral or high-dose oral replacement. Adequate supplementation results in resolution of megaloblastic anemia and resolution of or improvement in myelopathy.

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Figure 3. The Normal Mechanisms and Defects of Absorption of Vitamin B\textsubscript{12}.

The vitamin B\textsubscript{12} (Cbl) released from food protein by peptic action is bound to haptocorrin (HC) in the stomach and travels to the duodenum, where pancreatic proteases digest the HC, releasing Cbl to bind to intrinsic factor (IF). The IF-Cbl complex binds to a specific receptor in the distal ileum (the cubam receptor) and is internalized, eventually released from lysosomes, and transported into the blood. Both HC and transcobalamin (TC) bind Cbl in the circulation, although the latter is the cellular delivery protein. Adapted from Stabler.\textsuperscript{6}
### Table 2. Laboratory Testing in Vitamin B₁₂ Deficiency.*

<table>
<thead>
<tr>
<th>Test to detect deficiency</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum vitamin B₁₂ &lt;200 pg/ml or laboratory cutoff level</td>
<td>65–95% for proven clinical deficiency; 50% for detecting elevated level of methylmalonic acid</td>
<td>50–60% for clinical response; 80% for detecting elevated level of methylmalonic acid</td>
<td>Current vitamin B₁₂ assays are especially problematic in patients with anti–intrinsic factor antibodies</td>
</tr>
<tr>
<td>Serum vitamin B₁₂ &lt;350 pg/ml</td>
<td>90%</td>
<td>25% for detecting elevated level of methylmalonic acid</td>
<td></td>
</tr>
<tr>
<td>Holotranscobalamin &lt;20 to 45 pmol/liter‡</td>
<td>Insufficient data on sensitivity for clinical deficiency; 46–89% for detecting elevated level of methylmalonic acid</td>
<td>Insufficient data on specificity for clinical deficiency; 28–96% for detecting elevated level of methylmalonic acid</td>
<td>Levels of holotranscobalamin increase in renal failure; superior to measurement of total vitamin B₁₂ in pregnancy, when the total level decreases</td>
</tr>
<tr>
<td>Serum methylmalonic acid &gt;400 nmol/liter§</td>
<td>98% for clinical deficiency</td>
<td>Poor specificity for clinical response in patients with modest elevation of level of methylmalonic acid (300–1000 nmol/liter)¶</td>
<td>Renal failure and volume depletion may increase level of serum methylmalonic acid, but rarely to &gt;1000 nmol/liter</td>
</tr>
<tr>
<td>Serum or plasma total homocysteine &gt;21 µmol/liter</td>
<td>96% for clinical deficiency</td>
<td>Homocysteine level also increased in clinical folate deficiency and renal insufficiency</td>
<td></td>
</tr>
</tbody>
</table>

| Test to determine cause of deficiency‖ | | | |
| Pernicious anemia | | | |
| Anti–intrinsic factor antibodies | 50% | 100% | Must be tested >7 days after vitamin B₁₂ injection to prevent false positive result |
| Anti–parietal-cell antibodies | 80% | 50–100% | |
| Atrophic body gastritis (antral sparing)** | | | |
| Fasting high serum gastrin level (>100 pmol/liter) | 85% | | |
| Low level of serum pepsinogen I (<30 µg/liter) | 90% | | |
| Endoscopy with pentagastrin-fast hypochlorhydra | 100% | | Rarely performed |
| Malabsorption of vitamin B₁₂†† | | | |
| Vitamin B₁₂ absorption test | | | Schilling test no longer available |
| Increase in serum holotranscobalamin level after oral loading | Unknown | Unknown | Promising preclinical data, but still experimental |

* To convert the values for vitamin B₁₂ to picomoles per liter, multiply by 0.7378.
† Available assays are largely chemiluminescent microparticle immunoassays performed with the use of automated analyzers that in general show higher values than the radiodilution and microbiologic assays used in past studies of clinically confirmed deficiency. Thus, these tests are likely to have lower sensitivities and specificities than the older assays.
‡ The holotranscobalamin assay has been studied widely in Europe but is not yet commercially available in the United States. The appropriate lower end of the reference range is still under debate. The values for sensitivity and specificity are reviewed in Heil et al.²⁹
§ Urinary methylmalonic acid has not been extensively studied, but values greater than 2.5 µmol per millimole of creatinine suggest deficiency.
¶ Elevated levels of methylmalonic acid fail with vitamin B₁₂ therapy, but an associated clinical response is highly variable, depending largely on the presence of vitamin B₁₂–related disease.
‖ Evidence of a causal pathologic process does not confirm coexisting B₁₂ deficiency, since underlying gastrointestinal disease may predate the deficiency by many years.
** The relationship between atrophic body gastritis (autoimmune gastritis) and infection with Helicobacter pylori is variable. Antral sparing is a type of atrophic body gastritis in which the cells in the antrum can produce high levels of gastrin.
†† There is malabsorption if clinically proven vitamin B₁₂ deficiency is present in a patient who eats meat, receives multivitamin therapy, or both.
Figure 4. Serum Methylmalonic Acid and Total Homocysteine Concentrations in 491 Episodes of Vitamin B<sub>12</sub> Deficiency.

The data shown have been combined from studies performed over a period of 25 years. Most of the patients with clinically confirmed vitamin B<sub>12</sub> deficiency had documented pernicious anemia and a proven response to vitamin B<sub>12</sub> therapy. Open circles indicate episodes in patients with a hematocrit lower than 38%, and solid circles indicate episodes in those with a hematocrit of 38% or higher. Patients without anemia had neurologic manifestations of vitamin B<sub>12</sub> deficiency and similar values of methylmalonic acid and total homocysteine. The axis for serum methylmalonic acid is plotted on a log scale. The dashed lines indicate values that are required large oral doses (>500 μg daily) to correct elevated levels of methylmalonic acid. Adapted from Stabler.

Areas of Uncertainty

Vitamin B<sub>12</sub> deficiency is the major cause of hyperhomocysteinemia in countries with folate-fortified food, such as the United States and Canada. Most of the patients with pernicious anemia showed that 0.5 to 4% of radioactively labeled oral vitamin B<sub>12</sub> can be absorbed by passive diffusion in both normal controls and patients with pernicious anemia. Thus, oral doses of 1000 μg deliver 5 to 40 μg, even if taken with food.

A randomized trial that compared an oral dose of 2000 μg daily with parenteral therapy (seven injections of 1000 μg of cyanocobalamin over a period of 1 month, followed by monthly injections) in patients with pernicious anemia, atrophic gastritis, or a history of ileal resection showed similar reductions in the mean corpuscular volume and increases in the hematocrit at 4 months in both groups. All participants (four in each group) with paresthesias, ataxia, or memory loss had resolution or improved with treatment. However, levels of methylmalonic acid after treatment were significantly lower with daily oral treatment (169 nmol per liter, vs. 265 nmol per liter with parenteral treatment) and vitamin B<sub>12</sub> levels were significantly higher (1005 pg per milliliter vs. 325 pg per milliliter [741.5 vs. 239.8 pmol per liter]). A more recent trial with a similar design involving a proprietary oral vitamin B<sub>12</sub> preparation also revealed significantly lower levels of methylmalonic acid in the oral-treatment group at the 3-month follow-up. In a randomized trial comparing oral with intramuscular vitamin B<sub>12</sub> (1000-μg doses, daily for 10 days, then weekly for 4 weeks, and monthly thereafter), the two groups had similar improvements in hematologic abnormalities and vitamin B<sub>12</sub> levels at 90 days. Case series of patients treated with oral vitamin B<sub>12</sub> have yielded variable results; elevated levels of methylmalonic acid, homocysteine, or both were reported in about half of patients with malabsorption who were treated with twice-weekly oral doses of 1000 μg, whereas normal homocysteine levels were reported in patients treated with 1500 μg daily after gastrectomy. Data are lacking from long-term studies to assess whether oral treatment is effective when doses are administered less frequently than daily. Studies involving older adults, many of whom had chronic atrophic gastritis, showed that 60% required large oral doses (>500 μg daily) to correct elevated levels of methylmalonic acid.

Proponents of parenteral therapy state that compliance and monitoring are better in patients who receive this form of therapy because they have frequent contact with health care providers, whereas proponents of oral therapy maintain that compliance will be improved in patients who receive oral therapy because of convenience, comfort, and decreased expense. High-dose vitamin B<sub>12</sub> tablets (500 to 1500 μg) are available in the United States without a prescription. Self-administered injections are also easily taught, economical, and in my experience, effective. Patients should be informed of the pros and cons of oral versus parenteral therapy, and regardless of the form of treatment, those with pernicious anemia or malabsorption should be reminded of the need for lifelong replacement.
Canada. Epidemiologic studies show significant associations between elevated homocysteine levels and vascular disease and thrombosis. However, large randomized trials of combined high-dose vitamin B therapy in patients with vascular disease have shown no reduction in vascular events.\(^4\) Vitamin \(B_{12}\) status should be evaluated in patients with hyperhomocysteinemia before folic acid treatment is initiated.

The potential role of mild vitamin \(B_{12}\) deficiency in cognitive decline with aging remains uncertain. Epidemiologic studies indicate an inverse association between vitamin \(B_{12}\) supplementation and neurodegenerative disease, but results of randomized trials have been largely negative.\(^5\)

Besides oral tablets, vitamin B is available in sublingual preparations, oral sprays, nasal gels or sprays, and transdermal patches. Data on the absorption and efficacy of these alternative preparations are lacking.

**GUIDELINES**

Nutritional guidelines for vitamin \(B_{12}\) intake are published by the Food and Nutrition Board,\(^4\) and nutritional guidelines for vegetarians are published by the American Dietetic Association.\(^40\) There are no recommendations from the American Society of Hematology for the diagnosis and treatment of vitamin \(B_{12}\) deficiency. The American Academy of Neurology recommends measurements of vitamin \(B_{12}\), methylmalonic acid, and homocysteine in patients with symmetric polyneuropathy.\(^51\) The American Society for Gastrointestinal Endoscopy recommends a single endoscopic evaluation at the diagnosis of pernicious anemia.\(^52\)

**CONCLUSIONS AND RECOMMENDATIONS**

The patient in the vignette has neurologic abnormalities that are consistent with vitamin \(B_{12}\) deficiency. Since vitamin \(B_{12}\) levels may be above the lower end of the laboratory reference range even in patients with clinical deficiency, methylmalonic acid, total homocysteine, or both should be measured to document vitamin \(B_{12}\) deficiency before treatment is initiated; the elevated levels in this patient confirm the diagnosis. In the absence of dietary restriction or a known cause of malabsorption, further evaluation is warranted—in particular, testing for pernicious anemia (anti–intrinsic factor antibodies). Either parenteral vitamin \(B_{12}\) treatment (8 to 10 loading injections of 1000 \(\mu g\) each, followed by monthly \(1000-\mu g\) injections), or high-dose oral vitamin \(B_{12}\) treatment (1000 to 2000 \(\mu g\) daily) is an effective therapy. I would review both options (including the possibility of self-injection at home) with the patient. Effective vitamin replacement will correct blood counts in 2 months and correct or improve neurologic signs and symptoms within 6 months.

Dr. Stabler reports holding patents (assigned to the University of Colorado and Competitive Technologies) on the use of homocysteine, methylmalonic acid, and other metabolites in the diagnosis of vitamin \(B_{12}\) and folate deficiency, but no longer receiving royalties for these patents. No other potential conflict of interest relevant to this article was reported.

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

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