

# CLINICAL REVIEW

## Vitamin B<sub>12</sub> deficiency

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Vitamin B<sub>12</sub> is an essential cofactor that is integral to methylation processes important in reactions related to DNA and cell metabolism, thus a deficiency may lead to disruption of DNA and cell metabolism and thus have serious clinical consequences.<sup>1</sup> Intracellular conversion of vitamin B<sub>12</sub> to two active coenzymes, adenosylcobalamin in mitochondria and methylcobalamin in the cytoplasm, is necessary for the homeostasis of methylmalonic acid and homocysteine, respectively.<sup>2,3</sup> Methylmalonic acid is converted into succinyl-CoA, of which vitamin B<sub>12</sub> is a cofactor for the reaction. Homocysteine is biosynthesised from methionine then resynthesised into methionine or converted into amino acid cysteine.

Vitamin B<sub>12</sub> (also referred to as cobalamin) deficiency is relatively common, with important and variable clinical consequences. This review presents a concise summary of the most up to date evidence on how to diagnose and manage vitamin B<sub>12</sub> deficiency.

### What causes vitamin B<sub>12</sub> deficiency?

Foods containing vitamin B<sub>12</sub> are derived only from animals: meat, fish, and dairy. The daily Western diet contains around 5-30 µg of vitamin B<sub>12</sub> daily, of which 1-5 µg is absorbed. The UK government recommends a daily intake of 1.5 µg of vitamin B<sub>12</sub>, with the European Union recommending 1 µg and the United States recommending 2.4 µg. Body storage is relatively high, about 1-5 mg. Therefore deficiency from diminished intake or absorption may not manifest for several years after the depletion of stores.<sup>1,5,6</sup> Box 1 outlines the common causes of vitamin B<sub>12</sub> deficiency.

### Who gets vitamin B<sub>12</sub> deficiency and how common is it?

Deficiency can manifest in different groups as a result of periods when requirements are increased, such as during growth in children and adolescence or in pregnancy. Certain groups may have reduced intake, such as those with poor nutrition, older people, or people who adhere to a vegan or vegetarian diet.

In the United Kingdom and United States the prevalence of vitamin B<sub>12</sub> deficiency is around 6% in people aged less than 60 years, and closer to 20% in those aged more than 60 years.

Across Latin America approximately 40% of children and adults have clinical or subclinical deficiency. The prevalence of deficiency is much higher in African and Asian countries—for example, 70% in Kenyan schoolchildren, 80% in Indian preschool children, and 70% in Indian adults.<sup>10</sup> In vegan and vegetarian groups the rates vary—in the United Kingdom, 11% of vegans are deficient in vitamin B<sub>12</sub> and in Ethiopia 62% of vegetarian pregnant women are deficient.<sup>11</sup>

### What is the pathophysiology of vitamin B<sub>12</sub> deficiency?

In serum, vitamin B<sub>12</sub> is bound to haptocorrin as holohaptocorrin (formally transcobalamin III) and to transcobalamin as holotranscobalamin. Holohaptocorrin accounts for 80-94% of endogenous plasma vitamin B<sub>12</sub>. Holotranscobalamin on the other hand accounts for 6-20% of bound vitamin B<sub>12</sub>. It is synthesised in enterocytes and, through receptor mediated endocytosis, is responsible for uptake of vitamin B<sub>12</sub> from the ileum into the blood as well as into other cells. Only vitamin B<sub>12</sub> bound as holotranscobalamin is presented for cellular uptake. The malabsorption of this holotranscobalamin protein-bound vitamin B<sub>12</sub> results in vitamin B<sub>12</sub> deficiency in several cases.<sup>12</sup>

Intrinsic factor is a protein, produced by the parietal cells of the cardiac and fundic mucosa of the stomach. It binds vitamin B<sub>12</sub> to allow its absorption through the gastrointestinal tract, by way of a receptor on the intrinsic factor that is specific to cells at the terminal ileum. If there is resection or disease of the gastric mucosa or terminal ileum this leads to vitamin B<sub>12</sub> deficiency as a result of malabsorption.

Pernicious anaemia is an autoimmune disease with atrophy of the gastric mucosa of the body and fundus of the stomach. This reduces the number of parietal cells that produce the intrinsic factor necessary for absorption of vitamin B<sub>12</sub>. Secretion of intrinsic factor parallels gastric acid; thus there will be reduced secretion in an alkaline environment created by the long term use of high dose proton pump inhibitors and similar drugs. Figure 1⇓ illustrates the normal mechanism of vitamin B<sub>12</sub> absorption.

**Summary points**

- Vitamin B<sub>12</sub> deficiency is a common but serious condition
- Clinical presentation may not be obvious thus leading to complex issues around diagnosis and treatment
- There is no ideal test to define deficiency and therefore the clinical condition of patients is of the utmost importance
- There is evidence that new techniques such as the measurement of holotranscobalamin and methylmalonic acid levels seem useful in more accurately defining deficiency
- If the clinical features suggest deficiency then it is important to treat patients to avoid neurological impairment even if there may be discordance between the results and clinical features

**Sources and selection criteria**

We searched PubMed and Google Scholar using the terms "vitamin B<sub>12</sub> deficiency" and "cobalamin deficiency", and hand selected the most relevant and appropriate articles. We also used evidence based guidelines from the British Committee for Standards in Haematology; however, evidence, especially in the form of randomised controlled trials, is lacking.<sup>4</sup>

**Box 1 Common causes of vitamin B<sub>12</sub> deficiency<sup>5-9</sup>***Impaired gastric absorption*

- Pernicious anaemia
- Gastrectomy—partial or total
- Zollinger-Ellison syndrome

*Impaired intestinal absorption*

- Ileal resection or disease—for example, Crohn's inflammatory bowel disease and tuberculous ileitis
- Blind loop syndrome
- Luminal disturbances: chronic pancreatic disease and gastrinoma
- Parasites: giardiasis, bacterial overgrowth, and fish tapeworm

*Pancreatic insufficiency**Decreased intake*

- Malnutrition
- Reduced intake of animal products
- Strict vegan diet

*Congenital/inherited*

- Intrinsic factor receptor deficiency/defect—Imerslund-Gräsbeck syndrome
- Congenital deficiency of intrinsic factor—"juvenile" pernicious anaemia
- Cobalamin mutation (C-G-1 gene)
- Transcobalamin deficiency

*Increased requirements*

- Haemolysis
- HIV

*Drugs*

- Alcohol
- Nitrous oxide
- Proton pump inhibitors
- H<sub>2</sub> receptor antagonists
- Metformin
- Colchicine
- Slow K (potassium chloride) preparations
- Cholestyramine

**What are the clinical features of vitamin B<sub>12</sub> deficiency?**

The clinical manifestations of vitamin B<sub>12</sub> deficiency (fig 2),<sup>3 5-7 9 13</sup> represent the effects of depletion on multiple systems and vary greatly in severity. The clinical manifestations are heterogeneous but can also be different depending on the degree and duration of deficiency.

Mild deficiency manifests as fatigue and anaemia, with indices suggesting B<sub>12</sub> deficiency but an absence of neurological

features. Moderate deficiency may include an obvious macrocytic anaemia with, for example, glossitis and some mild or subtle neurological features, such as distal sensory impairment. Severe deficiency shows evidence of bone marrow suppression, clear evidence of neurological features, and risk of cardiomyopathy. However, it is important to recognise that clinical features of deficiency can manifest without anaemia and also without low serum vitamin B<sub>12</sub> levels. In these cases treatment should still be given without delay.<sup>4 13</sup>

## Bone marrow

The bone marrow is most commonly affected. Anaemia may range from mild to severe, with symptoms of fatigue on exertion, dyspnoea, palpitations, and pallor. All cell lines can be affected, with macrocytic anaemia, low white cell count or neutropenia, and thrombocytopenia.

## Tissues and organ dysfunction

Epithelial changes with vitamin B12 deficiency include skin hyperpigmentation and glossitis. Reproductive tissue can be affected, manifesting as infertility. Deficiency can also result in osteoporosis, with reduced bone derived alkaline phosphatase and plasma osteocalcin. Rarely, cardiomyopathy can occur.<sup>3</sup>

## Neurological features

Neurological impairment includes motor disturbances, sensory loss, abnormal balance and reflexes, cognitive impairment, and memory loss. Extreme cases may present with stupor or psychosis. An estimated 20% of patients with neurological signs do not manifest anaemia.<sup>13</sup> Clinical features of anaemia may be minimal and the blood indices may not reflect important anaemia. Neurological symptoms can occur in isolation so it is important to consider a diagnosis of vitamin B<sub>12</sub> deficiency in the presence of neurological symptoms of unknown cause, as neurological features may progress and become irreversible.

Subacute combined degeneration of the spinal cord involves demyelination of the posterior and lateral tracts. Initial bilateral peripheral neuropathy can progress to axonal degeneration and neuronal death if left untreated. This is followed by disturbances of proprioception, vibratory sense, and areflexia. Patients may mention clumsiness, poor coordination, and difficulty walking. Without treatment, weakness and stiffness may develop, manifesting as spastic ataxia. Damage to peripheral nerves results in sleepiness, altered taste and smell, and optic atrophy. In severe deficiency or advanced stages, a dementia-like illness may be seen, and frank psychosis with hallucinations, paranoia, and severe depression.<sup>9 13</sup>

## Which investigations should be carried out to determine vitamin B<sub>12</sub> deficiency?

Several investigations reflecting physiological, static, and functional B<sub>12</sub> status are available (table 1). Box 2 outlines when testing for vitamin B<sub>12</sub> deficiency should be considered.

There is still no ideal test for measuring vitamin B<sub>12</sub> deficiency. Measuring the serum cobalamin level remains the preferred choice. Second line tests include measuring plasma methylmalonic acid levels, which can help clarify uncertainties of underlying biochemical and functional deficiencies. Serum holotranscobalamin has an indeterminate “grey area” and therefore should be correlated with testing for methylmalonic acid. Testing for plasma homocysteine may be helpful but is less specific than for methylmalonic acid.<sup>4</sup> Furthermore, given the variety of laboratory techniques and assay, reference ranges are established locally, resulting in an inability of definitive definitions for clinical and subclinical deficiency states.

## Physiological investigations

Investigations that may allude to physiological changes reflecting vitamin B<sub>12</sub> deficiency include full blood count (mean cell volume and haemoglobin), reticulocyte count, blood film, and lactate dehydrogenase. Macrocytosis is the most common trigger for checking vitamin B<sub>12</sub> status. Bone marrow biopsy is

rarely required but may be indicated in selective cases where the diagnosis is unclear or blood indices show that the patient is not responding to adequate treatment (fig 3).

Patients with a concomitant iron deficiency may not develop the morphological features of vitamin B<sub>12</sub> deficiency until the iron deficiency has resolved. Macrocytosis may also be absent or masked by thalassaemia trait.<sup>5-7</sup> The diagnostic algorithm in figure 4 can be used to interpret the results of the full blood count and haematitic tests.

## Vitamin B<sub>12</sub> level

Vitamin B<sub>12</sub> level is actually a measurement of serum cobalamin. Measurement of vitamin B<sub>12</sub> in serum is the most common assay used to evaluate vitamin B<sub>12</sub> levels. The test, however, also measures both serum holohaptocorrin and serum holotranscobalamin, and as such may mask true deficiency or falsely imply a deficient state. The test is widely available at low cost and uses an automated method and competitive-binding immune chemiluminescence.<sup>2 4 14-17</sup>

The clinically normal level for serum cobalamin is not completely clear. It has been suggested that serum cobalamin <148 pmol/L (200 ng/L) would be sensitive enough to diagnose 97% of patients with vitamin B<sub>12</sub> deficiency. It is not clear what level of serum cobalamin may represent subclinical deficiency.<sup>4</sup>

## Holotranscobalamin

Holotranscobalamin, the metabolically active form of vitamin B<sub>12</sub>, can be measured by immunoassay. Reference ranges depend on the individual assay, according to the specific laboratory. The theoretical merits of measuring the levels of holotranscobalamin have been known for many years but it is only recently that an assay suited to routine use, known as active B<sub>12</sub>, has become available. Emerging evidence indicates that a low level of holotranscobalamin is a more reliable marker of impaired vitamin B<sub>12</sub> status than is a low level of serum vitamin B<sub>12</sub>.<sup>17</sup> Holotranscobalamin may be the earliest marker for vitamin B<sub>12</sub> depletion. This test is increasingly being adopted; however, discrepancies remain about mode of application and assignment of cut-off values. A second confirmatory test, such as for methylmalonic acid levels, is recommended if the result is in the intermediate range.<sup>18</sup>

## Methylmalonic acid

The conversion of methylmalonic acid to succinyl-CoA requires B<sub>12</sub> as a cofactor and hence accumulation occurs if B<sub>12</sub> is not available. An increase in methylmalonic acid level is an indicator of vitamin B<sub>12</sub> deficiency in tissue and this persists for several days even after replacement is started.<sup>2</sup> Measurement of methylmalonic acid may be the most representative marker of metabolic vitamin B<sub>12</sub> insufficiency. The interpretation in older patients (>65 years) and those with impaired renal function is, however, potentially challenging because levels can be falsely increased. High levels of plasma methylmalonic acid, however, usually indicate cobalamin deficiency. Methylmalonic acid is measured using gas chromatography mass spectrometry, a high cost test.

## Total homocysteine

Plasma total homocysteine levels are increased in B<sub>12</sub> deficiency. Plasma total homocysteine can increase early in the course of deficiency. It is a sensitive but non-specific marker and it is also high in folate deficiency, B<sub>6</sub> deficiency, renal failure, and hypothyroidism. Most laboratories regard levels >15 µmol/L as

**Box 2 When to consider testing for vitamin B<sub>12</sub> deficiency**

- Anaemia
- Macrocytosis mean cell volume >100 fl
- Clinical symptoms of vitamin B<sub>12</sub> deficiency
- Known gastrointestinal disorder associated with vitamin B<sub>12</sub> deficiency
- Vegan diet

high, although the reference range depends on the individual technique. The sample must be processed within two hours, which may inhibit the usefulness of the test.

**Identifying the cause of vitamin B<sub>12</sub> deficiency**

Once a diagnosis of vitamin B<sub>12</sub> deficiency is identified, history taking and examination are important (see fig 3). If there is no obvious dietary lack of vitamin B<sub>12</sub> or malabsorption, tests for intrinsic factor and antiparietal cell antibodies should be performed to exclude pernicious anaemia.<sup>19</sup>

**How is vitamin B<sub>12</sub> deficiency treated?****Timing of treatment**

It is usually acceptable to start treatment within a few days of a confirmed diagnosis (box 3 summarises treatment and management). If there are neurological disturbances then treatment should be expedited and started without delay. Specialist input should be sought in the event of neurological features, including impaired cognitive state (box 4). Neurological presentation may occur in the absence of haematological changes, with early treatment essential to avoid permanent neurological disability. Emergency treatment with packed red cell transfusion may be required for major anaemia in the presence of cardiovascular compromise.<sup>24</sup>

**Parenteral treatment**

Data from randomised controlled trials and observational studies for parenteral treatment are lacking; however, the expert consensus for standard treatment in the United Kingdom is to begin parenteral treatment with intramuscular hydroxocobalamin. This bypasses the possibility of the debate about whether the treatment will be adequately taken, absorbed, and metabolised. Standard initial treatment for patients without neurological involvement is 1000 µg intramuscularly three times a week for two weeks. If there are neurological symptoms then 1000 µg intramuscularly on alternate days should be continued for up to three weeks or until there is no further improvement.<sup>4 25</sup> In irreversible cases, for example, pernicious anaemia, the treatment should be continued for life. For temporary causes, such as pregnancy, the treatment can be reviewed when the patient is fully replete and the causative agent removed.

Hydroxocobalamin is generally well tolerated. Rarely, side effects include itching, exanthema, chills, fever, hot flushes, nausea, dizziness, and very rarely anaphylaxis. There can be a cross over reaction to cobalamin; if there is concern about this then the drug should be administered in a place where hypersensitivity can be managed, with hydrocortisone and chlorphenamine cover available.<sup>4 26 27</sup>

**Oral treatment**

Cyanocobalamin is an oral preparation that can be given at a dose of 50-150 µg daily, as licensed and outlined in the *British National Formulary*.<sup>25</sup> The duration is determined by the cause of the deficiency. If the cause is irreversible then parenteral

therapy should be continued for life. This is a drug preparation requiring conversion to metabolically active cobalamins. A Cochrane review of two randomised controlled trials in 108 people with vitamin B<sub>12</sub> deficiency found that high oral doses of B<sub>12</sub> (1000 µg and 2000 µg daily) were as effective as intramuscular treatment in achieving haematological and neurological responses.<sup>28</sup> However, UK national consensus is that there are arguments against the use of oral cobalamin in severely deficient patients and those with malabsorption. High dose oral cobalamin may be a suitable alternative in selective cases, where intramuscular injections are not tolerated and compliance is not a problem, as previously described.<sup>3</sup>

Oral treatment may be considered in certain situations—for example, in mild or subclinical deficiency with no clinical features and when absorption and compliance are definitely not a problem.<sup>4 28</sup>

Treatment with vitamin B<sub>12</sub> leads to the production of new erythrocytes, which results in an intracellular influx of potassium. This may produce severe hypokalemia, which requires monitoring and appropriate treatment.

**Treating concomitant deficiencies**

If there is concomitant vitamin B<sub>12</sub> and folic acid deficiency then vitamin B<sub>12</sub> must be started first to avoid precipitating subacute combined degeneration of the spinal cord. In patients with isolated vitamin B<sub>12</sub> deficiency and anaemia, additional folic acid supplementation is recommended until vitamin B<sub>12</sub> is replete, to prevent subsequent folate deficiency after replenishment of B<sub>12</sub> stores.<sup>7 12 23 24</sup> Iron deficiency can be treated with oral ferrous sulphate (or suitable alternative) 200 mg three times daily with vitamin C supplementation. If this is not tolerated or effective then referral to a specialist may be required.

**How is response to treatment assessed?**

Patients often have a sense of improvement within the first 24 hours of treatment; however, the haematological response can take several days before the effects are first noticed and up to two months to complete response.<sup>3</sup>

Initially, a full blood count and reticulocyte count after 7-10 days of treatment is useful to document the response, and a further check should be done after eight weeks to confirm a normal blood count. When there is inadequate reticulocytosis, an incorrect diagnosis may be responsible. Within eight weeks of treatment the mean cell volume should have normalised (77-95 fL). Iron and folate status should be checked because coexisting deficiency is often obscured in vitamin B<sub>12</sub> deficiency.<sup>22 29</sup>

Homocysteine or methylmalonic acid should normalise during the first week of treatment. Failure to do so suggests an incorrect diagnosis, unless renal failure or other causes of increases in the metabolites coexist. Cobalamin and holotranscobalamin levels are not helpful because they increase with vitamin B<sub>12</sub> influx regardless of the effectiveness of treatment,<sup>24</sup> and retesting

**Box 3 Summary of treatment and management of vitamin B<sub>12</sub> deficiency**

- Identify and treat the cause
- Administer hydroxycobalamin (parenteral vitamin B<sub>12</sub>) 1 mg intramuscularly on alternate days for two weeks if there is no neurological involvement
- Start folic acid 5 mg daily after B<sub>12</sub> supplementation
- Provide supportive care; including, if required, rest, oxygen, red cell transfusion
- Monitor and treat hypokalaemia, which can occur with B<sub>12</sub> treatment
- Prescribe lifelong maintenance parenteral vitamin B<sub>12</sub> treatment with hydroxycobalamin 1 mg intramuscularly every three months if no neurological deficit, or every two months if there is neurological deficit to all those with irreversible malabsorption or after gastric surgery, pernicious anaemia, and any other irreversible cause
- Follow up long term, especially for treatment of the cause
- Educate patients and families, especially when parenteral lifelong treatment is needed in pernicious anaemia
- Consider concomitant deficiencies if diet is poor, and treat as required
- Refer to specialist if required (box 4)

**Box 4 Who to refer for specialist care<sup>23</sup>***Referrals to haematologist*

- If diagnosis is uncertain or unclear
- If the cause of B<sub>12</sub> deficiency is unclear
- If response to treatment is inadequate
- If the patient is pregnant or neurological symptoms are present, including cognitive impairment
- If the suspected cause is haematological malignancy or another blood disorder
- If the mean cell volume is persistently >105 fL

*Referrals to gastroenterologist*

- For malabsorption or inflammatory bowel disorder as required
- Suspected gastric malignancy with anaemia secondary to gastrointestinal blood loss should be referred appropriately through the "urgent two week referral" and not a routine haematology referral

is not usually required. They can be tested 1-2 months after starting treatment or if there is no response to treatment.

Neurological recovery may take some time; improvement begins within one week and complete resolution usually occurs between six weeks and three months. Progression should prompt reassessment of the diagnosis. Patients with delayed improvement should be referred for rehabilitation, including physiotherapy. Residual disability is seen in up to 6% of patients. Damage is likely to be irreversible if diagnosis and treatment are delayed by six months.<sup>3 24</sup>

**Recent advances and future developments**

One study successfully identified the plasma protein that binds transcobalamin saturated with vitamin B<sub>12</sub>. Further investigation into the genetic variability of this transcobalamin receptor and its interaction with transcobalamin polymorphisms may yield useful insights into variable responses to treatment.<sup>30</sup>

**Can vitamin B<sub>12</sub> deficiency be prevented?**

It is not currently possible to prevent vitamin B<sub>12</sub> deficiency from pernicious anaemia. Deficiency due to gastric and terminal ileum disease should be anticipated and supplemented before clinical presentation of deficiency. Breakfast cereals are fortified with vitamin B<sub>12</sub>, as a non-animal based dietary source. This may be useful for older people (>65 years) and those with a restricted diet. Each portion contains approximately 25% of the recommended daily intake of vitamin B<sub>12</sub>.<sup>31</sup> For patients taking long term metformin and proton pump inhibitors the use of oral cyanocobalamin could be considered, or increased screening and vigilance of vitamin B<sub>12</sub> deficiency.<sup>9</sup>

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**Additional educational resources***Information for healthcare professionals*

National Institute for Health and Care Excellence. Clinical knowledge summaries. Anaemia—B<sub>12</sub> and folate (<http://cks.nice.org.uk/anaemia-b12-and-folate-deficiency>)—provides a clear overview of the topic, which is easily navigated

Patient.co.uk. Pernicious anaemia and B12 deficiency ([www.patient.co.uk/doctor/pernicious-anaemia-and-b12-deficiency](http://www.patient.co.uk/doctor/pernicious-anaemia-and-b12-deficiency))—an easy to understand overview with ability to print the literature in PDF format and with links to related issues

British Committee for Standards in Haematology. Haematology guidelines ([www.bcshguidelines.com/4\\_haematology\\_guidelines.html](http://www.bcshguidelines.com/4_haematology_guidelines.html))—guidelines for the diagnosis and treatment of cobalamin and folate disorders

*Information for patients*

NHS Direct Wales ([www.nhsdirect.wales.nhs.uk/encyclopaedia/ch/article/anaemia,vitaminb12andfolatedeficiency/](http://www.nhsdirect.wales.nhs.uk/encyclopaedia/ch/article/anaemia,vitaminb12andfolatedeficiency/))—a clear concise overview of vitamin B<sub>12</sub> deficiency with some related links

NHS Choices. Vitamin B12 or folate deficiency anaemia. ([www.nhs.uk/conditions/anaemia-vitamin-B12-and-folate-deficiency/Pages/introduction.aspx](http://www.nhs.uk/conditions/anaemia-vitamin-B12-and-folate-deficiency/Pages/introduction.aspx))—a clear overview, with access to patient forums

Patient.co.uk. Vitamin B<sub>12</sub> deficiency anaemia ([www.patient.co.uk/health/vitamin-b12-deficiency-and-pernicious-anaemia](http://www.patient.co.uk/health/vitamin-b12-deficiency-and-pernicious-anaemia))—contains information similar to that provided by doctors during consultations

Pernicious Anaemia Society ([www.pernicious-anaemia-society.org](http://www.pernicious-anaemia-society.org))—a charitable organisation based in the United Kingdom and providing a support forum for people with pernicious anaemia worldwide

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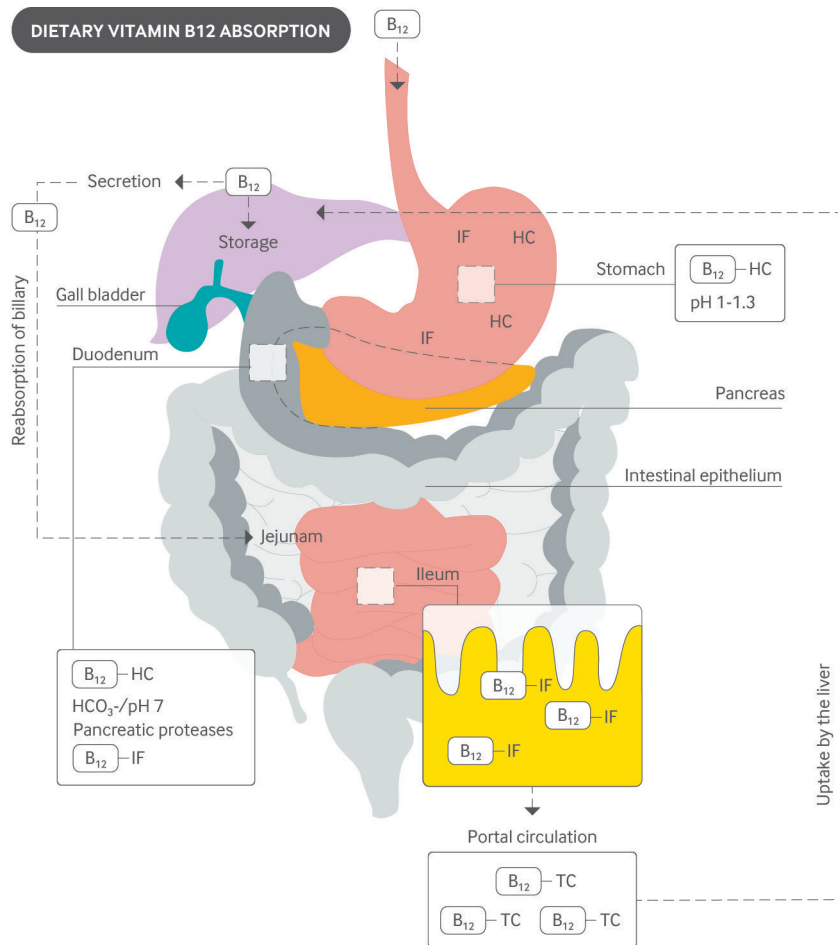
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## Table

Table 1 | Investigations to assess vitamin B12 deficiency<sup>5-7, 12 20 21</sup>

Assessment and investigation	Finding	Major limitations/comments
<b>Physiological:</b>		
Mean cell volume	Normal or increased (>100 fL)	Usually reflects megaloblastic anaemia due to ineffective erythropoiesis. Mean cell volume alone has poor specificity and sensitivity. Mean cell volume may be normal in coexisting iron deficiency anaemia or thalassaemia
Haemoglobin	Normal or low (<130 g/L in males and <115 g/L in females)	Poor specificity and sensitivity
Reticulocyte count	Low (<50×10 <sup>9</sup> /L)	Poor specificity
Lactate dehydrogenase	Often noticeably increased (<450 IU/L although local reference ranges may apply)	Due to intramedullary haemolysis, which results in increased serum bilirubin and serum iron levels
Blood film	Anaemia, macrocytosis with or without ovalocytes, hypersegmented neutrophils (>5% of neutrophils with ≥5 lobes). Neutropenia and thrombocytopenia	Can sometimes be difficult to differentiate from dysplasia if deficiency is severe and prolonged
Bone marrow aspirate and trephine	Hypercellular marrow, decreased myeloid: erythroid ratio. Nuclear chromatin is diffuse and immature. White cells show megaloblastic features with large band forms and giant metamyelocytes. Nuclei may have abnormal stain appearance. Megakaryocytes may have increased nuclear lobulation or hypogranulation	Should only be performed if there is ambiguity about diagnosis or inappropriate response to adequate treatment, as interpretation is difficult in the context of deficiency
<b>Static:</b>		
Serum/plasma cobalamin concentration	Low (<150 ng/L)	Not a highly specific indicator of vitamin B <sub>12</sub> deficiency. Normal levels in some deficient patients. Insensitive to deficiency as a result of inborn errors of metabolism or in concomitant myeloproliferative disorders. Slight to moderately low levels may not reflect deficiency
Serum/plasma holotranscobalamin	Low (<5 pmol/L), replete (>50 pmol/L), intermediate (25-50 pmol/L)	Measures transcobalamin II bound vitamin B <sub>12</sub> . Test not yet widely available
<b>Functional:</b>		
Serum/plasma or urine methylmalonic acid	Increased (>350 nmol/L)	Levels increased in renal insufficiency and older people (>65 years) so could be misinterpreted in extreme cases
Plasma homocysteine	Raised (>15 μmol <sup>-1</sup> )	Levels increased in folate and vitamin B <sub>6</sub> deficiencies, renal insufficiency, hypothyroidism

## Figures

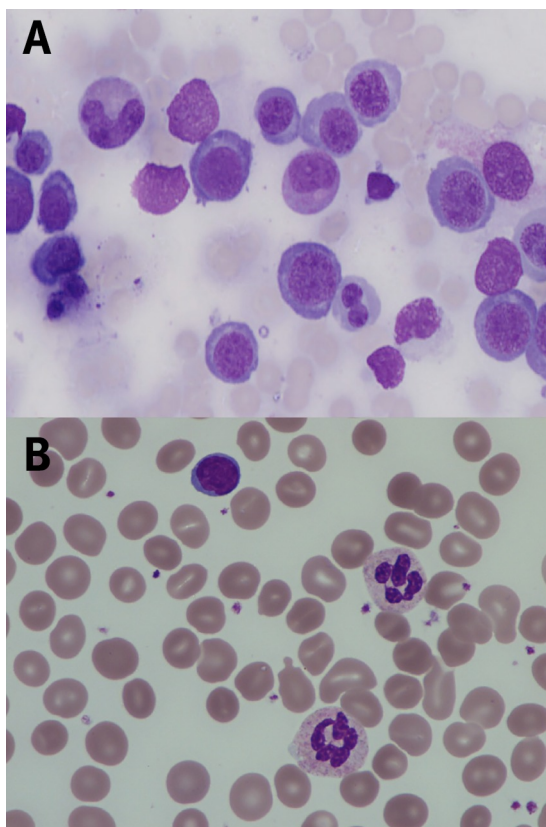


**Fig 1** Mechanism of dietary vitamin B<sub>12</sub> absorption. IF=intrinsic factor; TC=holotranscobalamin; HC=haptocorrin

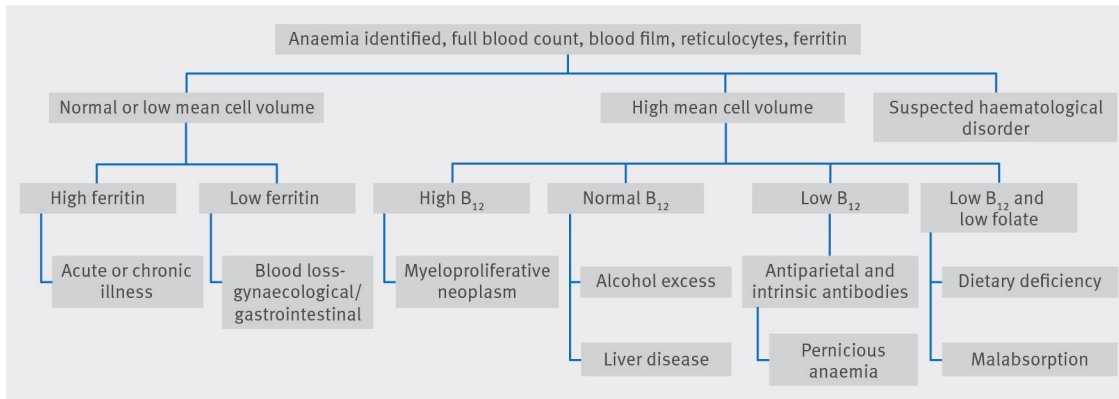




**Fig 2** Clinical features of vitamin B<sub>12</sub> deficiency



**Fig 3** Blood film and bone marrow aspirate indicating megaloblastic features



**Fig 4** Diagnostic algorithm for vitamin B<sub>12</sub> deficiency