Vitamin B12 Deficiency: New Diagnostic Assay

Clinical Setting

B12 deficiency is common especially in the elderly. Estimates of the prevalence of B12 deficiency vary depending on the population tested and the diagnostic test and cut-offs used; in European studies have shown deficiency in 1-10% of adults.

Vitamin B12 sufficiency requires adequate dietary B12 intake, intact gastric parietal cells for Intrinsic Factor (IF) and gastric acid production, and normal terminal ileum function to absorb the B12-IF complex via specific receptors.

Pernicious anaemia accounts for about 75% of B12 deficiency. It is estimated that about 2% of individuals over 60 years have undiagnosed pernicious anaemia. Dietary B12 is not absorbed by these individuals as they have intrinsic factor deficiency, due to an autoimmune gastritis characterised by elevated anti-parietal cell and anti-intrinsic factor antibodies. Younger people (especially Black African women) may also develop pernicious anaemia. Patients with autoimmune thyroid disease and type one diabetes mellitus are at increased risk of pernicious anaemia. Patients with pernicious anaemia have a higher incidence of gastric cancer and carcinoid tumours.

Other groups at risk of B12 deficiency are vegetarians (B12 is chiefly present in animal protein), alcoholics and the elderly on a poor diet, patients with inflammatory bowel disease, malabsorption (e.g. coeliac), post gastrectomy or ileal resection, and H. pylori and HIV infected patients. Patients on phenytoin, metformin and proton pump inhibitors may have impaired B12 absorption. Nitrous oxide, (available in some supermarkets to make whipped cream), inactivates B12 with resulting deficiency and nitrous oxide abusers include health professionals and experimenting youths.

Clinical Presentation can be insidious

B12 deficiency presents with predominantly haematological and neurological findings. The classical haematological features, in usual order of appearance, are hypersegmented neutrophils, macrocytosis, anaemia, leukopenia and thrombocytopenia. (See Figure 1.). These are due to impairment of DNA synthesis; however, folate deficiency may present identically and concurrent iron deficiency may mask macrocytosis. Furthermore, B12 deficiency may be present without any haematological abnormalities at all. Impaired DNA synthesis may similarly affect other rapidly dividing cells causing glossitis, gastrointestinal symptoms and infertility.

Neurological manifestations are common in B12 deficiency and may be the only presenting feature; 75%-90% of clinically affected B12 deficient patients have neurological features and of these, 25% have normal haematology. Neural damage is due to methionine deficiency as B12 is required to convert homocysteine to methionine. Methionine is required for myelin synthesis and hence demyelination may ensue. Presentation is classically with limb weakness, sore tongue and ascending paraesthesia (sensations of cold, numbness, tightness in the tips of toes and fingers, and occasionally lancinating pains; loss of pinprick and vibration, absent ankle jerks). Ataxia is a late development. Less common findings include neuropsychiatric symptoms (e.g. depression, memory loss, dementia psychosis), visual, autonomic, (e.g. erectile dysfunction, incontinence) light-headedness and impaired taste and smell. Vague constitutional symptoms e.g. fatigue, malaise and GIT symptoms occur in about 50% of patients. Many patients presenting to general practitioners are thus candidates for Vitamin B12 testing.

Developments in laboratory testing

Functional tests of B12 deficiency

Vitamin B12 is required for the further metabolism of methylmalonic acid and homocysteine, thus these compounds increase in concentration in the blood of patients with deficiency.
and vitamin B12 deficiency is unlikely in the presence of normal levels of these metabolites/compounds. Methylmalonic acid levels are a sensitive test of B12 deficiency but are also raised in renal impairment and require highly specialised and expensive methodology, (Gas Chromatography/Mass Spectroscopy), which is not readily available in routine laboratories. Homocysteine is more accessible but elevations are not specific as they also occur in folate and B6 deficiency as well as in renal failure and in the common genetic causes of hyperhomocysteinaemia. The Schilling test, which measures GIT absorption of B12, is no longer commercially available.

**Vitamin B12 forms in the blood**

Serum vitamin B12 is bound to two major carrier proteins; about 70%-90% is bound to Haptocorrin (HC, also called Transcobalamin 1, synthesised by granulocytes) and this form is metabolically inactive. 10%-30% of B12 is bound to Transcobalamin 2 (TC2); this is the bioavailable, active fraction as it binds to TC2-B12 receptors on cell membranes and is then taken up by these cells. The TC2-B12 complex is called holotranscobalamin or “Active” B12. (See Figure 2)

**“Standard” Vitamin B12 assays**

Standard Vitamin B12 assays, used in medical laboratories, measure total B12, i.e. both HC-B12 (inactive) and Active B12. This leads to sensitivity and specificity problems in diagnosing B12 deficiency, as selective fluctuations in HC-B12 may obscure the real (active) B12 status. A typical finding is a low B12 level which does not accord with the clinical setting. Metabolic studies show that between 15%-40% of patients with low B12 results do not have B12 deficiency.

Selective decreases of HC-B12 occur in inherited HC deficiency (present in 3% of the population and comprising 15% of the patients with low total B12 levels), in pregnancy /oestrogen therapy and in neutropenic states e.g. aplastic anaemia. These patients may have low total B12 levels but are not actually B12 deficient.

Selective increases of HC-B12 may occur in liver damage, the elderly, chronic renal failure and hypothyroidism and these patients may have high or normal total B12 levels which do not reflect their true B12 status.

**Active B12 (Holotranscobalamin)**

A new test which only measures Active B12 has recently become available. This test is not affected by fluctuations of HC-B12 and thus is not as prone to false positives and

negatives as is the standard (total) B12 assay. Early data shows it is a more specific, more sensitive and an earlier test of B12 deficiency than the (total) B12 assay. Active B12 has been shown to be a better predictor of elevated methylmalonic acid levels than is (total) B12. The major clinical advantage is that it allows confident diagnosis of B12 deficiency when the standard B12 assay result is in the low to low normal range. As Active B12 is only measurable in pmol/L, we will now be reporting all B12 measurements in pmol/L, rather than pg/mL. The lower reference limit for B12 of 270pg/mL corresponds to 200pmol/L in the new units.

In line with Clinipath Pathology’s commitment to providing a scientifically validated, high quality leading pathology service, we are now performing Active B12 testing on patients with (total) B12 levels of less than 200pmol/L (270 pg/mL) to assist in interpretation of low and low normal (total) B12 results. Patients with (total) B12 levels of 200pmol/L (270 pg/mL) and above are likely to be B12 replete and not require Active B12 testing.

**References:**


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