Pernicious Anaemia and B12 Deficiency

Vitamin B12 (cobalamin) is present in meat and animal protein foods. Absorption occurs in the terminal ileum and requires intrinsic factor (IF), a secretion of gastric mucosal (parietal) cells, for transport across the intestinal mucosa. Body stores of vitamin B12 are in the region of 2-3 mg, which is sufficient to last for 2-4 years.

Pernicious anaemia is an autoimmune process that involves gastritis, atrophy of all layers of both the body and fundus of the stomach and loss of normal gastric glands, mucosal architecture, and parietal and chief cells. These cause achlorhydria (absence of gastric hydrochloric acid) and lack of IF.[1]

*Helicobacter pylori* infection has been mooted to be an initiating factor, with subsequent autoimmune changes affecting the gastric mucosa. Genetic susceptibility to this process has been suspected.[2]

Aetiology[1]

Pernicious anaemia accounts for 80% of cases of megaloblastic anaemia due to impaired absorption of vitamin B12. Other causes of vitamin B12 deficiency include:

- Gastric causes: gastrectomy, gastric resection, atrophic gastritis, *H. pylori* infection or congenital IF deficiency or abnormality.
- Inadequate dietary intake of vitamin B12 - eg, a vegan diet.
- Intestinal causes - eg, malabsorption, ileal resection, Crohn’s disease affecting the ileum, chronic tropical sprue, HIV and any radiotherapy causing irradiation of the ileum.
- Drugs - eg, colchicine, neomycin, metformin, anticonvulsants.
- Long-term use of drugs that affect gastric acid production (eg, H₂-receptor antagonists, proton pump inhibitors) can worsen deficiency because gastric acid is needed to release vitamin B12 bound to proteins in food.

Epidemiology[1]

- The incidence of pernicious anaemia in the UK population is estimated to lie between 1-5/100,000 per annum.[3]
- The disease occurs in all races. The peak age is 60, although it is starting to be recognised in younger age groups.[4]
- A study of 3,511 people, 65 years of age or older, found a prevalence of vitamin B12 deficiency of around 5% in people 65-74 years of age, and more than 10% in people 75 years of age or older. Dietary vitamin B12 deficiency is unusual in younger people, except those eating strict long-term vegan diets.

Presentation[1]

Symptoms of anaemia may include fatigue and lethargy, dyspnoea, faintness, palpitations and headache. Vitamin B12 deficiency may present with unexplained neurological symptoms - eg, paraesthesia, numbness, cognitive changes or visual disturbance.

Findings on examination may include pallor, heart failure (if anaemia is severe), lemon tinge to the skin, glossitis and oral ulceration. Neuropsychiatric features may include irritability, depression, psychosis and dementia. Neurological features may include subacute combined degeneration of the spinal cord and peripheral neuropathy.

- Peripheral loss of vibratory sense and position are early indications of central nervous system (CNS) involvement, accompanied by reflex loss and mild-to-moderate weakness. Later stages may be characterised by spasticity, Babinski’s responses and ataxia.
- Other uncommon neurological symptoms include impairment of pain, temperature and touch sensations. The legs and feet are involved earlier and more consistently than the hands.
- Yellow-blue blindness may occur.
- Psychiatric symptoms (usually more prominent in advanced cases) may include depression, paranoia (megaloblastic madness), delirium, confusion and dementia.
- Severely anaemic patients may present with heart failure, often triggered by an infection. Hepatomegaly and splenomegaly may be present.

Differential diagnosis[1]

Causes of megaloblastic anaemia

- Folate deficiency - poor diet, goat’s milk, gluten-induced enteropathy, tropical sprue, pregnancy, prematurity, chronic haemolytic anaemias (eg, sickle cell anaemia), malignant disease, increased renal loss (congestive cardiac failure, dialysis), drugs (anticonvulsants, sulphasalazine).
- Functional vitamin B12 deficiency: neurological complications such as subacute combined degeneration of the spinal cord may occur despite normal serum B12 levels. Failure of intracellular transport of B12 by transcobalamin-2 can lead to functional B12 deficiency but with apparently normal serum levels.\textsuperscript{[5]}

Causes of macrocytosis\textsuperscript{[1]}
- Pregnancy and the neonatal period.
- Alcohol excess.
- Liver disease.
- Severe hypothyroidism.
- Reticulocytosis (eg, post-acute blood loss or haemolytic anaemia).
- Other blood disorders - red-cell aplasia, aplastic anaemia, myeloid leukaemia, myelodysplastic disorders.
- Changes in plasma proteins (eg, increased paraprotein secondary to multiple myeloma) may cause a spurious rise in mean cell volume (MCV) without the presence of macrocytes.
- Drugs that affect DNA synthesis - eg, azathioprine, hydroxyurea.

Investigations\textsuperscript{[1, 3]}
There is currently no 'gold standard' test for the diagnosis of vitamin B12 deficiency:
- Low vitamin B12 levels of uncertain significance may occur with nonspecific symptoms and no anaemia.
- Patients with strong clinical features of vitamin B12 deficiency may have serum vitamin B12 levels which lie within the reference range (false normal vitamin B12 level).
- Other tests (plasma homocysteine, plasma methylmalonic acid and serum holotransvitamin B12) may help to determine an underlying functional or biochemical deficiency but they are not currently widely available and the cut-off points to indicate deficiency vary between different laboratories.

FBC and blood film
Identification of hypersegmented neutrophils may suggest either vitamin B12 or folate deficiency; however, they are not sensitive in early vitamin B12 deficiency and are not specific.

Oval macrocytes, hypersegmented neutrophils and circulating megaloblasts in the blood film and megaloblastic change in the bone marrow are the typical features of clinical vitamin B12 deficiency. However, an elevated MCV is not a specific indicator of vitamin B12 deficiency and the possibility of underlying myelodysplastic syndrome has to be considered, as well as excluding alcohol excess, drugs and other causes of an elevated MCV.

The absence of a raised MCV cannot be used to exclude the need for vitamin B12 testing, as neurological impairment occurs with a normal MCV in 25\% of cases. Associated iron deficiency may result in the MCV being normal, in which case two types of red blood cells may be seen (a dimorphic blood film).\textsuperscript{[6]}
Biochemistry
There may be an increase in plasma unconjugated bilirubin due to increased destruction of red cell precursors in the marrow. LFTs, TFTs and protein electrophoresis may help in the differential diagnosis of macrocytosis.

Vitamin B12:
- Serum vitamin B12 is currently the standard initial routine diagnostic test. Establishment of reference ranges by individual laboratories can be challenging since the serum vitamin B12 level can be affected by many variables, including diet, pregnancy, vitamin supplements, contraceptive pill and metformin. Vitamin B12 and folate assays should be assessed concurrently due to the close relationship in metabolism.
- Plasma total homocysteine: deficiency of vitamin B12 results in elevation of plasma homocysteine, which is a sensitive biomarker of vitamin B12 deficiency and increases early in the course of deficiency, and progresses as the deficiency worsens. However, elevated levels also occur in folate deficiency, B6 deficiency and in patients with renal failure, hypothyroidism and as a result of certain genetic polymorphisms.
- Plasma methylmalonic acid is raised in vitamin B12 deficiency. However, the level may be falsely elevated in subjects with renal disease, small bowel bacterial overgrowth and haemoconcentration. Exceptionally high levels almost invariably indicate vitamin B12 deficiency.
- Holotranscobalamin is the ‘active’ fraction of plasma vitamin B12 and may be more specific than serum vitamin B12 levels. However, this test is not currently widely available.

Folic acid levels should be measured to exclude deficiency, which may co-exist with B12 deficiency. Red cell folate is a better guide to deficiency than serum folate. B12 deficiency may result in increased serum folate levels but reduced red cell folate levels, because of the effect on intracellular folate metabolism.

Investigations to determine the aetiology of vitamin B12 deficiency

Autoantibody screen
Pernicious anaemia is one of a number of autoimmune diseases, including Hashimoto's disease, type 1 diabetes, vitiligo and hypoadrenalism, which may coexist together. Antibodies against specific tissue antigens can help to diagnose specific conditions.

Pernicious anaemia is characteristically diagnosed by the presence of intrinsic factor antibodies (IFABs). However, autoimmune profiles done in patients as part of overall assessments of various endocrinopathies and other autoimmune disorders can reveal antibodies which may be associated with pernicious anaemia (IFAB, anti-parietal cell antibody), raising the possibility of co-existent pernicious anaemia.

During investigation of pernicious anaemia, other autoimmune disorders may be found to co-exist (particularly thyroid disease and type 1 diabetes) and it has been suggested that investigation for these should be considered.

Intrinsic factor antibody (IFAB)
Has high specificity with a high positive predictive value (95%) for the presence of pernicious anaemia if positive, and a low false positive rate (1-2%). It identifies those patients who need lifelong vitamin B12 replacement therapy. IFAB has low sensitivity and is positive in 40-60% of cases. A negative IFAB assay does not therefore rule out pernicious anaemia. High titre IFAB may interfere with assays of vitamin B12, leading to a false normal serum vitamin B12. Testing for IFAB is therefore advised in patients with strong clinical features of deficiency such as megaloblastic anaemia or subacute combined degeneration of the cord despite a normal serum vitamin B12 level.

Gastric anti-parietal cell antibodies
Have a low specificity for the presence of pernicious anaemia since, despite being positive in 80% of pernicious anaemia subjects, they are also positive in 10% of normal individuals. Positive gastric parietal cell antibodies may cause gastric acid achlorhydria and progression to pernicious anaemia may occur. However, a positive gastric parietal cell antibody test is not definitive for pernicious anaemia. Anti-gastric parietal cell antibody testing is therefore not recommended for diagnosing pernicious anaemia.

Schilling test
The test was used to measures the absorption of B12 by assessing increased urine radioactivity after an oral dose of radioactive B12. This test is no longer available.

Bone marrow aspiration
Bone marrow examination was historically recommended in situations where the clinical picture is unclear based on laboratory tests alone. However, some people with vitamin B12 deficiency have no overt haematological abnormalities and the value of a marrow examination is unknown.

However, bone marrow aspiration may be necessary to narrow the differential diagnosis, especially if myelodysplasia, aplastic anaemia, myeloma, or other marrow disorders are suspected. In B12 and folate deficiency, megaloblasts and giant metamyelocytes (early granulocyte precursors) are seen.\[9\]

Gastric secretions
Total gastric secretions are reduced to approximately 10% of the reference range; most patients have achlorhydria and absent IF.

Gastroscopy
People with pernicious anaemia who develop subsequent iron deficiency (indicating evidence of chronic atrophic gastritis) should be investigated with endoscopy due to the slight increased risk of gastric carcinoma; however, surveillance endoscopy is not recommended.

Associated diseases

People with pernicious anaemia are at increased risk of developing gastric cancer and there is an association with other autoimmune diseases, including primary hypothyroidism, thyrotoxicosis, Hashimoto's thyroiditis, Addison's disease, type 1 diabetes, hypoparathyroidism and vitiligo.

Management

Neurological presentation (peripheral neuropathy, subacute combined degeneration of the cord) may occur in the absence of haematological changes; early treatment is therefore essential to avoid permanent neurological disability.

Care should be taken not to give folic acid (instead of B12) to any patient who is B12-deprived, as this may result in fulminant neurological deficit.

The British National Formulary (Section 9.1.2 Drugs used in megaloblastic anaemias) states:

- Hydroxocobalamin has completely replaced cyanocobalamin as the form of vitamin B12 of choice for therapy.
- By intramuscular injection, pernicious anaemia and other macrocytic anaemias without neurological involvement, initially 1 mg 3 times a week for 2 weeks, then 1 mg every 3 months.
- Pernicious anaemia and other macrocytic anaemias with neurological involvement, initially 1 mg on alternate days until no further improvement, then 1 mg every 2 months.
- Prophylaxis of macrocytic anaemias associated with vitamin B12 deficiency, 1 mg every 2-3 months.

When to refer

- Seek urgent advice from a haematologist if the person has neurological symptoms, or is pregnant.
- Refer to a haematologist if the cause of vitamin B12 or folate deficiency is uncertain following investigations, or the suspected cause is haematological malignancy (urgently refer) or other blood disorder.
- Refer to a gastroenterologist if:
  - Malabsorption of vitamin B12 (other than due to pernicious anaemia) is suspected.
  - The person has pernicious anaemia and gastrointestinal symptoms, especially if there is a suspicion of gastric cancer (eg, co-existing iron deficiency). The urgency of referral will depend on the nature of the symptoms.

- Consider referral to a dietician if vitamin B12 deficiency is thought to be due to a poor diet.

Subclinical deficiency

- Low serum vitamin B12 without anaemia or other significant objective parameters may arise from testing for nonspecific symptoms such as tiredness, especially in the elderly population. This may be described as subclinical deficiency.
- This group may contain previously undiagnosed and 'latent' pernicious anaemia, patients with food malabsorption, patients on medications to reduce gastric acid production and patients on metformin.
- Management should be based on clinical judgement, with second-line tests to demonstrate deficiency in the small number of patients in whom a deficiency is strongly suspected.
- In most patients, the serum vitamin B12 assay should be repeated after 1-2 months. The serum level may then return as normal and no further investigation is recommended.
- In those where repeat sampling still falls within the 'subclinical' range, a blood sample should be taken for IFAB and a short trial of empirical therapy (oral cyanocobalamin 50 micrograms daily for four weeks) should be given while awaiting results of IFAB, with instructions to the patient to report immediately if symptoms of neuropathy develop, since this dose would be inadequate for a true pernicious anaemia.
- The short course of vitamin B12 may be of benefit since the elderly have a high incidence of food malabsorption, with some early studies suggesting possible cognitive improvement after vitamin B12 supplements.
- If IFAB is positive, the future management is lifelong therapy.
- If IFAB is negative, a further serum vitamin B12 level is recommended after 3-4 months. If well within the reference range, food malabsorption is a strong possibility and it should be managed accordingly. If still within the 'subclinical' range, consider investigation (plasma methylmalonic acid or holotranscobalamin) to confirm biochemical deficiency.
- In patients with a reduced serum vitamin B12 and normal holotranscobalamin and plasma methylmalonic acid no further action is required since a normal plasma methylmalonic acid and holotranscobalamin indicates an absence of a vitamin B12 deficient state.
- If subclinical deficiency is confirmed then lifelong therapy with vitamin B12 should be considered.

Complications

- Severe anaemia causes a risk of cardiopulmonary complications.
- Neurological changes can occur, even when there are no changes in the blood count. These include paraesthesia, ataxia, peripheral neuropathy (legs usually affected more than the arms), visual disturbance, psychiatric abnormalities and memory loss. Subacute combined degeneration of the spinal cord may also occur.
- Vitamin B12 deficiency predisposes to neural tube defects (such as spina bifida, anencephaly, and encephalocoele) in the fetus.
Deficiency of vitamin B12 or folate may cause ineffective production of any type of blood cells derived from bone marrow. Vitamin B12 or folate deficiency may cause sterility. This is reversible with appropriate vitamin supplementation. People with pernicious anaemia are at increased risk of developing gastric cancer. [1]

Prognosis

Before the advent of treatment with B12, the disease was fatal. Hence the name ‘pernicious’. However, pernicious anaemia responds rapidly to replacement therapy and most patients have a normal lifespan with little morbidity. If the deficiency has been severe and prolonged, any neurological complications may be irreversible. [1]

Further reading & references

1. Anaemia - B12 and folate deficiency; NICE CKS, July 2015 (UK access only)
6. Red blood cell morphology; Faculty of Health Sciences, Stellenbosch University, 2010
9. Hematopathology; Pictures of giant metamyelocytes
10. British National Formulary (BNF); NICE Evidence Services (UK access only)

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