Holotranscobalamin – An Early Marker for Laboratory Diagnosis of Vitamin B_{12} Deficiency

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Abstract

Vitamin B_{12} deficiency is widespread. Among the population groups at risk are older people, vegetarians, pregnant women and patients with renal or intestinal diseases. The neurological symptoms of vitamin B_{12} deficiency are unspecific and can be irreversible. Early detection is therefore important. This article reviews the diagnostic performance of the different laboratory markers for vitamin B_{12} status. Total serum vitamin B_{12} is a relatively insensitive and unspecific biomarker of deficiency that does not reflect recent variations in cobalamin status. Holotranscobalamin (holoTC), the metabolically active portion of vitamin B_{12}, is the earliest laboratory parameter that becomes decreased in case of a vitamin B_{12} negative balance. Concentration of methylmalonic acid (MMA) is a functional vitamin B_{12} marker that will increase when the vitamin B_{12} stores are depleted. Isolated lowering of holoTC shows vitamin B_{12} depletion (negative balance), while lowered holoTC plus elevated MMA (and homocysteine) indicates a metabolically manifested vitamin B_{12} deficiency, although there still may be no clinical symptoms. The diagnostic use of holoTC allows the initiation of therapeutic measures before irreversible neurological damage develops. Because the clinical manifestations of vitamin B_{12} deficiency are unspecific, people at risk should be identified and should regularly test their holoTC with or without MMA.

Keywords

Cobalamin, holotranscobalamin, diagnosis, deficiency

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Pernicious anaemia, which causes severe vitamin B_{12} (cobalamin) deficiency, used to be a fatal, non-curable disease. However, in 1926 George Minot and William Murphy discovered that pernicious anaemia can be treated by including large amounts of liver in patients' food. Vitamin B_{12} is synthesised exclusively in micro-organisms. Animals obtain vitamin B_{12} from foods contaminated with vitamin B_{12}-synthesising bacteria, and thus foods of animal origin represent the only source of vitamin B_{12} in the human diet.

Together with 5-methyltetrahydrofolate, vitamin B_{12} is an essential co-factor in methyl group transfer, cell division and catabolism of homocysteine (Hcy). In addition, vitamin B_{12} is required by all cells for one-carbon metabolism and DNA synthesis and maintenance. Only two vitamin B_{12}-dependent enzymes are known in humans: methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase mediates the formation of methionine from Hcy, and requires methylcobalamin as a co-factor. L-methylmalonyl-CoA mutase requires adenosylcobalamin, and catalyses the formation of succinyl-CoA from methylmalonyl-CoA. The latter compound can be converted into methylmalonic acid (MMA). Serum concentrations of MMA and Hcy are therefore considered metabolic indicators of vitamin B_{12} status.

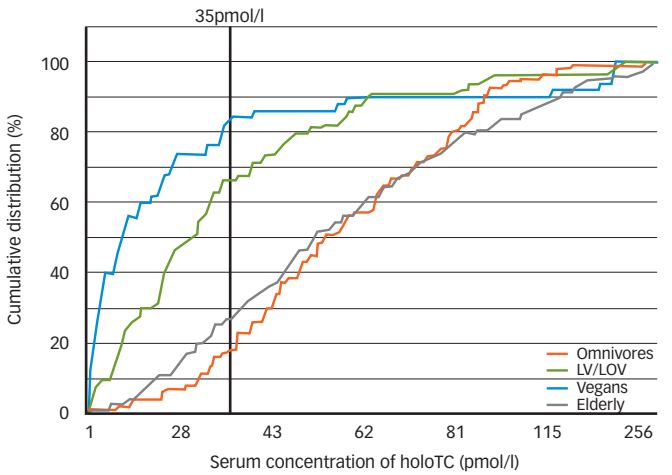
Vitamin B_{12} is conserved in humans through the enterohepatic circulation and via active re-absorption in the proximal tubule. The estimated stores of vitamin B_{12} in the human liver are relatively high.[1] Therefore, in the case of intact absorption, the depletion of the vitamin takes years to be expressed as a metabolic or clinical dysfunction. Acquired vitamin B_{12} deficiency can be related to prolonged insufficient intake, disturbed absorption, increased requirements or an accelerated loss of the vitamin.

Subtle Vitamin B_{12} Deficiency and Recommended Daily Amount

Subtle vitamin B_{12} deficiency is common in the general population and it is more widespread than has been assumed so far.[2–4] Chronic insufficient intake or disrupted absorption of vitamin B_{12} are the most common causes of cobalamin deficiency.

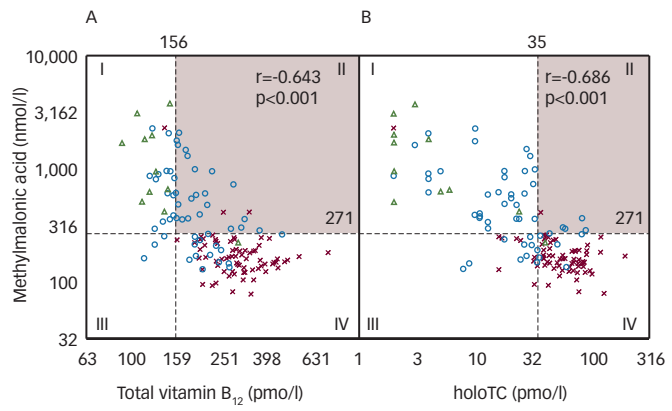
According to the recommended daily amount (RDA) guidelines from the National Research Council of the US National Academy of Sciences, adults should ingest 2.4µg daily (pregnant women up to 6µg), which can be met by a typical western diet.[5] The calculation of the required amount is based on the calculation of the amount of vitamin B_{12} necessary to sustain a normal haematological status (normal haemoglobin and mean corpuscular volume of erythrocytes [MCV]) and to maintain remission in pernicious anaemia. At the time when the RDA was set, functional metabolic markers of vitamin B_{12} had not been sufficiently investigated in clinical studies. Neurological manifestations are found at a relatively late stage of

Figure 1: Cumulative Distribution of Holotranscobalamin in Subjects with Different Diets



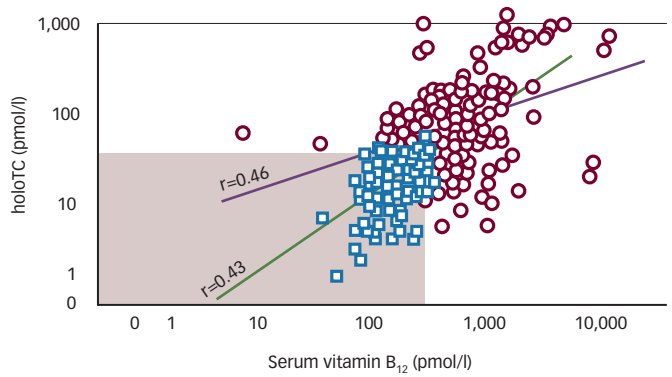
Omnivores n=109, lacto vegetarians (LV) + lacto-ovo vegetarians (LOV) n=114, vegans n=50. In addition to the distribution in elderly people (n=228, age 81 [range 69–90] years), the lower cut-off of holotranscobalamin (holoTC) of 35pmol/l is indicated.

Figure 2: Scatter Plots of Methylmalonic Acid and Total Vitamin B_{12} (A) and Methylmalonic Acid and Holotranscobalamin II (B) in the Subjects Not Taking Vitamins



x = omnivorous control subjects (n=79); lacto vegetarians and lacto-ovo vegetarians (n=53); and vegans (n=12). The dashed lines indicate the upper limit of the normal range for methylmalonic acid (271nmol/l) and the lower limit of the normal range for vitamin B12 (156pmol/l) and holotranscobalamin II (holoTC) (35pmol/l). The numbers on the axes are anti-log.

Figure 3: Correlation Between Vitamin B_{12} and Holotranscobalamin in Two Ranges of Vitamin B_{12}



vitamin B_{12} deficiency and are not unique. In addition, haematological signs are expressed by only a subpopulation. Therefore, the criteria used the define the RDA are too insensitive to detect cobalamin-deficient cases.

Recent investigations have suggested that this daily intake is too low to ensure normal blood cobalamin markers.[6] Recent data have shown that the plasma concentration of MMA and Hcy falls when vitamin B_{12} is ingested, whereas the concentration of holotranscobalamin (holoTC) as metabolically active B_{12} fraction rises.[6] At present, a daily intake between 4 and 6µg vitamin B_{12} is thought to be necessary to maintain the optimal plasma concentration of cobalamin biomarkers.[6] It is concluded that the recommended daily intake of B_{12} seems too low and should be newly determined, especially for older people.

Early Diagnosis of Vitamin B_{12} Deficiency

Since vitamin B_{12} deficiency can lead to irreversible neurological damage, early diagnosis is essential.[7–9] Early diagnosis of this disease is crucial for prevention of further complications. Moreover, vitamin B_{12} deficiency can cause hyperhomocysteinaemia, which has been related to the risk of vascular and cerebral diseases.[10] Studies have confirmed that serum concentrations of total vitamin B_{12} are insensitive in detecting early depletion of the vitamin.[11] Furthermore, people who express a benign low serum concentration of vitamin B_{12} related to low haptocorrin but who are not deficient are far from rare.[12] This implies that a low concentration of vitamin B_{12} in serum does not necessarily indicate a deficiency and indicates the need for a more specific and early laboratory marker. In addition, low serum concentrations of vitamin B_{12} are uncommon in patients with renal insufficiency.[13] However, this disagrees with the finding that renal patients show a marked MMA elevation that can be corrected by vitamin B_{12} supplementation,[13] indicating a pre-treatment deficiency.

In recent years, new and sensitive laboratory markers to determine vitamin B_{12} status have become available.[14] Cobalamin-saturated transcobalamin, also called holoTC, constitutes between 6 and 20% of total plasma vitamin B_{12}. This portion is the only one that can be delivered into all DNA-synthesising cells. Holohaptocorrin constitutes approximately 80% of total serum cobalamin and has no known function. Serum concentration of holoTC has been suggested as a sensitive marker for early changes (depletion or repletion) of vitamin B_{12}.

A study by our group[4] shows a clear difference in serum holoTC concentrations according to diet.[16] The holoTC values found in vegans were notably shifted towards the lower end of the distribution. On the other hand, the distribution of holoTC concentrations in lacto vegetarians/lacto-ovo vegetarians showed an intermediate pattern between that found in the vegans and the omnivores (see *Figure 1*). A low concentration of holoTC (<35pmol/l) was detected in 8% of the omnivores, 61% of the lacto/lacto-ovo vegetarians and 76% of the vegans. A combined two abnormal results (holoTC and MMA) were detected in 43 and 64% of the lacto/lacto-ovo vegetarians and vegan subjects, respectively. More importantly, about 45% of subjects with low holoTC and elevated MMA had normal serum vitamin B_{12}, again indicating the insensitivity of serum vitamin B_{12} (see *Figure 2*).[4]

Moreover, the distribution of holoTC in elderly subjects (see *Figure 1*) was only slightly shifted towards lower concentrations compared with healthy younger people. However, this did not explain higher serum concentrations of MMA compared with younger adults.[15] Approximately 20% of elderly subjects showed an elevated concentration of MMA but normal holoTC. This group had a significantly higher median serum concentration of creatinine compared with the group with normal MMA

and normal holoTC.[15] A combination of a low concentration of holoTC and an elevated MMA was found in approximately 16% of the elderly subjects; this represents subjects with a metabolic sign suggesting cobalamin deficiency.[15]

Taken together, available functional biomarkers such as MMA and holoTC facilitate the laboratory diagnosis of cobalamin deficiency. However, as before, there is no single 'gold standard' marker that can be applied for all clinical conditions.

Risk Groups

The prevalence of subclinical functional vitamin B_{12} deficiency is higher than expected when sensitive and relatively specific markers are used such as MMA, holoTC and Hcy.[15,16] Risk groups for vitamin B_{12} deficiency (see *Table 1*) include: patients with unexplained anaemia; patients with unexplained neuropsychiatric symptoms; patients with gastrointestinal manifestations, including stomatitis, anorexia and diarrhoea; elderly people;[15] vegetarians;[4] patients with gastrointestinal disorders, such as Crohn's disease or infection with *Helicobacter pylori*; and patients with stomach resection.[17] To date, the rate of people in the at-risk population who will develop clinical symptoms because of vitamin B_{12} deficiency has not been studied systematically.

In the general population, the prevalence of vitamin B_{12} deficiency in younger people is 5–7%.[18] Functional vitamin B_{12} deficiency – i.e. raised MMA and lowered holoTC – is common in old age and has been diagnosed in 10–30% of patients over 65 years of age.[16] A high prevalence of a slightly abnormal vitamin B_{12} status has been reported in elderly people despite intake of the recommended daily dose (>2.4µg/day). This deficiency is not presumed to be associated with dietary causes but rather with malabsorption.[19] Fifty-three per cent of elderly patients from Strasbourg who had vitamin B_{12} deficiency had malabsorption problems and 33% had pernicious anaemia; in only 2% was vitamin B_{12} deficiency related to insufficient dietary intake, and in 11% the aetiology of the vitamin B_{12} deficiency remained unexplained.[20] However, because the currently recommended dietary intake for vitamin B_{12} in elderly people is low, dietary deficiencies are underdiagnosed. Using synthetic vitamin B_{12} preparations can protect elderly persons from symptoms of deficiency.[21] However, dietary intake of vitamin B_{12} does not provide any information on vitamin B_{12} status because malabsorption is a common and important factor. Furthermore, elderly persons often have atrophic gastritis, pernicious anaemia or achlorhydria. Disorders that affect the gastrointestinal pH can also result in malabsorption and thus vitamin B_{12} deficiency. The incidence of *H. pylori* is high in elderly people and can lead to atrophic gastritis, and in turn to B_{12} malabsorption, owing to disrupted production of hydrochloric acid.[2] *H. pylori* was found in 56% of patients with vitamin B_{12} deficiency.[22] In 40% of patients, serum concentrations of B_{12} rose after treatment for *H. pylori* infection. According to recent reports, longer-term treatment of *H. pylori* (one year) resulted in a significant rise in mean vitamin B_{12} (from 146 to 271pmol/l) and a fall in mean Hcy concentrations (from 41 to 13µmol/l).[23] Vitamin B_{12} malabsorption owing to *H. pylori* infection can thus lead to vitamin B_{12} deficiency and hyperhomocysteinaemia.

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Table 1: Population Groups at Risk of Vitamin B_{12} Deficiency

Group	Causes/Comments
Vegetarians, vegans and macrobiotic diet	Low intake of vitamin B_{12}
Neonates from vegetarians or vitamin B_{12}-deficient mothers	Low vitamin B_{12} available during foetal life and later low intake via human milk from deficient mothers
Elderly	Pernicious anaemia, achlorhydia, gastrointestinal diseases or drugs that alter pH, ileum resection, *Helicobacter pylori*
Neurodegenerative and psychiatric diseases	Either causally related to the clinical symptoms or secondary to the disease because of low intake
Chronic atrophic gastritis	Malabsorption of vitamin B_{12}
Diseases of the terminal ileum	Crohn's disease, ileum resection, bacterial overgrowth
Macrocytic anaemia	Low B_{12} intake or pernicious anemia (antibodies against intrinsic factor)
Chronic alcoholism	Low intake, disturbed absorption of the vitamin
Medications	Proton pump inhibitors, H2-receptor antagonists, nitric oxide exposure
AIDS-associated myelopathy	Abnormal vitamin B_{12} utilsation

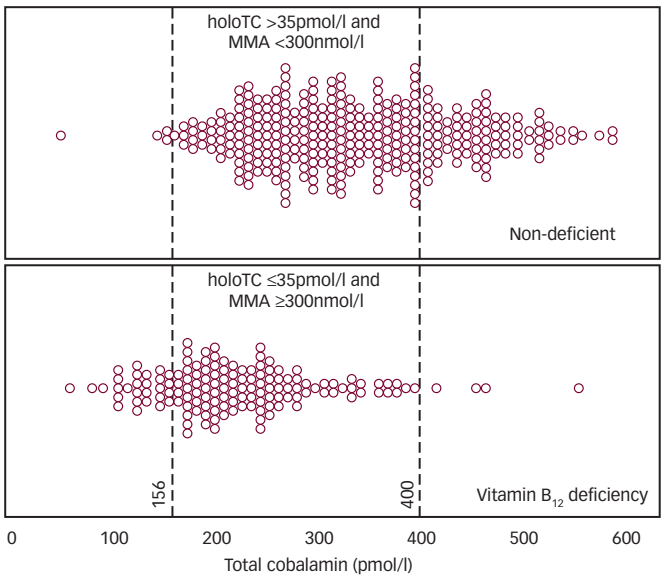
Vegetarians are at high risk of developing vitamin B_{12} deficiency because animal products are the main sources of vitamin B_{12}. A functional B_{12} deficiency (lowered holoTC, raised MMA and Hcy) is common in vegetarians and depends on the strictness of the diet and the amount of time for which the vegetarian diet has been followed.[4] Persons with an increased vitamin requirement – such as pregnant and breastfeeding women, patients with autoimmune disorders or persons with HIV infection – are a further risk group for vitamin B_{12} deficiency. Persons who regularly take proton pump inhibitors can also develop vitamin B_{12} deficiency.

Vitamin B_{12} deficiency is also widespread in patients with renal disorders.[13] In spite of normal plasma concentrations of vitamin B_{12} or holoTC, these patients often have raised serum concentrations of MMA and Hcy.[13] The likely cause is disrupted cellular absorption of holoTC, which results in intracellular vitamin B_{12} deficiency and raised metabolites. Studies have shown that patients with renal disorders may have higher concentrations of holoTC, which seems to contradict vitamin B_{12} deficiency.[13,24] This can be explained by the role of the kidney in transcobalamin filtration and resultant secondary accumulation of holoTC. The plasma concentration of holoTC in such patients therefore does not correctly reflect the functional vitamin B_{12} status.[13]

Utility of Holotranscobalamin as an Early Diagnostic Marker

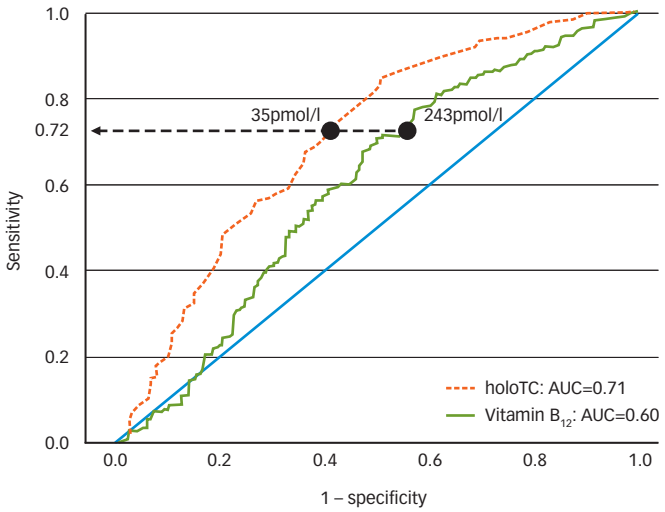
Recent studies have emphasised the need for testing the clinical utility of holoTC compared with vitamin B_{12} and MMA. The pros and cons of the different laboratory tests of cobalamin status have

Figure 4: Distribution of Concentrations of Total Cobalamin According to Combined Holotranscobalamin and Methylmalonic Acid in Patients with Normal Renal Function



Broken lines indicate the lowest cut-off of total cobalamin (156pmol/l) and a cut-off of 400pmol/l. holoTC = holotranscobalamin; MMA = methylmalonic acid.

Figure 5: Receiver Operator Characteristic Curves Testing the Performance of Holotranscobalamin and Vitamin B_{12} for Detecting Methylmalonic Above 300nmol/l (Individuals with Normal Renal Function)

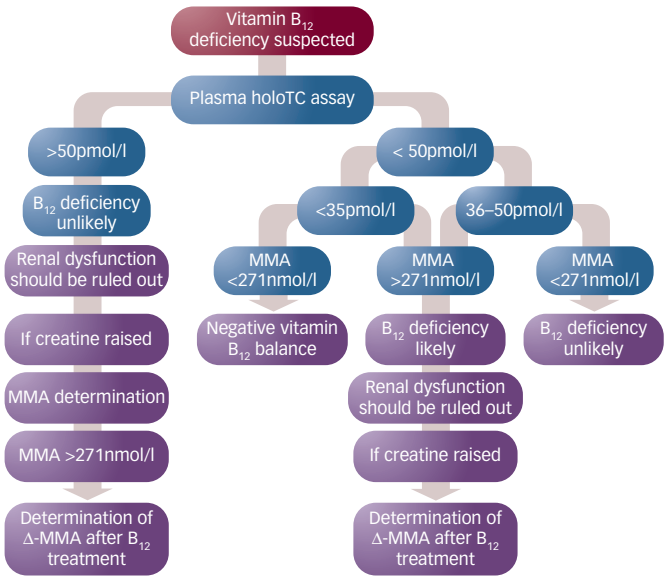


AUC = area under the curve; holoTC = holotranscobalamin.

recently been reviewed.[25–27] The development of automated methods for evaluating holoTC has made large-scale screening studies possible. With regard to the cost–benefit effect of early detection of vitamin B_{12} deficiency by using holoTC, it is anticipated that holoTC will soon replace vitamin B_{12} as a first-line laboratory parameter to screen for vitamin B_{12} deficiency.

Studies on vegetarians with different dietary habits have suggested that a lowered serum holoTC concentration is the earliest marker of vitamin B_{12} deficiency, and indicates that the body does not have sufficient available vitamin B_{12} and that vitamin B_{12} stores are emptying as a result of the negative balance of the vitamin.[4] At this stage, clinical or haematological symptoms may not yet be present.

Figure 6: Algorithm for Laboratory Diagnosis of Vitamin B_{12} Deficiency



The chart is our own suggestion for the early diagnosis of vitamin B_{12} deficiency. holoTC = holotranscobalamin; MMA = methylmalonic acid; Δ-MMA = reduction of MMA concentration by more than 250nmol subsequent to injection of vitamin B_{12}.

Lowered holoTC combined with raised MMA and Hcy levels is indicative of metabolically manifest vitamin B_{12} deficiency. Clinical signs may already be present, but can still be missing. At this stage, people may be clinically inconspicuous.[28]

The use of total vitamin B_{12} assay as a first-line parameter to screen for cobalamin deficiency has been disappointing, despite the relatively low costs of the assay. The limitations of the vitamin B_{12} assay are especially important in the lower range of serum vitamin B_{12}.[4,29] A significant positive correlation was found between the two parameters in the group with serum vitamin B_{12} concentrations >300pmol/l (r=0.46; p<0.001) (see *Figure 3*). However, the correlation line in the group with low serum vitamin B_{12} concentrations (<300pmol/l) was quite different from that seen in those with higher vitamin B_{12} concentrations; the slope of that curve was significantly steeper, indicating that the part of holoTC that contributes to total vitamin B_{12} is significantly decreased in the lower vitamin B_{12} concentration range to below 10%. Therefore, in the lower vitamin B_{12} concentration range (<300pmol/l), total vitamin B_{12} overestimates the cobalamin status.

In a recent study[27] on over 1,000 samples from patients referred for testing total serum vitamin B_{12}, we studied the distribution of serum concentrations of total vitamin B_{12} in subjects defined as cobalamin-deficient (holoTC ≤35pmol/l and MMA ≥300nmol/l) and those defined as non-deficient (holoTC >35pmol/l and MMA <300nmol/l) (see *Figure 4*). Only patients with normal renal function were included. The majority of patients with high MMA and low holoTC (cobalamin-deficient) had vitamin B_{12} concentrations between 156 and 400pmol/l and would be classified (utilising total vitamin B_{12} as a diagnostic tool) as false-normal. Few subjects had low concentrations of vitamin B_{12} and normal MMA and holoTC (false-positive).

The receiver operating characteristic (ROC) curves testing the performance of holoTC and vitamin B_{12} for detecting

concentrations of MMA above 300nmol/l in individuals with normal renal function showed a larger area under the curve (AUC) for holoTC compared with vitamin B_{12} (0.71 versus 0.60). This supports a better diagnostic sensitivity and specificity for holoTC compared with vitamin B_{12}. A 72% sensitivity could be expected by using a cut-off of 35pmol/l for holoTC and 243pmol/l for vitamin B_{12}.

Renal insufficiency constitutes a common and important exceptional condition for the interpretation of cobalamin markers. The artificial increase of serum concentrations of MMA and tHcy in some clinical settings is a major limitation of these parameters.[30] Both parameters correlate to serum concentration of creatinine and increase even in mild degrees of renal insufficiency.[31] In general, results of the metabolites should be interpreted with caution because it is difficult to determine the extent to which the impaired kidney function may participate in MMA and Hcy elevation.[31] Cobalamin deficiency is common in patients with renal dysfunction. Moreover, concentrations of holoTC in people with renal insufficiency are markedly elevated and they are not consistent with what we have learned about vitamin B_{12} deficiency. This one important exception is relatively common and may raise some uncertainty about using holoTC as a marker of cobalamin status in renal patients and in elderly people even with subclinical degrees of renal insufficiency. A laboratory diagnosis of cobalamin deficiency in renal patients is an important challenge that remains unresolved because of the simultaneous increase of both serum MMA and holoTC. Our observations of patients with renal dysfunction or elderly people with mild renal complications suggest that cobalamin deficiency in these patients can be ruled out only after cobalamin treatment. Whenever cobalamin deficiency is suspected in renal patients, cobalamin treatment should be started. A significant reduction of serum MMA (by approximately 250nmol/l) after cobalamin treatment indicates a pre-treatment deficiency. A residual increment of MMA is then related to renal dysfunction. We have suggested an algorithm for laboratory diagnosis of vitamin B_{12} deficiency (see *Figure 6*). This algorithm takes into account renal dysfunction as a common cause for elevated both holoTC and MMA.

Screening

At present there is no consensus regarding screening for vitamin B_{12} deficiency. Screening makes sense when the first signs of vitamin B_{12} deficiency can be detected before neurological or haematological anomalies develop. HoloTC and MMA are suitable screening tools; however, renal dysfunction should be kept in mind. ■

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