Diagnosis and treatment of vitamin B12 deficiency. An update

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We present an update on the diagnosis and treatment of vitamin B12 deficiency. Vitamin B12 deficiency should be suspected in all patients with unexplained anemia and/or neurological symptoms, as well as in patients at risk of developing vitamin B12 deficiency such as the elderly and patients with intestinal diseases. Measurement of plasma cobalamins is suggested as the primary analysis followed by measurement of plasma methylmalonic acid in uncertain cases. Accumulating evidence indicates that measurements of the biologically active cobalamin, plasma holotranscobalamin (holoTC), may be superior to plasma cobalamins, and these are currently being introduced into the clinical setting. No consensus exists concerning evaluation of the cause for vitamin B12 deficiency, and the pros and cons of the different tests mainly aiming at evaluation of the function of the gastric mucosa are presented. Once the diagnosis of vitamin B12 deficiency has been confirmed efficient treatment can be ensured either by injections every 2-3 months or by a daily dose of 1 mg vitamin B12.

Key words: cobalamins, vitamin B12 deficiency, holotranscobalamin, methylmalonic acid.

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Vitamin B12 deficiency is a major public health problem: however, there is no consensus on how to diagnose this deficiency. In the present review we attempt to give a balanced update. We include only issues related to diagnosing and treating vitamin B12 deficiency in the adult population and do not cover inherited diseases of vitamin B12 deficiency except for those related to classical pernicious anemia.

From a fatal disease to a biochemical condition

Classical pernicious anemia as described by Thomas Addison in the middle of the 19th century[1] does not usually give substantial diagnostic problems. Patients with the relatively rare, archetypical disease have symptoms of macrocytic anemia, glossitis, and neurological manifestations, such as paraesthesias and abnormal gait due to disturbances of vibratory sensation and proprioception.[2,3] The majority of these patients are easy to recognize and, if diagnosed and treated in due time, they can be completely cured. The major diagnostic challenge concerns patients who develop subtle vitamin B12 deficiency,[4] often without the presence of anemia.[3] It is difficult to diagnose these patients but at the same time important to do so since neurological manifestations may be irreversible if treatment is initiated too late.[5] This diagnostic challenge led to the search for early biochemical markers for vitamin B12 deficiency and thereby to the development of improved methods for measuring the two metabolites that accumulate if vitamin B12 is lacking: plasma methylmalonic acid (MMA) and plasma total homocysteine (tHcy).[6,7]

The introduction of MMA and tHcy assays has changed the understanding of vitamin B12 deficiency considerably. If vitamin B12 deficiency is defined as an increased concentration of MMA, vitamin B12 deficiency is no longer a relatively rare disease occurring with a prevalence of around 2% in the elderly[8] but a common condition affecting at least 15% of the elderly depending on the decision limits used.[9-11]

Causes of vitamin B12 deficiency

Lack of vitamin B12 may be caused by insufficient intake or by malabsorption of the vitamin[12] as outlined in Figure 1. Insufficient intake of vitamin B12 is seen in vegetarians and especially in vegans. Apart from these specific conditions, insufficient intake is not believed to be common in industrialized parts of the world. Malabsorption of vitamin B12 occurs in patients suffering from a number of gastrointestinal conditions. Common conditions are related to a decreased or abolished output of gastric intrinsic factor and/or hypo- or achlorhydria as seen in patients with destruction of the

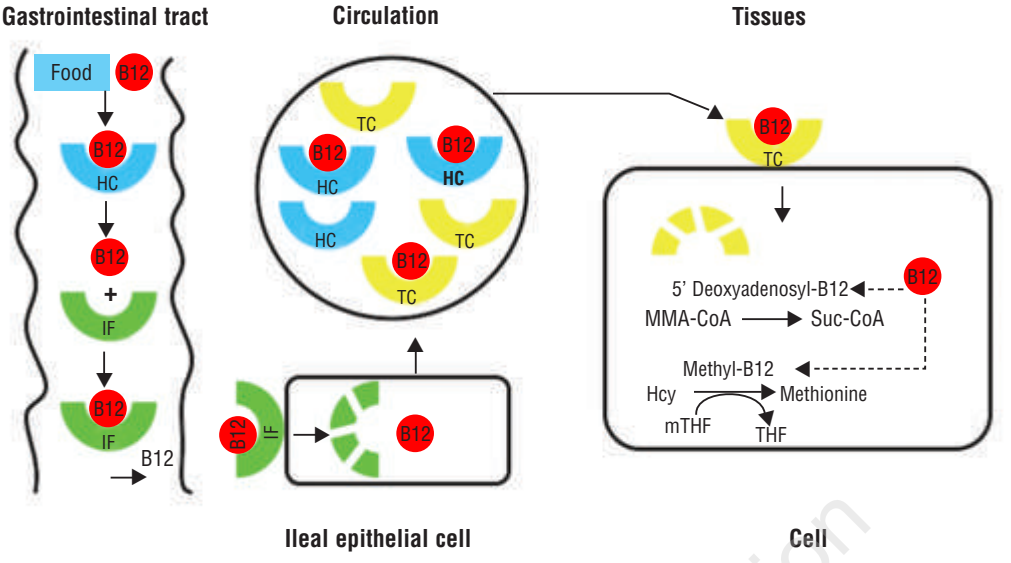


Figure 1. Vitamin B12 absorption, transport in the circulation, and function within the cell. Vitamin B12 is supplied by animal products (Food B12). After ingestion, dietary vitamin B12 enters the stomach bound to animal proteins and is released from the proteins by pepsin and hydrochloric acid. The free vitamin B12 is then bound to haptocorrin (*HC, light blue*) released from the salivary glands. In the small intestine, haptocorrin is degraded by pancreatic enzymes, and vitamin B12 is transferred to intrinsic factor (*IF, green*), a protein synthesized in the gastric parietal cell and secreted into the gastric juice. The IF-vitamin B12 complex is internalized in the distal part of the small intestine by the IF- vitamin B12 receptor-complex cubilin-amnionless[63,64] and thereafter IF is degraded by proteolysis.[12,65] Subsequently, only vitamin B12 enters the systemic circulation. Approximately 1% of the ingested vitamin B12 is believed to be taken up by passive diffusion in its free form,[12] a fact that explains why vitamin B12 deficiency can be treated by a large dose of oral vitamin B12. *In the circulation*, vitamin B12 is bound to two proteins, transcobalamin (*TC, yellow*) and haptocorrin (*HC, dark blue*). Vitamin B12 attached to transcobalamin is referred to as holotranscobalamin (holoTC). HoloTC represents the biologically active fraction that is delivered to all tissues of the body,[66,67] whereas the function of hapotocorrin is unknown. *After cellular uptake* of holoTC, transcobalamin is degraded,[67] and vitamin B12 functions as a co-enzyme for two enzymatic reactions: the conversion of methylmalonyl-CoA (MMA-CoA) to succinyl-CoA (Suc-CoA), and the conversion of homocysteine (Hcy) to methionine, which is accompanied, in the same enzymatic reaction, by the conversion of methyltetrahydrofolate (mTHF) to tetrahydrofolate (THF). Because THF is needed for normal DNA synthesis, vitamin B12 deficiency results in impaired synthesis of DNA.

gastric mucosa caused either by an autoimmune mechanism or by gastric atrophy. Importantly, a recent study suggests that in these cases vitamin B12 deficiency may be preceded by iron deficiency.[13] Achlorhydria hampers the extraction of vitamin B12 from protein food sources, and since intrinsic factor is needed for the intestinal internalization of vitamin B12, lack of this protein results in impaired vitamin B12 uptake. The prevalence of gastric atrophy increases with age,[14,15] which may well explain why malabsorption of vitamin B12 becomes more frequent with age. Malabsorption also occurs in pancreatic insufficiency because of lack of the enzymes needed to liberate vitamin B12 from haptocorrin, the protein that initially binds ingested vitamin B12.[12] Other reasons for malabsorption include gastric or ileal resection and disorders of the small intestine such as celiac disease, Crohn's disease, and ileitis.[12] Finally, long-term use of proton pump inhibitors,[16,17] histamine (H2) receptor antagonists,[18] or biguanides[19] may lead to reduced absorption of vitamin B12 and, possibly, vitamin B12 deficiency.[20]

When should vitamin B12 deficiency be suspected?

Vitamin B12 deficiency should be suspected in all patients with unexplained anemia, unexplained neuropsychiatric symptoms, and/or gastrointestinal manifestations, including sore tongue, anorexia, and diarrhea. Special attention should be paid to patients at risk of developing vitamin B12 deficiency. This includes mainly elderly people because of their high prevalence of atrophic gastritis,[14,15] vegetarians and vegans, and patients with intestinal diseases. Other groups may be considered at risk, including patients with autoimmune disorders such as Graves' disease, thyroiditis, and vitiligo as well as patients receiving proton pump inhibitors, histamine receptor antagonists, or biguanides for prolonged periods.

The question of screening for vitamin B12 deficiency is under debate. The rationale for screening is the possibility of detecting cases of subtle vitamin B12 deficiency, defined as a state with increased metabolites in patients without anemia.[21] We hesitate to recommend such screening since we do not find the specificity of the current tests sufficiently high for this purpose as several factors other than vitamin B12 deficiency may affect the results of the laboratory tests used for diagnosing vitamin B12 deficiency (Table 1). If some of the current tests were used for screening this would result in large-scale over-diagnosis and, in accordance with Carmel,[22] we do not find this justified as subtle vitamin B12 deficiency is

Table 1. Pros and cons of laboratory tests used to diagnose vitamin B12 deficiency.

Test, plasma	*Rationale and advantage*	*Disadvantage*
Cobalamins	Decreases in vitamin B12 deficiency Easily accessible Cheap	Variation in reference interval due to different methods Sensitivity and specificity debatable False positive if haptocorrin is reduced False negative if haptocorrin is increased (e.g. in chronic myeloid leukemia)
Methylmalonic acid	Increases in vitamin B12 deficiency High sensitivity	Not asily accessible Expensive Specificity debatable: False positive with reduced renal function
Total homocysteine	Increases in vitamin B12 deficiency High sensitivity	Special procedure for sample handling is required Low specificity: Influenced by life style factors (smoking, alcohol intake, coffee comsumption) False positive in folate deficiency False positive in vitamin B6 deficiency False positive with reduced renal function
Holotranscobalamin	Decreases in vitamin B12 deficiency Expected to have high sensitivity	Specificity needs to be further clarified

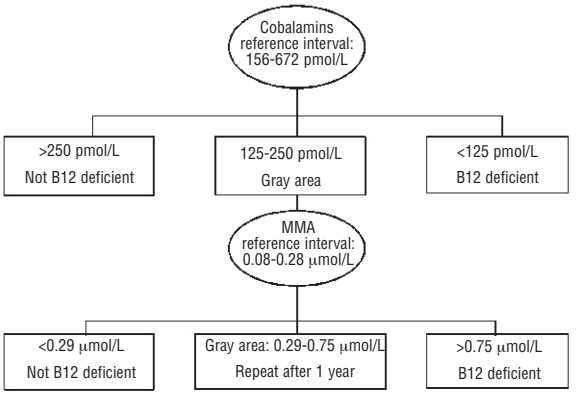
not a severe clinical problem and the benefit of treatment in these patients is still debatable.

Laboratory tests used for diagnosing vitamin B12 deficiency

We present a summary of the pros and cons regarding the use of the current biochemical markers of vitamin B12 deficiency and describe a feasible approach for diagnosing this vitamin deficiency.

Measurements of plasma cobalamins have been used worldwide since the 1950s. Despite limited specificity (Table 1) and controversy about their sensitivity,[23] these measurements are still the standard investigation for vitamin B12 deficiency.[24] Patients with plasma cobalamin concentrations well below the reference interval are generally considered to have B12 deficiency.[24]

The two metabolic markers MMA and tHcy are generally considered more sensitive indicators of vitamin B12 status than are plasma cobalamin levels;[23,25] however, Solomon recently reported that normal plasma cobalamin levels as well as normal MMA and tHcy levels were found in patients with clinical signs of vitamin B12 deficiency improving after treatment with the vitamin.[26] The specificity of the metabolic markers has been



[a]*Reference interval suggested by the manufacturer*

Figure 2. How to diagnose vitamin B12 deficiency. The flowchart shows a strategy for diagnosing vitamin B12 deficiency employing analysis of plasma cobalamins and plasma methylmalonic acid (MMA).

extensively discussed (Table 1)[27-29] and in general the specificity is considered rather low especially as regards tHcy. The major limitation concerning the metabolic markers is that tHcy also increases in patients with folate deficiency, and for MMA the complexity of the assay, the high cost, and often a slow turnaround time may be problematic.

Because of the limitations involved in the use of the metabolites for diagnosing vitamin B12 deficiency, there has been a continuous search for new biochemical markers. From a theoretical point of view, measurement of the amount of cobalamin available for the cells, which is cobalamins attached to transcobalamin (holoTC), would be a sensitive marker. Recently, suitable methods for measuring holoTC have been introduced,[30,31] and several studies evaluating the clinical performance of holoTC measurements[24,32-35] have shown that holoTC is an early marker of vitamin B12 deficiency. However, it is still debatable how much additional diagnostic value the measurement of holoTC provides beyond the already existing tests.[32] Further studies are also needed in order to decide whether holoTC can be used alone or should be used in combination with one of the other vitamin B12 markers.

Until the clinical usefulness of holoTC has been settled, we recommend the strategy shown in Figure 2. In this strategy the initial analysis consists in measuring plasma cobalamins. A plasma cobalamin level 25% lower than the bottom reference interval suggested by the manufacturer of the assay is considered to confirm the suspicion of vitamin B12 deficiency (<125 pmol/L if the lower limit of the reference interval is 156 pmol/L). If plasma cobalamin levels are in the gray area, an MMA assay is required, and based on the MMA levels and clinical signs, vitamin B12 deficiency can be confirmed

Table 2. Tests employed to investigate the cause of vitamin B12 deficiency.

Test	*Rationale and advantage*	*Disadvantage*
IF[1] antibodies	Pathognomic for pernicious anemia High specificity is ~ 100%	Relatively low sensitivity ~ 70% Methodologic diversity
Pepsinogen	Mirrors gastric function; increases in gastric atrophy High sensitivity	Low specificity
Gastrin	Mirrors gastric function; increases in gastric atrophy Relatively high sensitivity	Fasting sample needed Low specificity
Parietal cell antibodies	May be present in pernicious anemia	Very low specificity
Schilling's test	Considered to be the gold standard as a functional test of vitamin B12 absorption; measures absorption of free (I) or IF bound (II) vitamin B12 by measurement of the amount of labeled vitamin B12 excreted in the urine High specificity if lack of IF is the cause of reduced vitamin B12 absorption	Requires administration of radioactive vitamin B12 Requires collection of 24 h urine Requires use of IF False positive with reduced renal function Decreasing availability
CobaSorb	Functional test of vitamin B12 absorption; Measures absorption of free or IF-bound vitamin B12 by measuring the increase in holotranscobalamin and thus whether IF can correct a reduced vitamin B12 absorption Expected to have a high specificity if lack of IF is the cause of reduced vitamin B12 absorption	Requires availability of the holotranscobalamin assay Needs further evaluation before introduction into routine clinical practice

[1]*IF: intrinsic factor.*

or ruled out. If the level of MMA is also in the gray area, we advise repeating measurements of plasma cobalamins and possibly MMA a year later unless obvious clinical signs indicate vitamin B12 deficiency and thereby the need for treatment. This strategy involves two important points. In the first place the gray area for MMA concentrations is wide, meaning that the level of MMA has to be quite high (>0.75 μmol/L) before it is diagnostic of vitamin B12 deficiency. This point is supported by a previous, randomized placebo controlled study in which only limited clinical improvement was found after vitamin B12 treatment in patients with a moderately elevated MMA.[36,37] Secondly, the advice to wait a year before re-examining patients with an MMA concentration within the gray area – unless clinical signs are present – is based on the finding that in general a moderately increased MMA did not increase further during 1 to 4 years in patients not given vitamin B12.[38] Obviously, re-testing should be performed earlier if clinical signs appear. Abnormal blood hemoglobin concentration and erythrocyte mean cell volume support the suspicion of pernicious anemia, but normal levels do not rule out the presence of vitamin B12 deficiency.

Finding the cause of vitamin B12 deficiency

Once diagnosed, the next challenge is to find the cause of the vitamin B12 deficiency. Numerous tests aimed mainly at evaluating the function of the gastric mucosa have been developed over the years (Table 2), but none of them has been optimally standardized nor has any unified recommendation concerning their use been agreed upon. In the following, we present our view concerning the panel of examinations to consider once the diagnosis of vitamin B12 deficiency has been made. We recommend no further laboratory tests if a thorough history reveals dietary insufficiency (vegetarians or vegans), or gastric or ileal resection. Also in elderly patients, in whom the diagnosis of vitamin B12 deficiency is obvious according to the clinical findings and first line laboratory tests, we consider it debatable as to whether further tests need to be performed before treatment.

In all other patients we recommend further testing in order to clarify the cause of the vitamin B12 deficiency, and especially to establish whether the patient has classical pernicious anemia (Table 2). The presence of antibodies to intrinsic factor is pathognomonic for pernicious anemia: however, only about 70% of patients with pernicious anemia have these antibodies.[39] Determination of pepsinogen (pepsinogen I and pepsinogen I-pepsinogen II ratio) is a highly sensitive test,[39] but it is not specific.[40,41] Increased levels of plasma gastrin are suggestive but this test is not specific either.[42] Measurement of plasma parietal cell antibodies is diag-

nostically unreliable because of the low specificity.[39] All in all measurement of intrinsic factor antibodies, pepsinogen, and gastrin are considered useful for evaluating the cause of vitamin B12 deficiency,[39,43] but so far no firm recommendation concerning the use of these test can be offered.

For years, Shilling's test, in which labeled vitamin B12 is administered orally alone (Shilling's test I) or together with intrinsic factor (Shilling's test II), has been used to investigate whether lack of the vitamin is caused by lack of intrinsic factor,[44] and this test has been considered the gold standard for investigating vitamin B12 absorption. Shilling's test is, however, no longer readily available due to increasing difficulties in obtaining labeled vitamin B12 and intrinsic factor; the native human intrinsic factor for Shilling's test II is no longer available and substitutes such as hog intrinsic factor are difficult to obtain and only sparingly used because of safety concerns.

Because of the difficulties with Shilling's test, we recently proposed an alternative approach to measuring vitamin B12 absorption, named CobaSorb.[45,46] In CobaSorb an oral dose of non-radioactive vitamin B12 is administered, and active vitamin B12 absorption is reflected by an increase in holoTC, as demonstrated in healthy individuals.[45,46] No absorption, reflected as unchanged levels of holoTC, was observed in patients with Imerslund Grasbecks syndrome or inherited lack of intrinsic factor.[46] So far, CobaSorb has only been evaluated in a few patients with acquired vitamin B12 deficiency.[45,47]

Another theoretically feasible vitamin B12 absorption test was recently described by Carkett *et al.*[48] In this absorption test acceleration mass spectrometry is used to assess the absorption and kinetics of carbon-14-labeled vitamin B12. So far, this test has only been reported for one healthy human.[48]

Since patients with pernicious anemia have an increased risk of developing gastric cancer most experts recommend endoscopic screening for gastric malignancy about every 5 years in young patients with this disorder.[49,50]

Examination of the bone marrow is no longer a standard procedure when evaluating patients with vitamin B12 deficiency. In our opinion this should only be done if there is a need to rule out malignancy.

How and when to treat vitamin B12 deficiency

As outlined above it is not always easy to decide whether a patient suffers from vitamin B12 deficiency or not. In the following we first discuss treatment strategies in patients for whom a firm diagnosis has been established and then we present some reflections concerning what to do if the diagnosis of vitamin B12 deficiency is uncertain. Patients with vitamin B12 deficiency despite a normal absorption, such as vegetarians and vegans, only need a daily supplement in the form of a vitamin pill containing at least 6 μg of vitamin B12.[51] Patients with an irreversible cause of vitamin B12 deficiency are destined to lifelong treatment with a pharmacological dose of vitamin B12. Planning the strategy for treatment involves a decision on dose, route, and form of vitamin B12 to be employed as well as determination of the need for continuous follow-up.

Different forms of vitamin B12 can be used, including cyano,- hydroxy,- and methylcobalamin. Cyanocobalamin is the only form available in the USA. Hydroxycobalamin may have advantages due to a slower metabolism:[12,52] however, the depot preparation of cyanocobalamin (cyanocobalamin-tannin complex suspended in a sesame oil- aluminium monostearate gel) is metabolised even slower than hydroxycobalamin.[53,54] The co-enzyme form, methylcobalamin, is the preferred form in Japan,[12] but to our knowledge there is no evidence proving its superiority.

In most countries vitamin B12 is still given by intramuscular injection in the form of cyanocobalamin or hydroxycobalamin.[55] However, traditions concerning both dose and administration vary considerably. In the USA the usual treatment regime is injections with a daily dose of 1000 μg cyanocobalamin for the first week followed by weekly injections for the next month and after that monthly injections.[56] In Denmark, the recommended practice is to inject 1000 μg of the depot preparation of cyanocobalamin weekly for 4 weeks and thereafter every third month[53,54] or hydroxycobalamin every second month. For some, still unknown, reason some patients require maintenance treatment at even shorter intervals even when the depot preparation of cyanocobalamin is used.[57]

Some prefer to use oral vitamin B12, a route of treatment that accounted for more than 70% of the total vitamin B12 prescribed in Sweden in 2000.[55] Several studies report that a daily oral dose of at least 1000 μg of vitamin B12 is sufficient to maintain a normal vitamin B12 status[58-61] in patients with hematologic manifestations. However, the effect of oral vitamin B12 treatment in patients presenting with severe neurologic manifestations has not yet been adequately addressed.[62] Until this has been done, parenteral vitamin B12 treatment is still to be recommended for such patients.

When initiating treatment with vitamin B12, some points should be emphasized mainly as regards patients presenting with anemia. Once vitamin B12 has been administered, the increase in red cell production will increase the demand on iron stores and, therefore, it is important to monitor – and correct – any signs of iron deficiency.[12] Secondly, a folate deficiency may be unmasked as demonstrated by a drop in plasma folate after initiation of vitamin B12 treatment.[12]

There is no consensus concerning the need for continuous monitoring of the treatment effect once vitamin B12 treatment has been initiated. We recommend clinical eval-

uation of the treatment response. In patients with anemia due to vitamin B12 deficiency, we recommend hematologic monitoring (reticulocytes after 1 week and hemoglobin after 1 to 2 months), but apart from that, biochemical control is, in our opinion, not needed in patients receiving parenteral treatment. In patients given oral vitamin B12 plasma cobalamins, MMA or tHcy should be monitored if poor compliance is suspected.

A major challenge is to decide whether to treat or not patients whose clinical symptoms and laboratory test results are conflicting. In patients with severe signs of vitamin B12 deficiency we believe treatment should be given independently of the laboratory results, a point supported by the study of Solomon.[26] In patients with non-specific symptoms in combination with borderline results for plasma cobalamins and MMA one may choose to monitor the effect of vitamin B12 treatment, and then continue treatment provided a clinical improvement is evident. As described above and in Figure 2, one may also choose to re-examine the patient within a year or upon development of further symptoms.

An important direction for future research will be to clarify who, from among the group of patients with subtle vitamin B12 deficiency, would possibly benefit from treatment with vitamin B12. We believe that a large double-blinded intervention study is required in order to answer this question. Such a study would be of great importance both in order to clarify the need for active intervention and also in order to delineate a diagnostic strategy allowing identification of those in need of treatment.

Both authors have contributed equally in writing the paper.

Disclosures: Ebba Nexo is one of a group of researchers who holds patents for the production and use of recombinant human intrinsic factor produced in plants. Based on these patents the researchers together with investors established the firm Cobento A/S. A-MH has acted as a consultant for Cobento A/S. Other authors declare that they have no potential conflicts of interest.

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