Low vitamin B_{12}
What does it mean and what should I do?

A low vitamin B_{12} level is a common finding, and interpreting its significance can be difficult. A low level should never be ignored because deficiency may be responsible for occult haematological or neuropsychiatric disease.

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Serum levels of vitamin B_{12} (cobalamin) are commonly measured in clinical practice, usually in the assessment of a blood count abnormality (anaemia or macrocytosis) or in the context of neuropsychiatric symptoms or signs. There is significant overlap in the lower range of normal levels for vitamin B_{12}, and therefore a low level is not necessarily indicative of deficiency. A low vitamin B_{12} should be confirmed and deficiency excluded as far as possible, but this is not always easily achievable because definitive proof requires costly testing that may not be readily available. If a patient's vitamin B_{12} level is low and the clinical context is consistent with the possibility of vitamin B_{12} deficiency, further investigation for an underlying cause and replacement therapy are warranted.

Occult vitamin B_{12} deficiency may be more common than suspected, especially in the elderly and in patients taking gastric acid blocking medications.[1-3] Folate status may be a confounding factor. Primary folate deficiency may give rise to a low measured vitamin B_{12} level in the absence of true vitamin B_{12} deficiency – in such circumstances, the vitamin B_{12} level should be reassessed after folate replacement therapy. However, it should be borne in mind that folate supplementation may precipitate clinical disease if true vitamin B_{12} deficiency is present – this is important because folic acid supplementation is widely advocated. Vitamin B_{12} and folate should be assessed concurrently because they are metabolically inexorably bound – this is described in the box on page 20.

This article aims to assist the clinician faced with a patient who has a low vitamin B_{12} level. It presumes normal folate status and does not address the investigation of folate deficiency.

How common is vitamin B_{12} deficiency?

Accurate data on the prevalence of vitamin B_{12} deficiency in the community are not available. However, the frequency of clinical presentations (i.e. incidence) is known to increase with age. Despite the possible high prevalence of vitamin B_{12} deficiency, there is a need for more definitive

IN SUMMARY

- **Low vitamin B_{12} levels do not always indicate deficiency, but they should be investigated.**
- **Low vitamin B_{12} levels are common in elderly patients, but the clinical significance may be difficult to determine.**
- **It is possible that vitamin B_{12} deficiency may be responsible for cases of minor cognitive impairment, as well as the more classic neurological manifestations.**
- **There is concern that the widespread use of folic acid therapy and dietary supplementation may precipitate clinical disease in individuals with occult vitamin B_{12} deficiency**
- **Vitamin B_{12} assay results should always be interpreted in relation to the clinical context.**

continued

An overview of vitamin B_{12} metabolism

What is vitamin B_{12}?

The term 'vitamin B_{12}' is used to describe a group of compounds called cobalamins that contain cobalt in the centre of a corrin ring. They are some of the most complex molecules synthesised *de novo* in nature.

What is the role of vitamin B_{12}?

Vitamin B_{12} is an enzyme cofactor that, along with folate, is required for DNA synthesis and, to a lesser extent, for RNA and protein metabolism. A deficiency or disorder of metabolism in one of these substances will result in unbalanced cell development, with defective nuclear maturation and impaired cell division.

Two enzymatic reactions in humans are dependent on vitamin B_{12}:

- conversion of methylmalonyl–coenzyme A (CoA) to succinyl–CoA (vitamin B_{12} deficiency results in an increase in the serum methylmalonyl–CoA and its metabolic product, methylmalonic acid).
- synthesis of methionine from homocysteine (vitamin B_{12} deficiency results in accumulation of homocysteine).

An elevated methylmalonic acid level is a more specific marker for vitamin B_{12} deficiency than homocysteine (except in chronic renal failure and a number of other conditions). Homocysteine levels are increased in folic acid deficiency as well as in vitamin B_{12} deficiency.

How is vitamin B_{12} obtained?

Vitamin B_{12} is synthesised by micro-organisms, but not by higher plants or animals. Humans obtain it by ingesting the flesh or milk products of herbivores. These animals obtain vitamin B_{12} from bacteria that contaminate plants; further enrichment occurs in ruminants. Vitamin B_{12} is not present in vegetables or fruits.

How is vitamin B_{12} absorbed?

The absorption and transport of vitamin B_{12} is complex. The low pH in the stomach is required to release it effectively from food – this fact may be important in the elderly, in whom achlorhydria is common. After release from food, all vitamin B_{12} is initially bound to R-proteins, and the vitamin–protein complexes pass into the second part of the duodenum, where pancreatic proteases degrade the protein. Vitamin B_{12} is then rapidly bound to intrinsic factor (IF), an alkaline stable and thermolabile glycoprotein produced by the parietal cells of the gastric fundus and body, which is secreted in response to the same stimuli that lead to acid secretion. On reaching the ileum, the IF–vitamin B_{12} complex attaches to ileal receptors on the mucosal brush border, where conditions (e.g. pH >5.7) permit absorption to occur.

After absorption, metabolically active vitamin B_{12} is transported by transcobalamin and delivered to all cells that synthesise DNA, including those in the bone marrow and nervous system. It is rapidly cleared from the circulation, leaving transcobalamin 98% unsaturated.

Haptocorrin (formally transcobalamin I, an R-protein) binds most of the measurable serum vitamin B_{12}. However, it is not an active transport protein, and the bound vitamin B_{12} probably represents inert circulating storage vitamin. Vitamin B_{12} is mostly stored in the liver, but significant amounts are also bound by haptocorrin in the blood and in granulocytes.

What is the daily requirement of vitamin B_{12}?

The minimal daily dietary requirement of vitamin B_{12} is 0.6 to 1.2 µg. The NHMRC recommends a daily intake of 2 µg in healthy men and women.[4] In a patient commencing a deficient diet who does not have vitamin B_{12} malabsorption, normal body stores will last up to two years.

data before a case can be made for universal screening in the older population.

What are the consequences of vitamin B_{12} deficiency?

Vitamin B_{12} deficiency may be associated with haematological, neurological and psychiatric manifestations. Classically, it is a cause of macrocytic (megaloblastic) anaemia (Figure 1). Neurological sequelae include paresthaesias, peripheral neuropathy and subacute combined degeneration of the spinal cord. Although deficiency may rarely result in psychosis (megaloblastic madness), there is increasing recognition that it may be linked to lesser psychiatric disorders, including impaired memory, irritability, depression and dementia.

It is possible that vitamin B_{12} deficiency may also exert indirect cardiovascular effects. Serum homocysteine is elevated in patients with folic acid and/or vitamin B_{12} deficiency and it is an independent risk factor for atherosclerotic vascular disease. This is an area of active research – although folic acid has received more attention than vitamin B_{12}, it is yet to be established whether there is a role for folic acid supplementation as a preventive strategy for coronary artery disease and stroke by reducing homocysteine levels. This is important because folic acid supplementation may mask occult vitamin B_{12} deficiency and exacerbate or initiate neuropsychiatric manifestations. Furthermore, this raises the question of whether clinicians should consider excluding vitamin B_{12} deficiency before implementing folic acid therapy.

Investigating vitamin B_{12} deficiency

Testing for vitamin B_{12} deficiency is warranted when a patient has unexplained

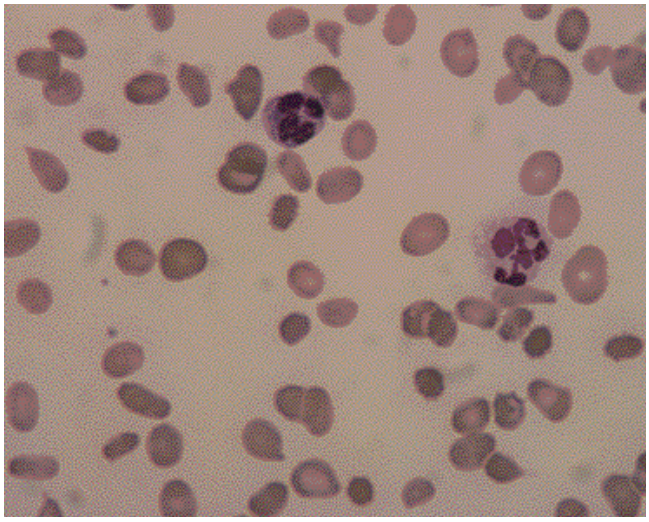

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Figure 1. Light micrograph of a blood smear in a patient suffering from pernicious anaemia.

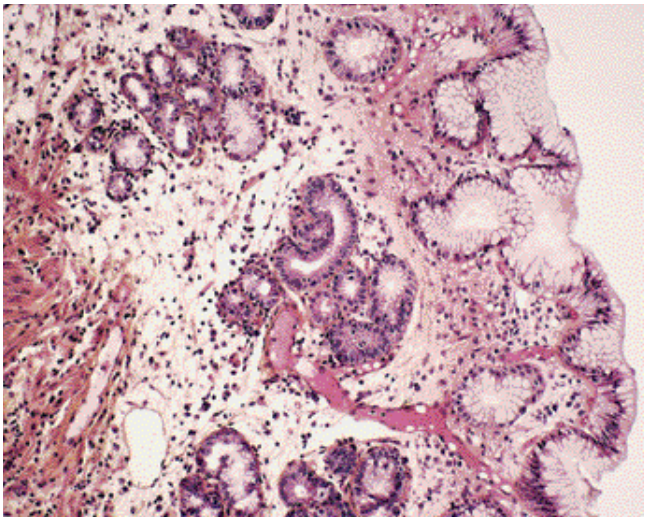

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Figure 2. Atrophic gastritis associated with pernicious anaemia.

anaemia, macrocytosis, peripheral neuropathy, subacute combined degeneration of the spinal cord, deterioration in cognitive ability, or some unexplained neurological or neuropsychiatric abnormalities. Clinical presentations in the elderly may be subtle but potentially serious, particularly the neurological and psychiatric manifestations.

The investigation of vitamin B_{12} deficiency may be divided into two phases:

- confirming the deficiency
- determining its cause (see Table).

Confirming vitamin B_{12} deficiency

A low vitamin B_{12} level should never be ignored, and an approach to investigation is shown in the flowchart on page 22. Until recently, diagnosing vitamin B_{12} and/or folate deficiency was considered relatively uncomplicated, but recent research has highlighted limitations in assays for these vitamins. With improvements in testing procedures, atypical and subclinical deficiency states have become recognised. Although there are pitfalls in assays for serum vitamin B_{12}, they remain the starting point for diagnosis. Reference ranges are generally set at 150 to 600 pmol/L (the actual range relates to the methodology used by individual laboratories).

Vitamin B_{12} assays have been problematic, with variable sensitivity and specificity (depending on the method used), but they are now generally reliable and reproducible. Normal or elevated levels of vitamin B_{12} in the presence of functional vitamin B_{12} deficiency (false negative results) may occur in the presence of elevated haptocorrin (e.g. active liver disease, lymphoma, autoimmune disease and myeloproliferative disorders). Low levels in the absence of deficiency (false positive results) can occur in the presence of folate deficiency, pregnancy, multiple myeloma, metformin therapy, excessive vitamin C intake and reduced haptocorrin. A result that does not appear compatible with the clinical findings should be repeated.

Table. Causes of vitamin B_{12} deficiency

Dietary factors
Poor nutrition, especially in the elderly
Veganism
Food fads
Breastfeeding (breastfed babies of mothers with vitamin B_{12} deficiency)

Intrinsic factor deficiencies
Addisonian pernicious anaemia (Figure 2)
Congenital intrinsic factor deficiency
Gastrectomy

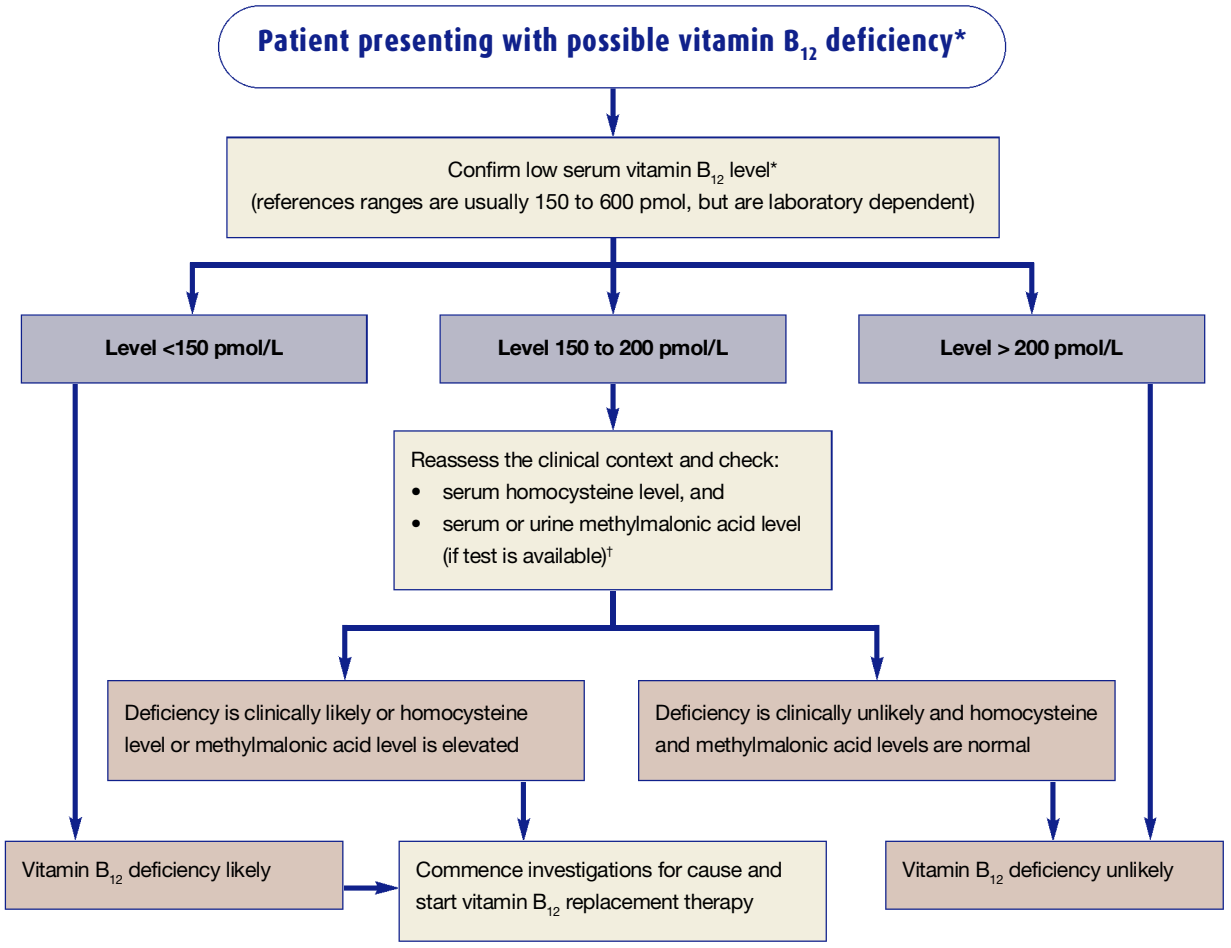
Gut infestations
Bacterial overgrowth
Fish tapeworm
Giardiasis

Small bowel diseases
Crohn's disease
Coeliac disease
Tropical sprue
Distal ileal resection (>50 cm)
Specific selective ileal malabsorption of vitamin B_{12} (Immerslund–Gräsbeck syndrome)

Rare and other possible causes
Hypothyroidism
Drugs (prolonged nitrous oxide exposure, ?gastric acid blocking medications)
Enzyme deficiencies in vitamin B_{12} metabolism
Inherited transcobalamin deficiency

continued

An approach to the investigation of vitamin B_{12} deficiency when folate status is normal



* Folate status is assumed to be normal.
† The methylmalonic acid test is rarely available and expensive.

Assay results of less than 150 pmol/L are generally indicative of low serum vitamin B_{12} levels. Such levels may, however, be seen in people (usually the elderly or patients receiving metformin therapy) without evidence of neurological, haematological, dietary or absorption abnormalities. The cause for the low levels in such patients is unclear, and follow up rarely reveals development of disorders associated with vitamin B_{12} deficiency.

Discrimination is poor, however, for vitamin B_{12} assay results between 150 and 200 pmol/L. For patients with values falling in this range, measurement of serum or urine methylmalonic acid and serum homocysteine levels is useful. A normal homocysteine level excludes significant folate or vitamin B_{12} deficiency. Methylmalonic acid levels are of value in excluding vitamin B_{12} deficiency and are generally more specific for vitamin B_{12} deficiency, but the tests are expensive and not readily available.

When serum vitamin B_{12} is measured using the assays that are currently available, the result reflects mainly metabolically inactive vitamin, and not holo-transcobalamin, the critical fraction that is available for developing cells. An assay for holo-transcobalamin is now available in kit form, and early results suggest that it may be a more sensitive and specific index of B_{12} deficiency.

Determining the cause

When establishing the cause of a low vitamin B_{12} level, malabsorption is the prime consideration. However, initial exclusion

of a dietary cause (e.g. veganism) is also important.

Traditionally, a Schilling's test was used to identify malabsorption of vitamin B_{12} and differentiate pernicious anaemia from small bowel causes, but because of concerns about the origin of the bovine intrinsic factor used in the test it is no longer available. Supportive evidence for pernicious anaemia can be obtained from blood tests for intrinsic factor antibodies, parietal cell antibodies and serum gastrin. The presence of intrinsic factor antibodies is generally diagnostic of pernicious anaemia, but these are detected in only 50% of cases. It is unlikely that the patient has pernicious anaemia if intrinsic factor and parietal cell antibodies are not detected and the serum gastrin level is not elevated.

If pernicious anaemia does not appear to be the cause and the history does not suggest dietary deficiency, further investigation for intestinal malabsorption is indicated (e.g. coeliac disease or bacterial overgrowth related to blind loops or small bowel diverticulum). Referral to a gastroenterologist may be necessary.

Conclusions

The assessment of vitamin B_{12} status has become increasingly difficult due to growing recognition of problems with the sensitivity and specificity of the commonly available assays. Folate has received close attention in recent years, with the problems of clinical and subclinical deficiency being effectively addressed. Vitamin B_{12} is increasingly the challenge, and it is difficult to make firm recommendations regarding assessment and management of patients with potential deficiency. The current dilemmas are well summarised in a recent editorial:[5]

'At this stage, it would be prudent to conclude that the currently available assays for identifying or excluding cobalamin deficiency, though potentially useful, should be used with full awareness of their possible limitations, at least until unresolved issues have been settled'. MT

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