Vitamin B\textsubscript{12} deficiency is more widespread in the population than has been assumed so far (1, 2). Since a deficiency in this vitamin can lead to irreversible neurological damage, early diagnosis is essential (3, e1, e2). In recent years, new and sensitive diagnostic markers to determine a person’s vitamin B\textsubscript{12} status have become available. It is therefore important to review the suitability of vitamin B\textsubscript{12} as a marker for the vitamin B\textsubscript{12} status. This article describes causes and effects of vitamin B\textsubscript{12} deficiency and presents the currently available laboratory markers for diagnosing vitamin B\textsubscript{12} deficiency disease.

**Methods**

This review article is based on a selective literature search. The authors searched PubMed using the following search terms: “diagnosing vitamin B\textsubscript{12} deficiency,” “symptoms of vitamin B\textsubscript{12} deficiency,” “metabolic markers of vitamin B\textsubscript{12} deficiency.” The authors used acknowledged references for their scientific and clinical work.

**Results and discussion**

Transport and metabolic function of vitamin B\textsubscript{12}

On the one hand, vitamin B\textsubscript{12} is a cofactor of L-methylmalonyl-CoA-mutase; as desoxyadenosylcobalamin it is involved in the isomerization of L-methylmalonyl-CoA to succinyl-CoA. On the other hand, as methylcobalamin it is a cofactor for methionine synthase (e3). This enzyme transfers a methyl group of 5-methyltetrahydrofolate to homocysteine during the synthesis of methionin. In case of intracellular deficiency of cobalamin, plasma concentrations of methyl malonic acid (MMA) and homocysteine will rise. Vitamin B\textsubscript{12} from food is made available through pepsin and gastric acid. It binds to R-binder (haptocorrins).
and is transferred to the intrinsic factor (IF) in the intestinal lumen by means of a pH dependent process. In the terminal ileum, the IF-B12 complex binds to IF receptors on the membrane surface of enterocytes and is then transferred through the ileal membrane. Vitamin B12 is subsequently released in the enterocytes and transferred to transcobalamin II (TC) (figure 1). The B12-TC complex—known as holotranscobalamin (holoTC)—arrives in the blood circulation and circulates until it is taken up by the cells. A maximum of 30% of circulating B12 is bound to TC, which represents metabolically active B12. The vitamin B12 that is bound to haptocorrin is thought to transport the surplus of vitamin B12 to the liver.

**Modern biomarkers for metabolic vitamin B12 deficiency**

Total vitamin B12 measurement is used cost effectively as the parameter of choice, but it has limited sensitivity and specificity, especially in persons with vitamin B12 concentrations <400 pmol/L (4, e4). If the total vitamin B12 concentration is in the lower reference range, 156 to 400 pmol/L, vitamin B12 deficiency cannot be ruled out. Clinical signs of vitamin B12 deficiency can be seen in persons with vitamin B12 concentrations within the reference range (>156 pmol/L) (5). Persons with normal concentrations of vitamin B12 may have raised concentrations of MMA (>300 nmol/L) and lowered concentrations of holoTC (<35 pmol/L), owing to intracellular, metabolically manifest (functional) vitamin B12 deficiency (4). By contrast, lowered concentrations of B12 and normal MMA indicate a false positive finding.

A lowered serum holoTC concentration is the earliest marker of vitamin B12 deficiency and signals that the body does not have sufficient available vitamin B12 and that the B12 stores are emptying as a result of the negative balance of B12 (4). At this stage, clinical or hematological symptoms might not yet be present.

Lowered holoTC combined with raised MMA and homocysteine levels are indicative of metabolically manifest vitamin B12 deficiency. Clinical signs may already be present but can still be missing—the patient may therefore still be clinically inconspicuous (6). Metabolically manifest B12 deficiency can affect the bone metabolism, for example, and stimulate osteoclasts (7). The exact prevalence of clinically significant B12 deficiency is not known; the range of symptoms is wide and the new markers enable the detection of vitamin deficiency notably more often.

Measuring MMA is expensive and requires special equipment, such as mass spectrometers. The holoTC immunoassay is available as an automated test. The costs are about double that of total vitamin B12. With regard to the cost-benefit effect of early detection of vitamin B12 deficiency by using holoTC, this test will become established as the laboratory parameter of choice to measure vitamin B12 status.

No consensus exists with regard to screening for vitamin B12 deficiency. Screening makes sense when first signs of B12 deficiency can be detected before neurological or hematological anomalies develop. For this reason, only the modern biomarkers, such as holoTC and MMA, are suitable screening tools. Although holoTC is a very early marker and MMA a functional biomarker for vitamin B12 deficiency, there is no universal marker for vitamin B12 status because limitations exist with regard to their diagnostic informative value (figure 2).

**Development and clinical presentation of vitamin B12 deficiency**

Insufficient intake or disrupted absorption of vitamin B12 will result in vitamin B12 deficiency. According to the recommended dietary intake (RDI) guidelines from the National Research Council of the US National Academy of Sciences, adults should ingest 2.4 μg daily, pregnant women up to 6 μg (8). The calculation of the required amount is based on the calculation of the amount of vitamin B12 that is necessary to sustain a normal hematological status (normal hemoglobin and mean corpuscular volume of erythrocytes [MCV]) and to maintain remission in pernicious anemia. At the time when the recommended dietary intake was set, no studies had investigated the direct link between vitamin B12 intake

![FIGURE 1](image-url)

Transport and cellular absorption of vitamin B12.

B12, vitamin B12; TC, transcobalamin II; MS, methionine synthase; Ado, desoxyadenosyl
and MMA concentrations. New data have shown that the plasma concentration of MMA and homocysteine falls when vitamin B12 is ingested, whereas the holoTC concentration rises (9). A minimum daily intake of 6 μg vitamin B12 results in an optimal plasma concentration of the investigated biomarkers (9). More recent studies have shown that the recommended daily intake of B12 should be newly determined and seems too low, especially for older people.

Vitamin B12 is important for DNA synthesis, and formation and maintenance of myelin sheaths, the synthesis of neurotransmitters, and erythropoiesis. Clinical vitamin B12 deficiency has two main manifestations: hematological and neuropsychiatric disorders. Symptoms often develop before a shortfall on the lower B12 reference limit (6). Macrocytic anemia is regarded as a late indicator of vitamin B12 deficiency.

The macrocytosis caused by B12 deficiency can be masked by concomitant iron deficiency, and the diagnosis is thus difficult (e5). Iron deficiency related microcytosis dominates over B12 deficiency related macrocytosis if the iron deficiency is more severe than the B12 deficiency (e6). The B12 deficiency can cause an additional loss of iron by means of a secondary effect on the enterocytes (e6).

Large vitamin B12 stores exist in the body, which is why a deficiency will become evident only after many years. In general, vitamin B12 deficiency develops in several stages:

- Depletion of stores
- Metabolic-functional disorder
- Clinical manifestation.

Hyperhomocysteinemia in vitamin B12 deficiency is important as an atherogenic risk factor but also as a sign of hypomethylation—for example, of DNA, RNA, myelin, phospholipids, or neurotransmitters. Hypomethylation occurs subsequent to the reduced availability of S-adenosyl-methionine (SAM), which is a universal methyl group donor. Vitamin B12 deficiency inhibits methionine synthase. The result is reduced methionine synthesis, with subsequent lowering of the SAM concentration. Funicular spinal cord disease (myelosis) is a common neurological sequela of vitamin B12 deficiency. The psychiatric and neurological disorders and the cognitive disorders, depression, or dementia that are observed in vitamin B12 deficiency can precede hematological anomalies by years, and sometimes such anomalies do not even develop.

Morphological changes to blood and bone marrow cells are among the main symptoms of vitamin B12 deficiency. Because of their high cell turnover rate, hematopoiesis reacts rapidly and sensitively to the blocked nucleic acid metabolism. Megaloblastic anemia in vitamin B12 deficiency develops as a result of disrupted DNA synthesis and the resultant maturation disorder of the cell nucleus, whereas the cytoplasm develops normally. In the periphery, macrocytic erythrocytes (MCV >110 fl) and hypersegmented neutrophils can be observed.

Risk groups

The prevalence of subclinical functional vitamin B12 deficiency is higher than hitherto assumed when sensitive and relatively specific markers are used—such as MMA, holoTC, and homocysteine (10, 11). Risk groups for vitamin B12 deficiency include (table)

- patients with unexplained anemia;
- patients with unexplained neuropsychiatric symptoms;
- patients with gastrointestinal manifestations, including stomatitis, anorexia, and diarrhea;
- elderly people (11);
- vegetarians (4);
- patients with gastrointestinal disorders, such as Crohn’s disease or infection with Helicobacter pylori, or patients with stomach resection (12).

The rate of people in the risk population who will develop clinical symptoms because of vitamin B12 deficiency has thus far not been studied systematically.
In the population, the prevalence of vitamin B12 deficiency in younger people is 5% to 7% (e8, 13). Functional vitamin B12 deficiency—that is, raised MMA and lowered holoTC—is common in old age and has been diagnosed in 10% to 30% of patients older than 65 years of age (10, 11, 14). A high prevalence of a slightly abnormal vitamin B12 status has been reported in elderly people, despite intake of the recommended daily dose (≥ 2.4 μg/day). This deficiency is not presumed to be associated with dietary causes but with malabsorption (15). 53% of elderly patients from Strasbourg who had vitamin B12 deficiency had malabsorption problems, 33% had pernicious anemia; in only 2% was vitamin B12 deficiency related to insufficient dietary intake, and in 11% the etiology of the vitamin B12 deficiency remained unexplained (16). However, because the currently recommended dietary intake for vitamin B12 in elderly people is low, dietary deficiencies are underdiagnosed.

Using synthetic B12 preparations can protect elderly persons from symptoms of deficiency (e8, 17). Dietary intake of B12, however, does not provide any information on the vitamin B12 status because malabsorption is a common and important factor. Further, elderly persons often have atrophic gastritis, pernicious anemia, or achlorhydria. Disorders that affect the gastrointestinal pH can also result in malabsorption and thus vitamin B12 deficiency. The incidence of Helicobacter pylori is high in elderly people and can lead to atrophic gastritis, and in turn to B12 malabsorption, owing to disrupted production of hydrochloric acid (1). Helicobacter pylori was found in 56% of patients with vitamin B12 deficiency (18). In 40% of patients, serum concentrations of B12 rose after treatment for Helicobacter pylori infection. According to recent reports, longer term treatment of Helicobacter pylori (1 year) resulted in a significant rise in mean vitamin B12 (from 146 pmol/L to 271 pmol/L) and a fall in mean homocysteine concentrations (from 41 μmol/L to 13 μmol/L) (19). B12 malabsorption owing to Helicobacter pylori infection can thus lead to vitamin B12 deficiency and hyperhomocysteinemia (e9).

Vegetarians are at high risk of developing vitamin B12 deficiency because animal products are the main sources of B12. A functional B12 deficiency (lowered holoTC, raised MMA and homocysteine) is common in vegetarians and depends on the strictness of the diet and how long the vegetarian diet has been followed. A study of lacto-vegetarians and ovo-lacto-vegetarians found raised MMA in 63% of subjects (>271 nmol/L), lowered holoTC concentrations (<35 pmol/L) in 73% , and hyperhomocysteinemia (>12 μmol/L) in 33%. In vegans, raised MMA was found in 86%, lowered holoTC in 90%, and hyperhomocysteinemia in 55% (4).

Persons with an increased vitamin requirement are a further risk group for B12 deficiency—for example, pregnant and breast feeding women, patients with autoimmune disorders, or persons with HIV infection. Persons who regularly take proton pump inhibitors can also develop vitamin B12 deficiency.

B12 deficiency is also widespread in patients with renal disorders (20). In spite of normal plasma concentrations of vitamin B12 or holoTC, these patients often have raised serum concentrations of MMA and homocysteine (20). These can be corrected with vitamin B12 substitution, which indicates a deficiency before starting treatment (20). The likely cause is a disrupted cellular absorption of holoTC, which results in intracellular vitamin B12 deficiency and raised metabolites. Studies have shown that patients with renal disorders may have higher concentrations of holoTC, which seems to contradict B12 deficiency (20, 21). This can be explained with the role of the kidney in transcobalamin filtration and resultant secondary accumulation of holoTC. The plasma concentration of holoTC in such

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TABLE

**Risk populations with high frequency of vitamin B12 deficiency, who should be tested at regular intervals (every 2 to 3 years)**

- **Group**: Vegetarian, vegan, and macrobiotic diet, Neonates and breast-fed infants of vegetarian mothers, Elderly people, Neurodegenerative and neuropsychiatric disorders, Chronic atrophic corpus gastritis, Disorders of the terminal ileum, Macrocytic anemia, Chronic alcoholism, Medication, AIDS associated myelopathy.
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- **Risk populations with high frequency of vitamin B12 deficiency**
  - Pregnant and breast feeding women
  - Patients with autoimmune disorders
  - Persons with HIV infection
  - Persons who regularly take proton pump inhibitors
  - Elderly people
  - Persons with an increased vitamin requirement
  - Persons with an increased vitamin requirement
  - Vegetarians
  - Patients with chronic atrophic corpus gastritis
  - Patients with disorders of the terminal ileum
  - Patients with macrocytic anemia
  - Patients with chronic alcoholism
  - Medication
  - Patients with AIDS associated myelopathy

**Notes**

- Risk populations with high frequency of vitamin B12 deficiency, who should be tested at regular intervals (every 2 to 3 years).
- In the population, the prevalence of vitamin B12 deficiency in younger people is 5% to 7% (e8, 13). Functional vitamin B12 deficiency—that is, raised MMA and lowered holoTC—is common in old age and has been diagnosed in 10% to 30% of patients older than 65 years of age (10, 11, 14). A high prevalence of a slightly abnormal vitamin B12 status has been reported in elderly people, despite intake of the recommended daily dose (≥ 2.4 μg/day). This deficiency is not presumed to be associated with dietary causes but with malabsorption (15). 53% of elderly patients from Strasbourg who had vitamin B12 deficiency had malabsorption problems, 33% had pernicious anemia; in only 2% was vitamin B12 deficiency related to insufficient dietary intake, and in 11% the etiology of the vitamin B12 deficiency remained unexplained (16). However, because the currently recommended dietary intake for vitamin B12 in elderly people is low, dietary deficiencies are underdiagnosed.
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The treatment of vitamin B12 deficiency depends on the underlying causes. Blocked or reduced oral bioavailability, such as occurs in pernicious anemia, requires injections of vitamin B12. If, however, there are no obvious reasons for an injection, oral substitution is a sensible strategy.

Vitamin B12 supplementation can be used for treatment or prevention depending on whether a person is at risk or already affected. In long standing vitamin B12 deficiency, dietary modifications are not sufficient; these patients require longer term B12 supplementation to normalize their metabolism (e10). Vegetarians and older persons receiving oral vitamin B12 supplementation (10 to 500 μg) have been shown to have lower concentrations of MMA and higher holoTC and B12 concentrations than persons not receiving supplementation. This indicates the metabolic efficacy of oral supplementation (4, 22). In randomized studies in elderly patients, a daily intake of 1 to 2 mg cyanocobalamin have resulted in normalization of the metabolic signs of the B12 deficiency and in improved neurological symptoms—e.g., in terms of memory power, gait, perception of vibrations, and paresthesias (6, 17). Vidal-Alaball et al. (23) have shown in randomized studies that compared with intramuscular application, high oral dosages of vitamin B12 (1 mg and 2 mg; daily at the start of treatment, then weekly, and later monthly) are of comparable efficacy in terms of improved hematological and neurological symptoms.

The therapeutic recommendations with regard to dosage and administration of B12 substitution treatment are divergent (24). In the United States, patients usually receive vitamin B12 injections of 1 mg daily in their first week of treatment. In the following month, they receive weekly injections and then monthly injections (25). In Denmark, patients receive injections of 1 mg cyanocobalamin weekly during the first month and every 3 months subsequently, or 1 mg hydroxocobalamin every other month (e11).

The optimal dose of B12 can be adjusted by testing B12 status in blood by means of laboratory parameters. Measuring homocysteine and MMA concentrations is helpful in monitoring vitamin therapy (17). The homocysteine concentration provides information on whether the intracellular methionine cycle is functioning (which depends on B vitamins), whereas MMA and holoTC are divergent (24). In the United States, patients usually receive vitamin B12 injections of 1 mg daily in their first week of treatment. In the following month, they receive weekly injections and then monthly injections (25).

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Corresponding author
Prof. Dr. med. habil. Dr. rer. nat. Wolfgang Herrmann
Universitätsklinikum des Saarlandes
Klinische Chemie und Laboratoriumsmedizin/Zentrallabor
Gebäude 57
66421 Homburg/Saar, Germany
prof.wolfgang.herrmann@uniklinikum-saarland.de

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Causes and Early Diagnosis of Vitamin B$_{12}$ Deficiency

Wolfgang Herrmann, Rima Obeid

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