Consult. Confirm. CONTROL:

with NovoSeven® (Recombinant Factor VIIa) in acquired haemophilia (AH)[1]

Henry, 78 years old, *presented with severe and extensive skin bruising and blood in the stool. Henry had no prior history of bleeding.*

This advertisement is intended for Healthcare Professionals

Your primary treatment objective in AH is to STOP THE BLEED*

NovoSeven® is one of the first-line treatment options in AH based on:†[2,3]

• Rapid bleed control with consistently high efficacy[4-11] • Established tolerability profile[1,4,12-15] • Simple, rapid reconstitution and administration‡ and convenient storage[1]

*Published guidelines also recommend eradicating the inhibitor with immunosuppressive therapy.
†Other first-line haemostatic treatments are also recommended.
‡NovoSeven® vial-to-vial reconstitution 2–5 mins to infuse.

Prescribing Information NovoSeven® Eptacog alfa (activated); recombinant Factor VIIa (rFVIIa) **Please refer to Summary of Product Characteristics for full information. Presentation:** Powder (vial) and solvent (pre-filled syringe) for solution for injection. Available in packs containing 1, 2, 5 or 8 mg rFVIIa (8 mg only available in the UK). **Uses:** Treatment of bleeding episodes and prevention of bleeding during surgery or invasive procedures in patients with:- congenital haemophilia with inhibitors to coagulation FVIII or FIX > 5 BU or who are expected to have a high anamnestic response to FVIII or FIX; - acquired haemophilia; - congenital FVII deficiency; - Glanzmann's thrombasthenia with past or present refractoriness to platelet transfusions, or where platelets are not readily available. **Dosage:** The rFVIIa is dissolved in the accompanying solvent before use. After reconstitution the solution contains 1 mg rFVIIa/ml. Administer by intravenous bolus injection over 2-5 minutes; must not be mixed with infusion solutions or given in a drip. NovoSeven® should be administered as early as possible after the start of a bleeding episode. *Haemophilia A or B with inhibitors or expected to have high anamnestic response* Initial dose of 90 µg/kg body weight. Duration of, and interval between, repeat injections dependent on severity of haemorrhage or procedure/surgery performed. Paediatric population: Clinical experience does not warrant a general differentiation in dosing between children and adults. Children have faster clearance than adults and higher doses may be needed to obtain similar plasma concentrations as in adults. For mild to moderate bleeding episodes (including home therapy): Two dosing regimens can be recommended: i) Two to three injections of 90 µg/kg body weight administered initially at 3-hour intervals. If further treatment is required, one additional dose of 90 µg/kg can be administered. ii) One single injection of 270 µg/kg body weight. Duration of home therapy should not exceed 24 hours. Only after consultation with the haemophilia treatment centre can continued home treatment be considered. For serious bleeding episodes, initial dose 90 µg/kg body weight; dose every two hours until clinical improvement. If continued therapy indicated, dosage interval can be increased successively. Major bleeding episode may be treated for 2-3 weeks or longer if clinically warranted. For invasive procedures/surgery administer initial dose of 90 µg/kg body weight immediately before the procedure. Repeat dose at 2-3 hour intervals for first 24-48 hours. In major surgery continue dosing at 2-4 hour intervals for 6-7 days. Dosage interval may then be increased to 6-8 hours for further 2 weeks. Treatment may be up to 2-3 weeks until healing has occurred. *Acquired haemophilia* Initial dose of 90 µg/kg body weight. Further injections may be given if required. Initial dose interval should be 2-3 hours. Once haemostasis achieved, the dose interval can be increased successively *Factor VII deficiency* For bleeding episodes and for invasive procedures/surgery, in adults and children, administer 15-30 µg/kg body weight every 4-6 hours until haemostasis achieved. Adapt dose and frequency to individual. Limited clinical experience in long term prophylaxis has been gathered in paediatrics below 12 years of age, with severe clinical phenotype. *Glanzmann's thrombasthenia* For bleeding episodes and for invasive procedures/surgery administer 90 µg/kg body weight (range 80-120 µg) every 2 hours (1.5-2.5 hours). At least three doses should be administered to secure effective haemostasis. For patients who are not refractory platelets are first line treatment. In all conditions the dose schedule should not be intentionally increased above the recommended doses due to the absence of information on the additional risk that may be incurred. **Contra-indications:** Known hypersensitivity to active substance, excipients, or to mouse, hamster or bovine protein may be a contraindication to the use of NovoSeven®. **Precautions:** Treatment should be initiated under the supervision of a physician experienced in the treatment of haemophilia and/or bleeding disorders. For severe bleeds NovoSeven® should only be administered in hospitals specialised in the treatment of patients with coagulation factor FVIII or FIX inhibitors or in close collaboration with a physician specialised in treatment of haemophilia. No clinical experience with administration of single dose of 270 µg/kg body weight in elderly patients. Home therapy should not exceed 24 hours. Possibility of thrombogenesis or induction of DIC in conditions in which tissue factor could be expected in circulating blood, e.g. advanced atherosclerotic disease, crush injury, septicaemia, or DIC. Since NovoSeven® may contain trace amounts of mouse, bovine and hamster proteins there is a remote possibility of the development of hypersensitivity. Monitor FVII deficient patients for prothrombin time and FVII coagulant activity; suspect antibody formation if FVIIa activity fails to reach expected level or bleeding not controlled with recommended doses. Thrombosis in FVII deficient patients receiving NovoSeven® during surgery has been reported but risk is unknown. Avoid simultaneous use of prothrombin complex concentrates, activated or not. Based on a non-clinical study it is not recommended to combine rFVIIa and rFXIII. **Interactions: (Irish requirement only)** Risk of a potential interaction between NovoSeven® and coagulation factor concentrates is unknown. Simultaneous use of prothrombin complex concentrates, activated or not, should be avoided. Anti-fibrinolytics have been reported to reduce blood loss in association with surgery in haemophilia patients, especially in orthopaedic surgery and surgery in regions rich in fibrinolytic activity, such as the oral cavity. Experience with concomitant administration of anti-fibrinolytics and rFVIIa treatment is however limited. **Fertility, pregnancy and lactation:** Only administer to pregnant women if clearly needed. Not known if excreted in human milk; a decision on whether to continue/discontinue breast-feeding or to continue/discontinue therapy with NovoSeven® should be made taking into account the benefit of breast-feeding to the child and the benefit of NovoSeven® therapy to the woman. Data from non-clinical studies as well as post-marketing data show no indication that rFVIIa has a harmful effect on male or female fertility. **Side Effects:** The frequencies of both serious and non-serious adverse drug reactions are: Uncommon (≥ 1/1,000, < 1/100): venous thromboembolic events (deep vein thrombosis, thrombosis at i.v. site, pulmonary embolism, thromboembolic events of the liver including portal vein thrombosis, renal vein thrombosis, thrombophlebitis, superficial thrombophlebitis and intestinal ischaemia); rash (including allergic dermatitis and rash erythematous); pruritus and urticaria; therapeutic response decreased - it is important that the dosage regimen of NovoSeven® is compliant with the recommended dosage; pyrexia. Rare (≥1/10,000, <1/1,000): disseminated intravascular coagulation and related laboratory findings including elevated levels of D-dimer and decreased levels of AT; coagulopathy; hypersensitivity; headache; arterial thromboembolic events (myocardial infarction, cerebral infarction, cerebral ischaemia, cerebral artery occlusion, cerebrovascular accident, renal artery thrombosis, peripheral ischaemia, peripheral arterial thrombosis and intestinal ischaemia); angina pectoris; nausea; injection site reaction including injection site pain; increased fibrin degradation products; increase in alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and prothrombin. Adverse drug reaction reported post-marketing only (i.e. not in clinical trials) are presented with a frequency of not known. Not known: anaphylactic reaction; intracardiac thrombus, flushing; angioedema. *Inhibitory antibody formation:* Post-marketing there have been no reports of inhibitory antibodies against NovoSeven® or FVII in patients with haemophilia A or B. Development of inhibitory antibodies to NovoSeven® has been reported in post-marketing observational registry of congenital FVII deficient patients. Patients with FVII deficiency, formation of antibodies against NovoSeven® and FVII is the only adverse drug reaction reported (frequency: common (≥ 1/100 to < 1/10)). Risk factors may have contributed to antibody development including previous treatment with human plasma and/or plasma-derived FVII, severe mutation of FVII gene, and overdose of NovoSeven®. Patients with FVII deficiency treated with NovoSeven® should be monitored for FVII antibodies. *Thromboembolic events:* When NovoSeven® is administered outside approved indications, arterial thromboembolic events are common (≥ 1/100 to < 1/10). A higher risk of arterial thromboembolic adverse events (5.6% in patients treated with NovoSeven® versus 3.0% in placebo-treated patients) has been shown in trials conducted outside current approved indications. Safety and efficacy of NovoSeven® have not been established outside approved indications; NovoSeven® should not be used in these cases. Thromboembolic events may lead to cardiac arrest. *Patients with acquired haemophilia:* Clinical trials showed certain adverse drug reactions were more frequent (1% based on treatment episodes): arterial thromboembolic events (cerebral artery occlusion, cerebrovascular accident), venous thromboembolic events (pulmonary embolism and deep vein thrombosis), angina pectoris, nausea, pyrexia, erythematous rash and investigation of increased levels of fibrin degradation products. The Summary of Product Characteristics should be consulted for a full list of side effects. **Marketing Authorisation numbers:** NovoSeven® 1 mg (50 KIU) EU/1/96/006/008 NovoSeven® 2 mg (100 KIU) EU/1/96/006/009 NovoSeven® 5 mg (250 KIU) EU/1/96/006/010 NovoSeven® 8 mg (400 KIU) EU/1/96/006/011 (UK only) **Legal Category:** POM **(UK ONLY)- Basic NHS Price:** NovoSeven® 1 mg £525.20 NovoSeven® 2 mg £1,050.40 NovoSeven® 5 mg £2,626.00 NovoSeven® 8 mg £4,201.60 For complete prescribing information, please refer to The Summary of Product Characteristics which is available: **For Ireland from** - www.medicines.ie or by email from info@novonordisk.ie or from Medical Department, Novo Nordisk Limited, 1st Floor, Block A, The Crescent Building, Northwood Business Park, Santry, Dublin 9, Ireland; Tel: 1 850 665 665 **For UK from** – www.medicines.org.uk or from Novo Nordisk Limited, 3 City Place, Beehive Ring Road, Gatwick, West Sussex, RH6 0PA; Tel: 01293 613555 or Fax: 01293 613535

Ireland only

Adverse events should be reported. Information about adverse event reporting is available at www.hpra.ie Adverse events should be reported to the Novo Nordisk Medical department; Tel: 1 850 665 665.

UK only

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard or search for MHRA Yellow Card in the Google Play or Apple App Store. Adverse events should also be reported to Novo Nordisk Limited (Telephone Novo Nordisk Customer Care Centre 0845 600 5055). Calls may be monitored for training purposes.

References: 1. NovoSeven® Summary of Product Characteristics. **2.** Huth-Kuhne A, et al. Haematologica 2009;94(4):566–575. **3.** Collins P, et al. BMC Res Notes 2010;3:161.**4.** Baudo F, et al. Blood 2012; 120(1):39–46. **5.** Borel-Derlon A, et al. Presented at the World Federation of Hemophilia (WFH) World Congress, July 24–28 2016, Orlando FL USA: Online poster PO-W-4. **6.** Bysted BV, et al. Haemophilia 2007;13(5):527–532. **7.** Fernández-Bello I, et al. Haemophilia 2017;23(1):868–876. **8.** Amano K, et al. Haemophilia 2017;23(1):50-58 **9.** Hay CR, et al. Thromb Haemost 1997;78(6):1463-1467. **10.** Sumner MJ, et al. Haemophilia 2007;13(5):451–461. **11.** Lentz SR, et al. J Blood Med 2014;5:1–3. **12.** Hedner U. Blood Rev 2015;29(S1):S4–S8. **13.** Tiede A, Worster A. Ann Hematol 2018;97(10): 1889–1901. **14.** Neufeld EJ, et al. Haemophilia 2018;24(4):e275-e277. **15.** Abshire T, Kenet G. Haemophilia 2008;14(5):898–902.

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NovoSeven® Recombinant Factor VIIa

Guidelines for the diagnosis and treatment of cobalamin and folate disorders

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Summary of key recommendations

1 The clinical picture is the most important factor in assessing the significance of test results assessing cobalamin status because there is no 'gold standard' test to define deficiency.
2 Serum cobalamin currently remains the first-line test, with additional second-line plasma methylmalonic acid to help clarify uncertainties of underlying biochemical/functional deficiencies. Serum holotranscobalamin has the potential as a first-line test, but an indeterminate 'grey area' may still exist. Plasma homocysteine may be helpful as a second-line test, but is less specific than methylmalonic acid. The availability of these second-line tests is currently limited.
3 Definitive cut-off points to define clinical and subclinical deficiency states are not possible, given the variety of methodologies used and technical issues, and local reference ranges should be established.
4 In the presence of discordance between the test result and strong clinical features of deficiency, treatment should not be delayed to avoid neurological impairment.
5 Treatment of cobalamin deficiency is recommended in line with the British National Formulary. Oral therapy may be suitable and acceptable provided appropriate doses are taken and compliance is not an issue.
6 Serum folate offers equivalent diagnostic capability to red cell folate and is the first-line test of choice to assess folate status.

Keywords: cobalamin, folate, holotranscobalamin, methylmalonic acid, intrinsic factor antibody.

Measurement of serum cobalamin and folate forms a considerable proportion of the volume of testing in routine haematology and clinical chemistry laboratories, performed for a wide spectrum of clinical conditions (outlined in Table I). The previous British Society for Haematology guidelines on investigation and diagnosis of cobalamin and folate deficiencies (British Committe for Standards in Haematology, 1994) were published 20 years ago and this update reflects changes in diagnostic and clinical practice.

These guidelines aim to provide an evidence-based approach to the diagnosis and management of cobalamin and folate disorders. However, such evidence, particularly in the form of randomized controlled trials, is lacking. As a result, these guidelines provide a pragmatic approach to the testing and treatment of cobalamin and folate disorders, with recommendations based, as far as possible, on the GRADE system (Appendix I: http://www.gradeworkinggroup.org/index.htm). In the majority of situations, the recommendations inevitably rely more on clinical judgement and consensus than objective laboratory data. Table II provides a clinical guide to patient evaluation in assessing cobalamin or folate deficiency.

The Guidelines Writing Group (GWG) reviewed publications up to 2013 identified via the Pubmed and Cochrane databases using index terms including cobalamin, vitamin B12, folate, methylmalonic acid (MMA), homocysteine, holotranscobalamin, and combined with deficiency, treatment, pregnancy, oral contraceptive, metformin, bariatric surgery and infancy. Initial review of the manuscript was performed by members of the General Haematology Task Force of the British Committee for Standards in Haematology (BCSH), the executive committee, and a sounding board drawn from UK haematologists.

Cobalamin deficiency

In patients with classical megaloblastic anaemia, the presence of a low serum cobalamin level and objective assessment of response in terms of the rise in haemoglobin concentration clearly outlines the treatment pathway. However, the majority of patients do not have such a clear-cut picture. Neurological presentation (peripheral neuropathy, sub-acute combined degeneration of the cord) may occur in the absence of haematological changes, and early treatment is essential to avoid permanent neurological disability. Low cobalamin levels of uncertain significance may occur with non-specific symptoms

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Table I. Summary of the causes of cobalamin and folate deficiency.

Population Sector	Cause	Cobalamin deficiency	Folate deficiency
All ages	Infections	*H.pylori*, *Giardia lamblia*, fish tapeworm	–
	Malabsorption	Pernicious anaemia	Poor bioavailability
	Medical conditions	Gastric resection for obesity or cancer, inflammation of small intestine due to coeliac disease, tropical sprue, Crohn disease	Inflammation of small intestine due to coeliac disease, tropical sprue, Crohn disease
	Inadequate dietary intake	Low intake of cobalamin-rich foods	Low intake of folate-rich or folic acid-fortified foods
Infants and children	Genetic disorders	Transcobalamin deficiency, Imerslund Grasbeck syndrome, other cobalamin mutations	Mutations in the SLC46A1 (PCFT; HGNC 30521) gene (proton-coupled folate transporter deficiency)
	Inadequate dietary intake	Maternal strict vegetarian (vegan) diet in pregnancy. Adherence to a vegan diet post-weaning	–
Women of child-bearing age	Pregnancy and lactation	Adherence to a low cobalamin diet during pregnancy may lead to metabolic signs of deficiency by the third trimester	Maternal deficiency secondary to high fetal and infant requirements
Older persons	Malabsorption	Achlorhydria due to atrophic gastritis and proton pump inhibitors: results in malabsorption of food-bound cobalamin. Symptoms may develop slowly because secretion of intrinsic factor continues, therefore the enterohepatic recycling of cobalamin is not affected	Poor bioavailability

and no anaemia. Furthermore, patients with strong clinical features of cobalamin deficiency may have serum cobalamin levels that lie within the reference range (false normal cobalamin level). As a result, other tests may be used to try and determine an underlying functional or biochemical deficiency (reviewed in Quadros, 2010; Fedosov, 2012). These tests, plasma homocysteine, plasma MMA and serum holotranscobalamin, may help but are not widely available currently and the cut-off points to indicate deficiency vary between different laboratories (Carmel, 2011; Heil *et al*, 2012). In addition, their role is not clearly defined in the routine diagnostic setting. Therefore, there is currently no 'gold standard' test for the diagnosis of cobalamin deficiency (Solomon, 2005; Herrmann & Obeid, 2012).

Given that the biochemical pathways of cobalamin and folate are closely intertwined, with patients showing similar clinical features for both deficiencies, assessment of cobalamin and folate status is usually performed concurrently. In the presence of true cobalamin deficiency, the serum folate is often normal or can be elevated. However, a low serum cobalamin level may be found in the presence of folate deficiency.

Tests to confirm/diagnose cobalamin deficiency

Mean cell volume and blood film examination. Identification of hypersegmented neutrophils, defined as >5% of neutrophils with five or more lobes and the presence of oval macrocytes, may suggest either cobalamin or folate deficiency, but they are not sensitive in early cobalamin deficiency (Carmel *et al*, 1996a) and are not specific for it (Westerman *et al*, 1999). Oval macrocytes, hypersegmented neutrophils and circulating megaloblasts in the blood film and megaloblastic change in the bone marrow are the typical features of clinical cobalamin deficiency. However, an elevated mean cell volume (MCV) is not a specific indicator of cobalamin deficiency (Galloway & Hamilton, 2007) and the possibility of underlying myelodysplastic syndrome has to be considered (having excluded alcohol excess, drugs and other causes of an elevated MCV).

The absence of a raised MCV cannot be used to exclude the need for cobalamin testing because neurological impairement occurs with a normal MCV in 25% of cases (Lindenbaum *et al*, 1988; Healton *et al*, 1991).

Serum cobalamin. A serum cobalamin assay is currently the standard initial routine diagnostic test. It quantitates both the 'inactive' forms (transcobalamin I- and transcobalamin III- bound, now referred to as holohaptocorrin) and the 'active' form (transcobalamin II-bound, now referred to as holotranscobalamin) of cobalamin in serum. It is widely available, low cost, automated method based on intrinsic factor binding of cobalamin and immune-chemiluminescence based assays. However, it lacks the specificity and sensitivity required of a robust diagnostic test (see Appendix II).

A number of assays are registered on the United Kingdom National External Quality Assessment Service (UK NEQAS)

Table II. Clinical features to guide clinicians in cases of suspected cobalamin or folate deficiency.

Clinical feature	Possible cause of cobalamin or folate deficiency
Anaemia	Exclude other causes, including haematological disorders (e.g malignancy, myelodysplasia, haemolysis), hypothyroidism, chronic liver disorders, etc.
Evaluation of diet	Dietary deficiency
Is patient vegan or vegetarian?	
Is patient anorexic or has food fads/poor diet?	
Personal and family history of autoimmune disease	Positive family history or personal autoimmune conditions increase pre-test probability of pernicious anaemia
Does patient, parent or sibling have vitiligo, hypothyroidism or pernicious anaemia?	
History of glossitis or mouth ulceration	Glossitis is a common presentation of low cobalamin, and mouth ulcers may reflect folate deficiency
History of parasthesiae, unsteadiness, peripheral neuropathy (particularly proprioception)	Wide differential including cobalamin deficiency, diabetes, carpal tunnel, paraproteinaemia, other causes. Note that neurological presentation of cobalamin deficiency may occur despite normal haematological indices
Features of malabsorption	Steatorrhoea may be due to pancreatic disease, or small bowel disease, such as coeliac disease
Ask about pale stool, bowel movements at night, abdominal pain, mouth or perianal ulceration.	Terminal ileal disease with mouth and perianal ulceration may be due to Crohn disease
Consider history of pancreatitis due to alcohol excess	Gastrectomy will result in gradual depletion of cobalamin stores, with deficiency occurring 1–2 years post-surgery
Ask about previous stomach surgery including partial gastrectomy, bariatric surgery, or small bowel resection, which may have occurred as part of a hemicolectomy	
Ask about drug history	
Prolonged proton pump inhibition	Omeprazole or equivalent result in a gastric pH of 3·0, this may affect cobalamin release from food and cause low cobalamin levels
Metformin	Metformin may cause malabsorption and low cobalamin levels
Oestrogen contraceptive pill	May be associated with mild reduction in cobalamin levels
Pregnancy	Pre-pregnancy folate supplements are important to reduce risk of neural tube defects
	Low cobalamin levels found in third trimester may be physiological
Neurocognitive impairment in the elderly	Low cobalamin levels of uncertain significance in the elderly may be associated with neurocognitive impairment. Low cobalamin or folate levels may reflect poor diet as part of poor general condition

scheme, and the overall coefficient of variation of performance between different assays is 5–15%, with inter-method biases of plus 10% or minus 20% from the all-laboratory trimmed mean. Some assays may give false normal results in sera with high titre anti-intrinsic factor antibodies (Hamilton *et al*, 2006, 2010; Carmel & Agrawal, 2012). It is not entirely clear what should be regarded as a clinically normal serum cobalamin level, although it has been proposed that a serum cobalamin of <148 pmol/l (200 ng/l) would have a sensitivity of diagnosing 97% of true cobalamin deficiency (Snow, 1999; Carmel & Sarrai, 2006). It is even less clear what levels of serum cobalamin represent 'subclinical' deficiency, i.e., a low serum cobalamin in the absence of clinical symptoms. An additional problem when comparing different assays and results from different laboratories, is the units for reporting of serum cobalamin levels. Some report in ng/l and some in pmol/l (1 pmol/l = 1·355 ng/l). Despite the absence of an international reference method of measurement, an international standard is available (Thorpe *et al*, 2007) which can facilitate harmonization of reporting in the future.

Establishment of reference ranges by individual laboratories can be challenging because the serum cobalamin level can be affected by many variables i.e. diet, pregnancy, vitamin supplements, contraceptive pill, metformin etc. They are best conducted by robust studies, accounting for such variables, by the manufacturer. The laboratories may then report their results adjusted in relation to assay bias of the consensus mean in UK NEQAS surveys. Exploration of assay sensitivity and specificity by a laboratory may be done by receiver-operating characteristic curve analysis, using MMA or an alternative appropriate metabolic marker, to define evidence of metabolic disturbance due to cobalamin deficiency [as in (Valente *et al*, 2011)].

Plasma total homocysteine (tHcy). Deficiency of cobalamin results in elevation of plasma total homocysteine (tHcy). Plasma tHcy is a sensitive biomarker of cobalamin deficiency and increases early in the course of deficiency, sometimes preceding symptoms, and progresses as the deficiency worsens. However, tHcy is not specific to cobalamin deficiency as concentrations of tHcy are also elevated in folate deficiency,

B6 deficiency and in patients with renal failure, hypothyroidism and as a result of certain genetic polymorphisms.

In the clinical laboratory, plasma tHcy is measured by a variety of techniques. There is no consensus on the reference range although most laboratories regard a concentration above 15 μmol/l as indicative of hyperhomocysteinaemia. Nevertheless, individual laboratories should determine their own reference ranges.

A major drawback to the clinical utility of tHcy is that sample collection and processing is critical as the plasma sample must be kept cool and then centrifuged and removed from the red cells within 2 h of collection.

Plasma methylmalonic acid (MMA). Plasma MMA is raised in cobalamin deficiency. However, it also may be falsely elevated in subjects with renal disease, small bowel bacterial overgrowth and haemoconcentration. Despite these limitations, exceptionally high levels of plasma MMA (>0·75 μmol/l) almost invariably indicate cobalamin deficiency.

Plasma MMA is quantified using gas-chromatography-mass spectrometry. Hence, this is a high cost test, which has prevented its widespread use. There is no routine national quality assessment scheme for plasma MMA assays in Britain.

High cut-off normal reference intervals for plasma MMA vary from 0·27 to 0·75 μmol/l (Heil *et al*, 2012). The GWG recommends collaborative national studies to determine reference ranges.

Holotranscobalamin. Holotranscobalamin (HoloTC), the 'active' fraction of plasma cobalamin, may be more specific than serum cobalamin levels, and an immunoassay for this fraction is now available. In clinical research studies, the HoloTC assay performs better than the serum cobalamin assay in assessing deficiency based on MMA levels (Miller *et al*, 2006; Nexo & Hoffmann-Lucke, 2011) and red cell cobalamin levels (Valente *et al*, 2011) as reference assays. However, arguments have been raised against accepting this (Schrempf *et al*, 2011; Carmel, 2012a), given that even MMA or red cell cobalamin may not be regarded as gold standard tests for determining deficiency. Despite this, the assay has a smaller 'grey zone' (uncertainty range) than serum cobalamin assays and better sensitivity and specificity characteristics.

The expected values for HoloTC in healthy individuals are 35–171 pmol/l. Lower and upper reference intervals for plasma HoloTC range from 19–42 pmol/l and 134–157 pmol/l, respectively (Refsum *et al*, 2006; Brady *et al*, 2008; Sobczynska-Malefora *et al*, 2014). A recent multicentre study suggested a cut-off point of 32 pmol/l of HoloTC for screening for cobalamin deficiency based on a MMA level >0·45 μmol/l (Heil *et al*, 2012). The GWG recommends that individual laboratories should either determine their own reference ranges dependent upon the particular HoloTC assay used or implement the manufacturer's reference range where a suitable study has been conducted.

Further studies are needed to evaluate the clinical utility of HoloTC in assessing cobalamin deficiency in a routine high output laboratory testing. It may cut down the percentage of indeterminate results, particularly in patients over the age of 65 years. There is the added advantage of use in pregnancy and in patients on oral contraceptives as the HoloTC fraction of cobalamin does not seem to be subject to the physiological drop seen in total serum cobalamin over the course of pregnancy (Greibe *et al*, 2011).

Given that samples for HoloTC analysis do not need any special pre-analytical preparation, and can be stored for batch analysis, it appears to be a strong candidate for future routine first-line assessment of cobalamin deficiency, particularly if costs of the test become favourable.

Bone marrow examination. Bone marrow examination was historically recommended in situations where the clinical picture is unclear based on laboratory tests alone (BCSH guidelines, 1994). However, some cobalamin deficient patients have no overt haematological abnormalities and the value of a bone marrow examination, in this context, is unknown.

Recommendations

1 **A blood film showing oval macrocytes and hypersegmented neutrophils in the presence of an elevated MCV may alert the clinician to the presence of underlying cobalamin or folate deficiency (Grade 2B).**
2 **Cobalamin and folate assays should be assessed concurrently due to the close relationship in metabolism (Grade 1A).**
3 **The writing group recommends adoption of reporting for cobalamin assay results in pmol/l (Grade 2C).**
4 **A serum cobalamin cut-off level of either 148 pmol/l (200 ng/l) or one derived from a local reference range should be used as evidence of cobalamin deficiency in the presence of a strong clinical suspicion (Grade 2B).**
5 **The report providing the result of a serum cobalamin assay should include the following:**
   - **a The interpretation of the result should be considered in relation to the clinical circumstances.**
   - **b Falsely low serum cobalamin levels may be seen in the presence of folate deficiency or technical issues.**
   - **c Neurological symptoms due to cobalamin deficiency may occur in the presence of a normal MCV (Grade 1B).**
6 **Plasma tHcy and/or plasma MMA, depending on availability, may be considered as supplementary tests to determine biochemical cobalamin deficiency in the presence of clinical suspicion of deficiency but an indeterminate serum cobalamin level (Grade 2B).**
   - **a Although plasma tHcy is a sensitive marker of cobalamin deficiency, plasma MMA is more specific.**

b Both assays have to be interpreted in relation to renal function.

7 HoloTC is suggested as a suitable assay for assessment of cobalamin status in a routine diagnostic laboratory in the future (Grade 1B).

Tests to determine the aetiology of cobalamin deficiency

Pernicious anaemia is one of a number of autoimmune diseases, including Hashimito disease, type 1 diabetes, vitiligo and hypoadrenalism, which may coexist together (Chanarin, 1972; Toh *et al*, 1997; Perros *et al*, 2000; Dittmar & Kahaly, 2003). Antibodies against specific tissue antigens can help to diagnose specific conditions.

Pernicious anaemia is characteristically diagnosed by the presence of anti-intrinsic factor antibodies (anti-IFABs) (Annibale *et al*, 2011; Bizzaro & Antico, 2014). However, autoimmune profiles, performed in patients as part of the overall assessment of various endocrinopathies and other autoimmune disorders, can reveal antibodies that may be associated with pernicious anaemia (anti-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 (Chanarin, 1972; Ottesen *et al*, 1995; De Block *et al*, 2008). There are no guidelines on screening for pernicious anaemia in other autoimmune disorders and each case has to be judged on individual clinical features.

Anti-intrinsic factor antibody (anti-IFAB). The finding of a low total serum cobalamin level may be further evaluated by testing for anti-IFAB. If positive, the test has a high positive predictive value (95%) for the presence of pernicious anaemia (Toh *et al*, 1997), with a concurrent low false positive rate (1–2%) i.e. a high specificity. It identifies those patients with a need for lifelong cobalamin replacement therapy. IFAB is positive in 40–60% of cases (Ungar *et al*, 1967), i.e., low sensitivity, and the finding of a negative IFAB assay does not therefore rule out pernicious anaemia (hereafter referred to as AbNegPA). In addition, the positivity rate increases with age (Davidson *et al*, 1989) and in certain racial groups [Latino-Americans and African-Americans; (Carmel, 1992)].

The incidence of pernicious anaemia in the UK population is estimated [extrapolated from National Health and Nutrition Examination Survey (NHANES) reports in the United States] to lie between 1–5/100 000 per annum, i.e., a rare disease. Reflex testing of all low cobalamin samples in a routine diagnostic laboratory is therefore expensive with a low detection rate. A history of other autoimmune disease e.g. hypothyroidism, and family history (Banka *et al*, 2011) increases the pre-test probability of pernicious anaemia.

High titre IFAB may interfere with assays of cobalamin, leading to a false normal serum cobalamin level. 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 cobalamin level. In these cases, pre-treatment serum should be stored for investigation with an alternative assay (HoloTC or MMA) to confirm the presence of a severe deficiency.

IFAB assays, based on automated chemiluminescence cobalamin binding, are vulnerable to give false positive IFAB results if the patient has had a recent cobalamin injection. Manufacturer product literature warns that these assays are only suitable for samples with a specified upper limit of serum cobalamin levels, and laboratories must comply with this advice. Results of assays using these methods should not be reported if the serum cobalamin is >295 pmol/l (400 ng/l) based on UK NEQAS survey data. True immunoassays for IFAB, based on porcine or recombinant intrinsic factor binding, can be used for post-treatment samples.

Gastric anti-parietal cell antibody. Gastric parietal cell (GPC) antibodies have a low specificity for the presence of pernicious anaemia as, despite being positive in 80% of pernicious anaemia subjects, they are also positive in 10% of normal individuals. Positive GPC antibodies may cause gastric acid achlorhydria and progression to pernicious anaemia may occur. However, a positive GPC antibody test is not definitive for pernicious anaemia (Khan *et al*, 2009).

Others. In the past, a proportion of low cobalamin normal IFAB (antibody-negative pernicious anaemia; AbNegPA) patients might have undergone a Schilling test, which is no longer available. Recently, another test to assess cobalamin absorption based on serum HoloTC levels (Hvas *et al*, 2011) has been described but requires further evaluation.

Pernicious anaemia patients 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, but surveillance endoscopy is not recommended.

Recommendations

1 All patients with anaemia, neuropathy or glossitis, and suspected of having pernicious anaemia, should be tested for anti-IFAB regardless of cobalamin levels (Grade 1A).

2 Patients found to have a low serum cobalamin level in the absence of anaemia and who do not have food malabsorption or other causes of deficiency, should be tested for IFAB to clarify whether they have an early/latent presentation of pernicious anaemia (Grade 2A).

3 Anti-GPC antibody testing for diagnosing pernicious anaemia is not recommended (Grade 1A).

Treatment of cobalamin deficiency

Current clinical practice within the UK is to treat cobalamin deficiency with hydroxocobalamin in the intramuscular form (outlined in the British National Formulary, BNF, http://www.medicinescomplete.com/mc/bnf/current/PHP5867-drugs-used-in-megaloblastic-anaemias.htm). Standard initial therapy for patients without neurological involvement is 1000 μg intramuscularly (i.m.) three times a week for 2 weeks. The BNF advises that patients presenting with neurological symptoms should receive 1000 μg i.m. on alternate days until there is no further improvement. However, the GWG recommends a pragmatic approach in patients with neurological symptoms by reviewing the need for continuation of alternate day therapy after 3 weeks of treatment.

Patients presenting with severe anaemia may develop a transient hypokalaemia following treatment, the clinical significance of which is unknown (Carmel, 1988), and potassium replacement therapy may be considered. In patients presenting with anaemia, a reticulocyte response should be evident by 7–10 d provided the patient has adequate levels of iron and folate. If a haematological response is not achieved the initial diagnosis should be reviewed. A suboptimal response may indicate previously masked iron deficiency or presence of another co-existing cause of anaemia.

Maintenance treatment for patients presenting without neurological deficit is with hydroxocobalamin 1000 μg i.m. every 3 months. Those with initial neurological deficit should receive hydroxocobalamin 1000 μg i.m. every 2 months. No further testing for cobalamin levels is required. Although there is little evidence that more frequent dosing is harmful, specific objective studies demonstrating clinical benefit are absent, and the GWG cannot make specific recommendations.

Hydroxocobalamin is generally well tolerated, though side effects include itching, exanthema, chills, fever, hot flushes, nausea, dizziness and, exceptionally, anaphylaxis (Hovding, 1968; James & Warin, 1971). This may be due to hypersensitivity to cobalt or any of the other components of the medication (Grant *et al*, 1982). Acneiform eruptions (Dupre *et al*, 1979) have been reported rarely. Due to cross-sensitivity of hydroxocobalamin and cyanocobalamin, treatment of patients may be a challenge. Skin patch testing may help to choose an appropriate product (Heyworth-Smith & Hogan, 2002). If absolutely necessary, treatment may be considered under hydrocortisone cover in a hospital setting where severe hypersensitivity can be managed.

High dose oral cyanocobalamin (1000–2000 μg) is licenced for use in several countries outside the UK and is widely available via the internet. Passive, intrinsic factor-independent absorption of a small fraction of such large doses should suffice to meet daily requirements. A Cochrane review on the use of oral cobalamin suggests it is as effective as intramuscular vitamin B12, with benefit of fewer visits to health centres and reduced discomfort of injections (Vidal-Alaball *et al*, 2005). However, the efficacy and cost–effectiveness of oral treatment in wider population-based settings has yet to be established. There are arguments against the use of oral cobalamin in initiation of cobalamin therapy in severely deficient individuals who have poor absorption, especially due to pernicious anaemia. High dose oral cobalamin would be a reasonable alternative as maintenance in patients unable to tolerate intramuscular injections provided there is good compliance with treatment (Stabler, 2013). On the other hand, some patients may prefer intramuscular injection therapy in order to assure effective treatment.

Low dose oral cyanocobalamin (BNF, 50 μg) is licenced within the UK and may improve serum cobalamin and biochemical markers in borderline cases. Their role in the treatment of subclinical deficiency is under active research. Care must be taken if low dose supplements are prescribed, as such an approach risks the suboptimal treatment of latent and emerging pernicious anaemia with possible inadequate treatment of neurological features.

Recommendations

1 **Treatment of established cobalamin deficiency should follow the schedules in the BNF (Grade 1A).**
2 **Initial treatment with oral cobalamin may not be appropriate in pernicious anaemia, but may be considered in maintenance or correction of suboptimal levels in asymptomatic patients (Grade 2C).**

Clinical approach to investigation and treatment of cobalamin-associated disorders

An approach to investigation and management of patients with suspected cobalamin deficiency can be found in Algorithms 1 and 2 (Figs 1 and 2).

Low serum cobalamin and anaemia or strong objective clinical features of glossitis or peripheral neuropathy [Algorithm 1]. These patients are likely to have pernicious anaemia.

Recommendations

1 **Patients suspected of having pernicious anaemia should be tested for IFAB. Patients found to be positive should have lifelong therapy with cobalamin (Grade 1A).**
2 **Patients negative for IFAB, with no other causes of deficiency, may still have pernicious anaemia and should be treated as anti-IFAB-negative pernicious anaemia. Lifelong therapy should be continued in the presence of an objective clinical response (Grade 2A).**

Borderline or normal serum cobalamin, in the presence of anaemia or other symptoms (false normal cobalamin levels). Serum cobalamin levels around the normal range have been reported

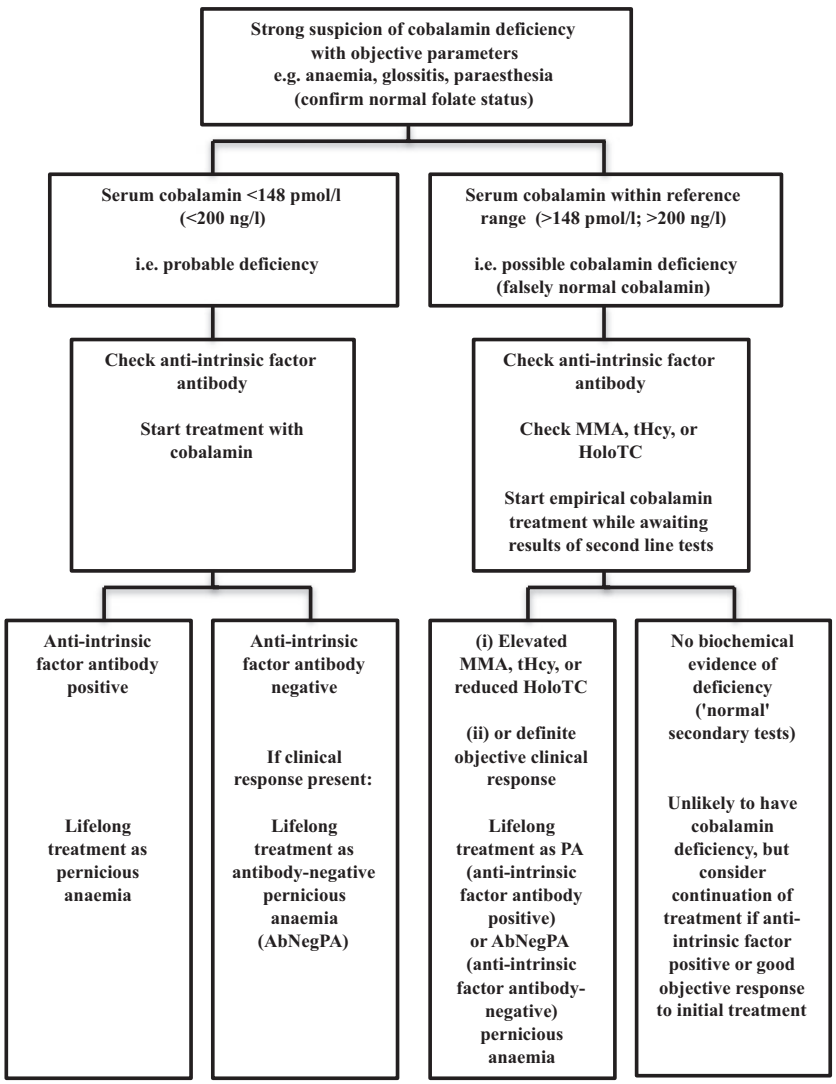

Fig 1. Algorithm 1. Investigation and management of patients presenting with a strong clinical suspicion of cobalamin deficiency and objective parameters to support this. MMA, methylmalonic acid; tHcy, total homocysteine; HoloTC, holotranscobalamin; PA, pernicious anaemia.

in the presence of significant anaemia (Devalia, 2006; Galloway & Hamilton, 2007) or other problems (Mahood, 1977). This may be a technical problem with the assay due to the presence of high levels of intrinsic factor antibodies (Hamilton *et al*, 2006). Further confirmation of tissue deficiency may be obtained by checking plasma MMA and homocysteine, and empirical treatment (as for pernicious anaemia) initiated immediately pending the results of second-line tests. If possible, pre-treatment serum should be stored in case future further investigation is necessary by the laboratory or UK NEQAS. Confirmed false normal results should be reported to the Medicines and Heathcare products Regulatory Agency (MHRA).

Recommendation

1 **Serum cobalamin level of >148 pmol/l (200 ng/l) in the presence of a strong clinical suspicion of cobalamin deficiency should be evaluated further with MMA, tHcy or HoloTC and a trial of hydroxocobalamin given to ascertain any clinical improvement (Grade 1C).**

Low serum cobalamin without anaemia or other significant objective parameters (low cobalamin of uncertain significance) [Algorithm 2]. This group arises from testing for non-specific symptoms (tiredness, neuropsychiatric symptoms, 'screening'), largely in the elderly population. This may be described as subclinical deficiency with the level of serum cobalamin possibly falling between 110–148 pmol/l, i.e., an 'indeterminate zone' within 25% below the lower reference range (Hvas & Nexo, 2006). This highly heterogeneous group poses challenges in further investigation and management, with added patient anxiety regarding the consequences of a low cobalamin level.

In general, this group may contain previously undiagnosed and 'latent' pernicious anaemia (Karnad & Krozser-Hamati, 1992), patients with food malabsorption, patients

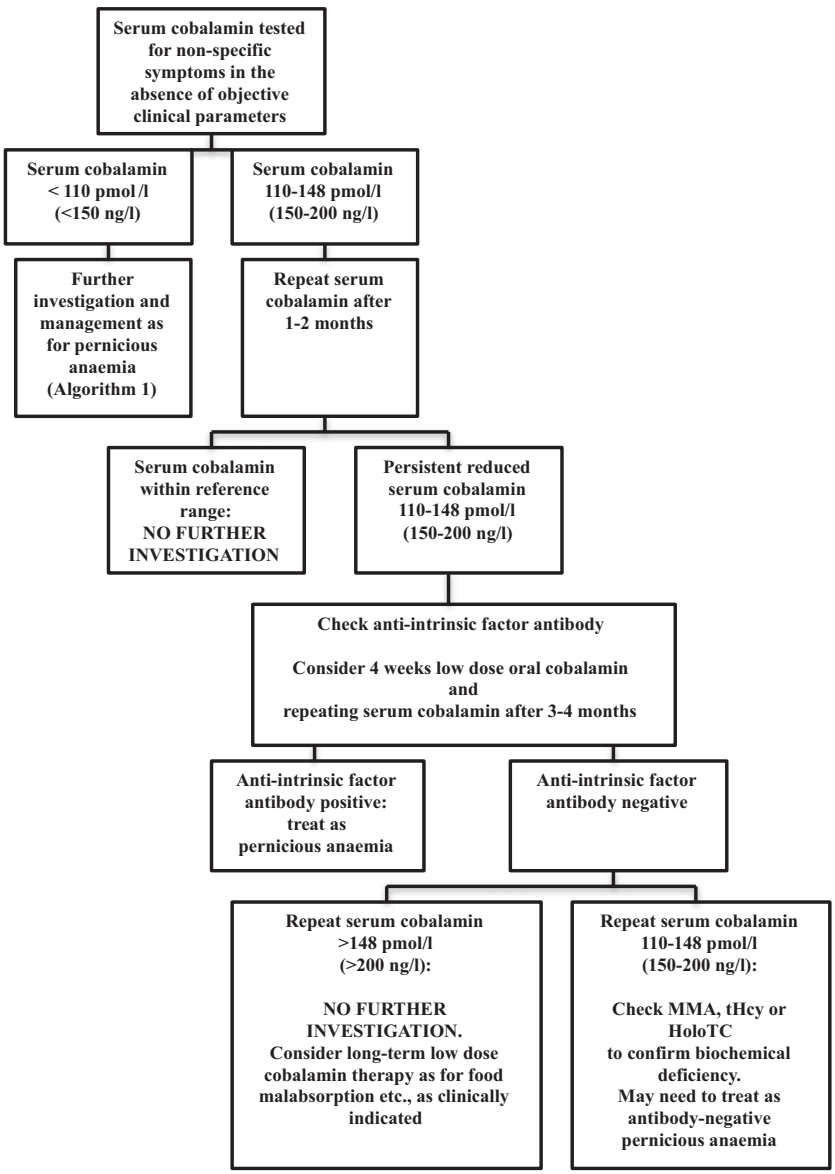

Fig 2. Algorithm 2. Investigation of low serum cobalamin in patients without objective clinical parameters. MMA, methylmalonic acid; tHcy, total homocysteine; HoloTC, holotranscobalamin.

on medications to reduce gastric acid production and patients on metformin. These patients with a low serum cobalamin of unknown significance lack specific symptoms, and even in this group, there are two subgroups of patients, namely those with biochemical evidence of deficiency and those with none. 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 cobalamin assay should be repeated after 1–2 months. In a proportion, this will be normal and no further investigation is recommended in these cases. In those where repeat sampling still falls within the 'subclinical' range, a blood sample should be taken for anti-IFAB and a short trial of empirical therapy (oral cyanocobalamin 50 μg daily for 4 weeks) should be given (while awaiting results of the IFAB test), with instructions to the patient to report immediately if symptoms of neuropathy develop as this dose would be inadequate for a true pernicious anaemia. The short course of cobalamin may be of benefit because the elderly have a high incidence of food malabsorption, with some early studies suggesting possible cognitive improvement after cobalamin supplements with or without Folate/B vitamins (van Asselt *et al*, 2001; Nilsson *et al*, 2001; Smith & Refsum, 2009; Smith *et al*, 2010; de Jager *et al*, 2012). In cases of a positive anti-intrinsic factor, the future management is lifelong therapy. In those negative for this, a further serum cobalamin level assessment is recommended after 3–4 months. If the level is well within the reference range, food malabsorption is a strong possibility and the patient should be managed accordingly (see section below). If still within the 'subclinical' range, consider investigation

(MMA or tHcy) to confirm biochemical deficiency and management as AbNegPA (see above).

In patients with a reduced serum cobalamin and normal tHcy and MMA no further action is required, as a normal MMA and tHcy indicates an absence of a cobalamin deficient state. Some patients with paraproteinaemia may have a low serum cobalamin but normal MMA and tHcy, which, on treatment of an underlying myeloma, will return to normal, suggesting the initial low value was due to assay interference (Vlasveld, 2003).

Recommendation

1 **In patients with serum cobalamin levels of 'subclinical deficiency' on two occasions, an empirical trial of treatment with oral cyanocobalamin (50 μg daily for 4 weeks) should be given. Strict instructions should be given to patients to seek immediate medical attention if symptoms of neuropathy develop. The cobalamin level should be rechecked after 3 months, and second-line tests considered if there is no improvement (Grade 2c).**

Low serum cobalamin on metformin. Use of metformin in type II diabetes is associated with reduced serum cobalamin levels (Tomkin *et al*, 1971; Leung *et al*, 2010), related to dose and duration of treatment (Ting *et al*, 2006). The mechanism is unknown but malabsorption may be one factor (Adams *et al*, 1983) and may be alleviated by increased intake of calcium (Bauman *et al*, 2000).

Biochemical evidence of cobalamin deficiency (increase in plasma tHcy) with metformin use has also been shown (Wulffele *et al*, 2003; de Jager *et al*, 2010) with no resultant clinical impact. In contrast, precipitation and progression of peripheral neuropathy has been reported (Bell, 2010; Wile & Toth, 2010). More recently, it has been suggested that despite a low serum cobalamin, the intracellular cobalamin metabolism is improved in patients taking metformin (Obeid *et al*, 2013).

The management dilemmas are similar to those of 'subclinical' deficiency, and short empirical treatment could be considered (Solomon, 2011; Mazokopakis & Starakis, 2012) in the presence of strong clinical suspicion of deficiency to prevent onset/progression of peripheral neuropathy, which may prove irreversible otherwise.

Recommendations

1 **No definitive advice can be given on the desirable frequency of monitoring of serum cobalamin in patients with type II diabetes mellitus on metformin therapy, but it is recommended that serum cobalamin is checked in the presence of strong clinical suspicion of deficiency (Grade 2B).**
2 **If serum cobalamin levels are reduced, patients should have tests for anti-IFAB because the concurrence of pernicious anaemia with diabetes should be considered. If positive, the patient should have lifelong treatment with replacement cobalamin. If negative, the reduced level may be purely as a result of metformin, although underlying AbNegPA cannot be excluded. Treatment with oral cobalamin may be considered (50 μg for 1 month); subsequent monitoring of serum cobalamin after 6 months and then at yearly intervals is suggested (Grade 2C).**
3 **Currently no recommendations can be given on prophylactic administration with oral cobalamin in patients taking metformin.**

Patients on hormone replacement therapy (HRT) and oral contraception. Oral contraceptive use causes a reduction in serum cobalamin levels (Wertalik *et al*, 1972; Lussana *et al*, 2003), but may not be significant when using 'low dose' oral contraception, i.e., containing 20 μg ethinyl estradiol (Sutterlin *et al*, 2003). The effect of HRT is not conclusive, given that both a reduction of cobalamin (Lacut *et al*, 2004) and no significant effect on cobalamin levels (Carmel *et al*, 1996b) have been reported. However, a cross-sectional study of young women taking oral contraceptives did not show any biochemical evidence of impaired cobalamin status (measuring MMA and tHcy) despite a reduction of serum cobalamin and HoloTC (Riedel *et al*, 2005) of up to 25%. The same study did not show any effect of HRT on serum cobalamin, holoTC, or metabolic markers.

Testing of serum cobalamin is suggested only in the presence of strong clinical suspicion of deficiency because the interpretation of results can be challenging.

Recommendation

1 **Asymptomatic women taking oral contraception or HRT with mildly reduced serum cobalamin (110–148 pmol/l; 150–200 ng/l) do not require further investigation but should be advised to review their dietary intake of cobalamin-rich foods, and cobalamin supplements may be considered (Grade 1B).**

Pregnancy. Pregnancy causes a lowering of serum cobalamin (Baker *et al*, 2002; Chery *et al*, 2002; Koebnick *et al*, 2002). In normal pregnancy, total serum cobalamin levels fall by 30% by the third trimester.

In a longitudinal study of healthy pregnant women, the biochemical marker MMA and tHcy showed a moderate increase in the third trimester, indicating a functional intracellular depletion of cobalamin (Chery *et al*, 2002; Murphy *et al*, 2007), suggesting that pregnancy itself can cause a stress on the cobalamin status of the mother. However, the HoloTC level has been shown to remain unchanged (Morkbak *et al*, 2007), suggesting that this is a better test to assess cobalamin status in pregnancy than serum cobalamin. In the absence of symptoms (parasthesiae, neuropathy or

macrocytic anaemia) serum cobalamin levels are impossible to interpret.

Serum cobalamin levels of 110–148 pmol/l (150–200 ng/l) in pregnancy may be physiological, and other biochemical tests to determine tissue deficiency are unproven. However, in the presence of strong clinical suspicion, anti-intrinsic factor antibodies should be checked and treat as pernicious anaemia if positive. If a low cobalamin result has been found in the presence of negative anti-IFAB, but with strong clinical suspicion of deficiency, in order to limit extensive investigation with resultant anxiety and to treat potential fetal deficiency, three injections of hydroxocobalamin are suggested to cover the pregnancy, with serum cobalamin levels being checked 2 months post-partum to ensure resolution to normal levels.

Recommendations

1 **Serum cobalamin levels fall during pregnancy and are less reliable in determining underlying deficiency (Grade 1A).**
2 **During pregnancy, in the presence of strong suspicion of underlying deficiency, a short course of empirical hydroxocobalamin should be given, with further investigations post-partum (Grade 2C).**
3 **HoloTC may be more reliable than serum cobalamin in determining deficiency in pregnancy, and is recommended as the test of choice, if available (Grade 1B).**

Vegetarians. Various studies show that vegetarians, especially vegans, have a lower cobalamin status than omnivores (Stabler & Allen, 2004). Biochemical markers of cobalamin status may reach significantly abnormal levels, resulting particularly in hyperhomocysteinaemia, which may have cardiovascular health consequences, and therefore it has been suggested that cobalamin status should be monitored in vegetarians (Refsum *et al*, 2001; Herrmann *et al*, 2003). Strict vegans are at increased risk of deficiency, especially during pregnancy/breast feeding, and oral supplements during this period are recommended.

Recommendations

1 **Vegetarians, particularly strict vegans, should be considered for monitoring of their cobalamin level according to clinical assessment (Grade 2C).**
2 **Dietary alterations or oral supplementation may be considered according to the clinical situation (Grade 2C), particularly during pregnancy and breast-feeding.**

Poor absorption due to gastrointestinal surgery or disease.

1 Patients who have had gastric surgery have a high prevalence of cobalamin deficiency (Sumner *et al*, 1996), and more recently, treatments for obesity including gastric banding and gastric bypass surgery have also led to cobalamin deficiency (Ledoux *et al*, 2006; Vargas-Ruiz *et al*, 2008). Recommendations for prevention and treatment of nutritional deficiencies have been published (Ziegler *et al*, 2009), which include monitoring of levels and prophylactic supplementation. Oral treatment may not be adequate (Donadelli *et al*, 2012) and lifelong compliance may be poor (Edholm *et al*, 2012). There are no studies of prospective intramuscular cobalamin supplementation in bariatric surgery.
2 'Food-bound cobalamin malabsorption' has been used to define a group of disorders characterized by gastric hypochlorhydria due to age-related gastric atrophy or secondary to drugs such as the proton pump inhibitors (Carmel, 1995; Bradford & Taylor, 1999; Lam *et al*, 2013).
   - **a** In such conditions the cobalamin may not be separated from food protein and therefore is unavailable to the intrinsic factor. This is associated with 30–40% of cases of sub-clinical cobalamin deficiency (Carmel, 2012b) (see above).
   - **b** The degree of this form of malabsorption can vary and the dose of oral cobalamin necessary to correct it cannot be prescriptive. Dose finding studies using 2·5–1000 μg of oral cyanocobalamin have shown good response in terms of serum cobalamin using lower doses but lesser response in terms of tHcy and MMA (Rajan *et al*, 2002; Seal *et al*, 2002; Eussen *et al*, 2005; Andres *et al*, 2009). A pragmatic approach would suggest starting with a low dose and increasing it as necessary. Future studies should help to clarify the parameters that should be used when assessing replacement therapy.
3 Exocrine pancreatic disease results in failure of release of cobalamin from haptocorrin in the duodenum, resulting in cobalamin deficiency (Gueant *et al*, 1990). This is relatively rare and no specific recommendations can be given.

Recommendations

1 **Patients who have had bariatric surgery should have their cobalamin status monitored and are likely to need cobalamin supplementation via a route depending upon the type of surgery (Grade 1B).**
2 **Patients with food-bound cobalamin malabsorption may benefit from low dose oral replacement (Grade 2C).**

Infancy. The typical symptoms in infancy are failure to thrive, movement disorders, developmental delay and megaloblastosis. Neurological symptoms and signs can develop without haematological abnormalities. Early recognition and treatment of cobalamin deficiency is important because, despite treatment, long-term consequences may remain in

the form of poor intellectual performance (Graham *et al*, 1992; Bjorke-Monsen & Ueland, 2011).

True cobalamin deficiency, with significant clinical problems, is considered to be rare in infants in developed countries and limited data exists on its prevalence. It is largely due to maternal deficiency (Bjorke Monsen *et al*, 2001; Guez *et al*, 2012), particularly when mothers have adhered to a strict vegetarian diet (Specker *et al*, 1990; Allen, 1994; Rasmussen *et al*, 2001). Rare cases of occult maternal pernicious anaemia causing cobalamin deficiency in infancy have also been reported (Banka *et al*, 2010).

The cobalamin status undergoes changes during infancy and childhood and a biochemical profile of cobalamin deficiency is thought to be relatively common in breastfed infants (Bjorke-Monsen & Ueland, 2011). Breastfeeding is associated with a low cobalamin status (Hay *et al*, 2008) in asymptomatic infants, particularly with prolonged exclusive breast feeding of 4 months or longer duration, in mothers who are replete of cobalamin levels (Greibe *et al*, 2013). This is thought to be due to reduced cobalamin levels in the breast milk with time. However, the clinical significance of this is unknown. It is not known if any replacement is necessary and future studies should, hopefully, clarify the issue.

Recently, a study in infants with feeding difficulties or developmental delay in association with impaired cobalamin status showed benefit from treatment (Torsvik *et al*, 2013) and this too needs further clarification given that varying degrees of such symptoms are common.

Various rare hereditary defects of cobalamin absorption and cellular uptake have been reported (Whitehead, 2006) resulting in cobalamin deficiency, which may present as early as 1–3 months of age or later, at 12–18 months, when cobalamin stores acquired *in utero* are exhausted (Linnell & Bhatt, 1995). As the serum cobalamin level may be within reference limits in a number of the cases in this subgroup, further clarification of intracellular cobalamin deficiency has to rely on biochemical tests of MMA, tHcy and others. In addition, identifying the underlying abnormality requires specialist fibroblast culture and molecular biology techniques. Treatment of the inborn errors of cobalamin metabolism is with intramuscular cobalamin, although the response varies greatly in different disorders. In conditions where hyperhomocysteinaemia is a feature, betaine can be added to cobalamin supplementation. In conditions with methylmalonic aciduria, total protein or valine, isoleucine, methionine and threonine restriction, carnitine supplementation, lincomycin and metronidazole may help to decrease methylmalonylCoA levels within cells.

Interestingly, asymptomatic newborns have been identified as having biochemical cobalamin deficiency by newborn screening programmes (Sarafoglou *et al*, 2011), mostly due to unsuspected maternal deficiency, but also due to known and recently discovered hereditary disorders (Quadros *et al*, 2010).

Recommendations

1 **Reduced serum cobalamin levels in infancy in the presence of clinical features should be treated promptly to prevent long term neurological sequelae (Grade 1A).**
2 **In the presence of clinical suspicion of underlying cobalamin deficiency, even in the presence of normal serum cobalamin levels, further biochemical tests, including MMA and tHcy, are recommended (Grade 1B). The role of HoloTC in this context is undefined. Further investigation to define any possible genetic abnormalities should be referred to a specialist centre. No specific recommendation can be made regarding treatment because each case has to be judged individually.**
3 **No specific recommendations can currently be made in relation to breastfeeding-associated biochemical low cobalamin status in asymptomatic infants.**

Folate deficiency

Folate is the term encompassing all the different biologically active forms of the vitamin and folic acid is the synthetic form used in supplements, fortified foods and for treatment. Both types are absorbed from the proximal small intestine and almost half of the body folate is found in the liver. The bioavailability of dietary folate is influenced by many factors within the intestinal lumen. Natural folates in food are also vulnerable to a variable degree of degradation by cooking processes (McKillop *et al*, 2002). These factors do not tend to affect folic acid and there is general consensus that the bioavailability of food folates is, on average, about 50% lower than folic acid (Gregory, 1995).

A low intake or poor absorption of folate leads to a low serum folate level, which then leads to low tissue levels. As folate is required for DNA synthesis, the earliest signs of deficiency are seen in rapidly proliferating cells, such as those of the bone marrow and gastrointestinal tract. Severe folate deficiency can cause pancytopenia and megaloblastic anaemia.

Given that serum folate reflects recent dietary intake, concerns have been raised about any assay performed after any oral folate intake, and therefore, masking underlying deficiency and giving a 'false negative' result. In addition, a 'false positive' reduced serum folate may be found in patients with anorexia, acute alcohol consumption, normal pregnancy and patients on anticonvulsant therapy (Beck, 1991). It is important to consider the result of the serum folate level within the context of the full clinical picture.

There is no clearly defined progression from the onset of inadequate tissue folate status to development of megaloblastic anaemia. Both biochemical and clinical evidence of folate deficiency can be observed in the absence of clinical symptoms. With increasing adoption of food fortification, the incidence of folate deficiency has declined significantly

(Joelson *et al*, 2007), and interpretation of folate status in patients has to be made in relation to presence of known risk factors causing deficiency (Vinker *et al*, 2013).

Tests to diagnose folate deficiency

Serum folate. The serum folate concentration reflects recent folate status and intake. Most clinical laboratories today measure serum folate by competitive folate binding protein (FBP) assays using chemiluminescence or fluorescence detection systems. Despite considerable variation in performance between assays, using the isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) international reference methods, there was close correlation of the consensus mean in the UK NEQAS surveys (Blackmore *et al*, 2011). This offers the prospect of harmonization of reference ranges for serum folate assays.

There is no clear consensus on the level of serum folate that indicates deficiency. Conventionally, clinicians have used serum folate lower than 7 nmol/l (3 μg/l) as a guideline because the risk of megaloblastic anaemia greatly increases below this level. However, there is a sizeable 'indeterminate zone' [between approximately 7 and 10 nmol/l (3 and 4·5 μg/l)]. Therefore, a low serum folate level should be taken as suggestive of deficiency rather than as a highly sensitive diagnostic test.

Red cell folate. The red cell folate level gives an assessment of the tissue folate status over the lifetime of the red cells and is therefore regarded as an indicator of longer term folate status than the serum folate assay.

A red cell folate level below 340 nmol/l (150 μg/l) has been regarded as consistent with clinical folate deficiency (Joelson *et al*, 2007) in the absence of cobalamin deficiency. Whether serum folate or red cell folate is better for assessing body folate status has been extensively argued. A pathology benchmarking review concluded that serum folate measurement provides equivalent information to red cell measurement (Galloway & Rushworth, 2003). However, it is suggested that in about 5% of patients the measurement of red cell folate may be useful in patients with macrocytosis who have a normal serum folate.

Current red cell folate assays are affected by pre-analytical and analytical variables, which preclude them as robust assays. There is a lack of standardization of current commercial red cell folate assays, which show very poor inter-method agreement in UK NEQAS red cell folate surveys. A recent review also suggested serum folate measurement may be better than red cell folate because it is affected by fewer pre-analytical and analytical variables (Farrell *et al*, 2013).

Homocysteine (in relation to folate disorders). Elevated plasma tHcy is a sensitive indicator of folate status and is strongly correlated with serum folate levels in the low physiological range [i.e. serum folate levels below about 10 nmol/l (4·5 μg/l)]. tHcy arises as a by-product of methionine metabolism and is normally present in plasma at concentrations below 12 μmol/l, depending on age, gender, renal function, genetic factors and the nutritional status of several other vitamins. It is not, therefore, a specific marker of folate status. Furthermore, it has stringent requirements regarding sampling and technical analysis. As a result, it is not used for routine testing.

Recommendations

1 **A serum folate level <7 nmol/l (3 μg/l) is indicative of folate deficiency (Grade 1B).**
2 **Routine red cell folate testing is not necessary because serum folate alone is sufficient in most cases (Grade 1A).**
3 **In the presence of strong clinical suspicion of folate deficiency, despite a normal serum level, a red cell folate assay may be undertaken, having ruled out cobalamin deficiency (Grade 2B).**
4 **Plasma tHcy can be measured to confirm suspected folate deficiency only in special circumstances; a level above 15 μmol/l could be indicative of folate deficiency but must be assessed in relation to local reference ranges (Grade 2B).**

Clinical approach to investigation and treatment of folate associated disorders

There is usually a decrease in serum folate within several weeks of folate deprivation but it is not clear how low the serum folate must be in order to cause knock-on biological and haematological effects.

Conditions mimicking cobalamin deficiency. In practice, cases of isolated clinical folate deficiency are extremely rare in the developed world and a diagnosis of folate deficiency should be made with consideration of a circumstance leading to shortage or malabsorption of multiple nutrients. Furthermore, the metabolic roles of folate and cobalamin are closely linked, and deficiency of either vitamin can result in the same clinical manifestations. In addition, a low serum folate may be associated with a low serum cobalamin, in which case treatment is initiated with cobalamin therapy before adding in folate therapy. Also, serum folate seems to be the preferred marker for folate status in cobalamin deficiency because the red cell folate may be lower in the presence of cobalamin deficiency (Klee, 2000).

Anaemia due to folate deficiency. Anaemia due to folate deficiency is now most often seen in cases of poor diet, severe alcoholism and in certain gastrointestinal diseases (Allen, 2008). It may be a presenting feature of coeliac disease.

1 *Dietary deficiency*: The folate status is likely to be poorer in diets reliant on foods not fortified with folic acid and

consuming low amounts of legumes and green leafy vegetables. If the habitual dietary folate intake is low (<100 μg/day) supplementing with 400 μg/d of folic acid is adequate to maintain folate status even during pregnancy (Institute of Medicine, 2000).

Goat's milk has a much lower folate concentration than cow's milk (Gregory, 1975), and infants fed exclusively on this may become deficient, unless commercial folic acid fortified goat's milk is used.

2 *Alcoholism*: The risk of developing deficiency is increased when >80 g of ethanol is consumed daily (Gloria *et al*, 1997), with the presence of liver disease aggravating the situation (Savage & Lindenbaum, 1986). Poor diet (Eichner & Hillman, 1971) and increased urinary excretion also contribute to this (Russell *et al*, 1983).

3 *Pregnancy*: Pregnancy and lactation are associated with increased folate requirements, and preferential delivery of folate to the fetus may result in severe maternal deficiency in the presence of normal folate status in the baby (O'Connor *et al*, 1997). Multiparity and hyperemesis gravidarum increase the risk of developing deficiency in the mother.

As women are advised to take folate supplements in the preconception period and in early pregnancy, there is no requirement to measure serum folate in pregnancy apart from poor diet, suspected malabsorption, hyperemesis, or development of macrocytic anaemia.

4 *Increased requirements*: Folate requirements increase in peripheral red cell destruction or abnormal haemopoiesis, manifested by increased red cell requirements or thrombocytopenia, e.g. sickle cell disease (Liu, 1975; Kennedy *et al*, 2001) and haemolytic anaemias.

Exfoliative skin diseases also increase folate requirements (Gisondi *et al*, 2007).

5 *Haemodialysis*: Given that serum folate levels are reduced in the immediate post-dialysis period, red cell folate may be the preferable assay soon after dialysis (Heinz *et al*, 2008). However, for pre-dialysis assessment, the serum folate assay may be preferable (De Vecchi *et al*, 2000).

Other folate associated disorders. Low blood folate status is seen following chronic use of some medications (Reynolds, 1967; Neubauer, 1970; Lindenbaum, 1983; Mountifield, 1985; Linnebank *et al*, 2011; Wilson *et al*, 2011), particularly anticonvulsants (Eren *et al*, 2010; Linnebank *et al*, 2011) and in individuals homozygous for a well-described functional polymorphism in the *MTHFR* gene (HGNC:7436) which encodes the folate metabolizing enzyme 5,10-methylenetetrahydrofolate reductase. The 677 C→T variant leads to a more labile enzyme form that is associated with lower folate status and has been linked to higher risk of several chronic disease conditions and of neural tube defects (Brouns *et al*, 2008). However, there are no specific recommendations for treatment of low folate levels in individuals who are homozygous for this polymorphism i.e. all patients with low folate levels are treated in the same way regardless of their genotype.

Assessment of folate status with respect to the risk of neural tube defects, cardiovascular and cerebrovascular risk is beyond the scope of these guidelines.

Recommendations

1 **Folate status is generally checked in clinical situations similar to those of cobalamin deficiency (Grade 1A).**
2 **Consultation of the BNF and Summary of Product Characteristics is recommended for clarifying any suspicion of low serum folate levels associated with prescribed medications.**

Treatment of folate deficiency

The dose of folic acid necessary for treatment depends on the cause of the deficiency. A meta-analysis showed that daily doses of 0·8 mg or more of folic acid are typically required to achieve the maximal reduction in plasma homocysteine concentrations produced by folic acid supplementation (Homocysteine Lowering Trialists' Collaboration, 2005). Doses of 0·2 and 0·4 mg are associated with 60% and 90% respectively, of this maximal effect (Homocysteine Lowering Trialists' Collaboration, 2005). However, more recently, a dose of 0·2 mg/d over at least 6 months was shown to have optimal effects (Tighe *et al*, 2011).

The BNF has outlined the treatment of folate deficiency as follows (http://www.medicinescomplete.com/mc/bnf/current/PHP5867-drugs-used-in-megaloblastic-anaemias.htm):

Folate deficient megaloblastic anaemia (due to dietary insufficiency, pregnancy or antiepileptics): 5 mg of folic acid daily is taken for 4 months, except in pregnancy where it is continued until term, and up to 15 mg daily for 4 months is suggested in malabsorptive states.

Chronic haemolytic states and renal dialysis: the prophylactic dose suggested is 5 mg daily to weekly, depending on the diet and rate of haemolysis.

Pregnancy: the prophylactic dose suggested is 200–500 μg daily.

Recommendation

1 **Treatment of folate disorders should follow the schedules in the BNF (Grade 1A).**

Recommendations for future research

1 Well-designed reference range studies for cobalamin, folate, homocysteine, methymalonic acid and holotranscobalamin in target populations, addressing variables such as diet, gender, pregnancy, vitamin intake etc.
2 Prospective long term studies on the natural history of subclinical cobalamin deficiency or low serum cobalamin of unknown significance, with or without low dose cobalamin therapy.
3 Research on discovery of more sensitive and specific tests is needed.
4 Research on ways of assessing adequate replacement would be of benefit in titrating the treatment with clinical efficacy.

Recommendations for audit

1 Laboratory performance of serum cobalamin assay in relation to UK NEQAS.
2 Clinical reason for serum cobalamin request as specified on request forms.
3 Haemoglobin concentration and MCV, if available, taken at the same time as serum cobalamin in the deficiency level and 25% below the local laboratory reference range cut-off levels.
4 Clinical audit investigating deficiency states in relation to local reference ranges.
5 Establishment of clinical cut-off points for deficiency with holotranscobalamin assay.

Disclaimer

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Appendix I
Strength of recommendation and quality of evidence

Strength of recommendations

Strong (grade 1): Strong recommendations (grade 1) are made when there is confidence that the benefits do or do not outweigh harm and burden. Grade 1 recommendations can be applied uniformly to most patients. Regard as 'recommend'.

Weak (grade 2): Where the magnitude of benefit or not is less certain a weaker grade 2 recommendation is made. Grade 2 recommendations require judicious application to individual patients. Regard as 'suggest'.

Quality of evidence

The quality of evidence is graded as high (A), moderate (B) or low (C). To put this in context it is useful to consider the uncertainty of knowledge and whether further research could change what we know or our certainty.

(A) High. Further research is very unlikely to change confidence in the estimate of effect. Current evidence derived from randomized clinical trials without important limitations.

(*B) Moderate*. Further research may well have an important impact on confidence in the estimate of effect and may change the estimate. Current evidence derived from randomized clinical trials with important limitations (e.g. inconsistent results, imprecision – wide confidence intervals or methodological flaws – e.g. lack of blinding, large losses to follow up, failure to adhere to intention to treat analysis), or very strong evidence from observational studies or case series (e.g. large or very large and consistent estimates of the magnitude of a treatment effect or demonstration of a dose–response gradient).

(C) Low. Further research is likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate. Current evidence from observational studies, case series or just opinion.

Appendix II
Problems associated with serum cobalamin assays

In the case of cobalamin testing there are three pitfalls:

1 The definition of a disease state is not clear since individuals identified with low levels or 'deficient' includes three categories:
   - a Individuals with clinical disease state, e.g., megaloblastic anaemia or neuropathy.
   - b Individuals with no symptoms but with evidence of metabolic disturbance, i.e., subclinical deficiency identified by metabolic changes but no clinical features.
   - c Individuals with levels below two standard deviations from the mean of the population studied but no clinical or identifiable metabolic abnormality

2 The diagnostic cut-off point may be chosen to identify only 1(a) or to include 1(a) and 1(b). The cut off point of 148 pmol/l (200 ng/l) results in 95% of category 1(a) being identified, i.e., 95% sensitivity, however 50% of individuals with results below this cut off point will have no identifiable clinical sequelae of cobalamin deficiency, i.e., specificity of 50%. Choosing a lower cut-off point e.g. 110 pmol/l (150 ng/l) will reduce the sensitivity of the test to approximately 75% but will enhance the specificity reducing the false positives and enhancing the true positives. The choice of cut off point can be explored by the use of receiver-operated characteristic curves, in studies where a gold standard test can be used to identify deficient patients – however, no entirely suitable gold standard test exists.

3 The definition of a reference range is the mean of a suitably large number of individuals in a study population, plus or minus two standard deviations and may be easily determined by taking the entire database of cobalamin results and taking the 2·5th and 97·5th centile as lower and upper cut-off points. The distribution is not normally distributed, unless log transformed. Some cobalamin distributions are bimodal, suggesting interference with the assay at high levels or vitamin supplementation. The range of cobalamin results in the study population itself is affected by age, ethnicity and dietary differences, e.g., low levels in vegetarians and vegans, pregnancy, the contraceptive pill and drug therapy, e.g., metformin, or in long-term gastric acid suppression by proton pump inhibitors.