

Chapter 2 How to diagnose vitamin B12 deficiency

*You will not have to do anything but stay calm.
The Lord will do the fighting for you.*

Exodus 14:14

Message from a patient to the world

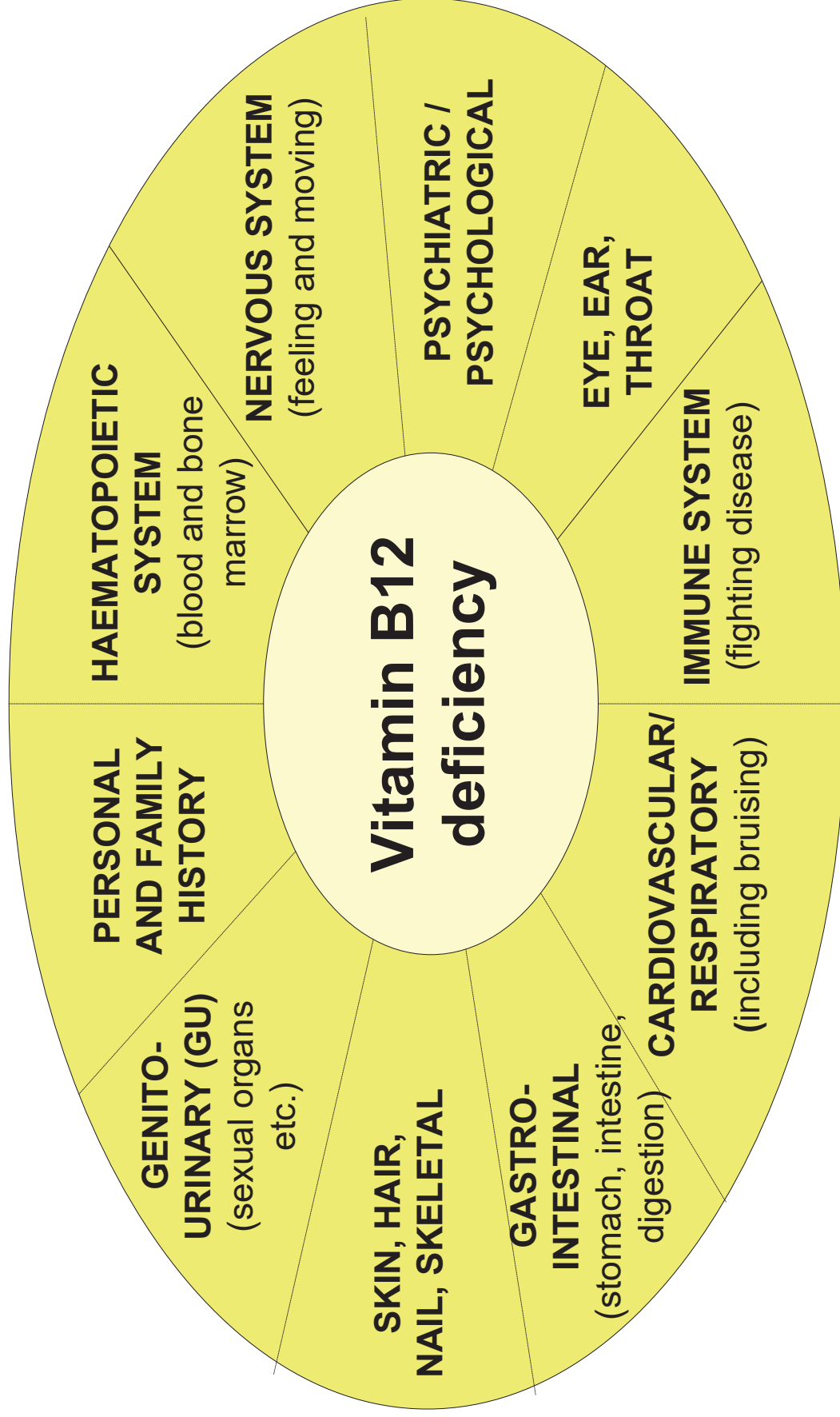
Is Dr Joseph Chandy a miracle worker? YES, I believe he is – yet he alone cannot perform miracles. His work is in The Hands of God!

For no less than 25 years this man has more or less single-handedly conducted research into medical dilemmas. Dr J. Chandy is a warm-hearted family man who cares deeply for the population of today – now that his work is being recognised we must keep up the fight. As a long term B12 sufferer I say, “we must unite together to get the treatment we deserve”. In my condition I’d do almost anything to have a full head of hair and full use of my hands. Maybe in my future this will come but until that time I’m relying on my B12 injections and I’ll back Dr J. Chandy, his family and dedicated members of staff all the way in his quest for B12.

My greatest thanks to all involved.

Jannette Chapman, 2 November 2006

Figure 2-1 The range of categories of vitamin B12 deficiency signs and symptoms



2.1 Background to diagnosis of vitamin B12 deficiency

The traditional means of diagnosing vitamin B12 deficiency has been by a serum B12 blood test to determine the patient's B12 level, and the presence of the signs and symptoms of pernicious anaemia (PA) – the disease historically associated with causing lack of this vitamin. These signs are: macrocytosis (enlarged red blood cells), other changes in the blood and the presence of anti-Intrinsic Factor (IF) and anti-gastric parietal cell (GPC) antibodies.

In our experience, and in the views today of many experts and researchers of B12 referenced in this book, this is an outdated means of diagnosing B12 deficiency. It has a serious flaw in that it does not capture the many sufferers who do not have anaemia at all, or any other haematological signs. They may even have a serum B12 blood level which, according to the widely accepted cut-off point of 200 nanograms per litre (ng/L), would be considered to fall within the “normal” range. Instead, they have varying degrees of neuropsychiatric and neurological symptoms. Cases where the deficiency is “subtle” (i.e. not yet strongly manifested) are particularly at risk of being missed. As one expert says: “The proscription that cobalamin deficiency should not be diagnosed unless megaloblastic changes are found is akin to requiring jaundice to diagnose liver disease” (Carmel quoted in Smith & Refsum, 2011).

In this chapter we consider the limitations of the classical method of diagnosis and present our own diagnostic method, based mostly on signs and symptoms, and refined over three decades of clinical practice. This began as a formal “Pathway” in 2004 which we originally developed using our experience and following the recommendations in *Harrison's Principles of Internal Medicine*, the *BMJ Best Practice* guide and United Kingdom National Quality Assessment Scheme for Haematinic Assays (NEQAS) which advise that it must be a *clinical* decision to undertake a therapeutic trial in the suspicion of cases when a patient presents with classic signs and symptoms of B12 deficiency (see Introduction). The Pathway was further refined in the light of new knowledge and overseen by a senior haematologist from the Freeman Hospital, Newcastle-upon-Tyne. It was accepted as an official Pathway by Easington Primary Care Trust in 2006. This Pathway (since evolved into our Protocol) provides sufficient guidance for a clinician to diagnose vitamin B12 deficiency at an early stage and also enables patients to recognise their own symptoms through the **One Minute Health Check**. The Protocol is given in full in Appendix 1. To our knowledge, there is as yet no set of guidelines, other than our own, which specifically emphasises the neuropsychiatric and neurological signs and symptoms of B12 deficiency. The most helpful guidance we have otherwise encountered can be found in *BMJ Best Practice* [online] and in the *British Journal of Haematology* (Devalia et al., 2014). Our Protocol has been successfully used to diagnose and treat many B12-deficient patients.

2.1.1 Traditional link with anaemia

In Chapter 1 of this book we saw that vitamin B12 was first discovered in relation to PA, which was highly prevalent in the late eighteenth and early nineteenth centuries when nutrition was poor. To recap, two haematologists, George R. Minot and William P. Murphy, discovered by accident in 1926 that eating liver could cure, or at least alleviate, this disorder – which was at the time almost always fatal (hence its name “pernicious”) – and concluded that liver contained a substance vital for human health. Two decades later, the vitamin B12 compound was isolated.

This particular historical trajectory had a number of consequences (Wailoo, 1997). Because vitamin B12 was discovered in relation to PA and by haematologists (who were subsequently awarded a Nobel Prize for their work), B12 deficiency came within the “scope and expertise” of haematologists

(Green, 2017). The diagnostic markers of B12 deficiency came to be, in the classical view, the haematological signs of PA. Collectively, these are: macrocytosis, other blood cell abnormalities, and the presence in the blood of signs of autoimmune attack. Although PA was known to be accompanied by neurological and neuropsychiatric signs and symptoms, these were not given foremost importance and were not viewed as indicators of B12 deficiency if present without macrocytosis and anti-GPC or anti-IF antibodies.

This was despite the fact that the early literature documenting this type of anaemia had described its neurological associations (Leichtenstern, 1884; Lichtheim, 1887; cited in Reynolds, 2006), and that these were recognised by James Samuel Risien Russell (1863-1939) in the early twentieth century when he described Subacute Combined Degeneration of the spinal cord – SCD (Russell et al., 1900). For discussion of SCD, see Chapter 6 of this book.

This connection with anaemia has persisted in traditional methods of diagnosis even though it has become clear that B12 deficiency has many other symptoms and that it may not be accompanied by anaemia at all.

The criteria for diagnosis of B12 deficiency which developed out of diagnosis of PA are still commonly found in traditional guidelines (see, for example, ‘Anaemia – B12 and folate deficiency’ in (NICE CKS, 2018a), ‘Vitamin B12 or folate deficiency anaemia’ in the (NHS, 2016c) online and ‘Megaloblastic Anemias’ (Babior & Bunn, 2005) in the medical textbook *Harrison’s Principles of Internal Medicine*). They are known as the “Addisonian criteria”, named after Thomas Addison (1793-1860) who is credited with the first description of PA in 1849, before it was given the name which identified it as fatal (Pearce, 2004). These criteria are summarised in Figure 4-1 at the beginning of Chapter 4. They have various drawbacks (described below) and are, we believe, a complicated diagnostic method which often leads to misdiagnosis of B12 deficiency or the diagnosis being entirely missed.

2.1.2 B12 deficiency *without* anaemia is common

Not only is the Addisonian system complicated, but its main drawback is that it makes no allowance for patients presenting with neurological and/or neuropsychiatric symptoms *without* anaemia. There is plenty of evidence in the medical literature that this type of presentation is far more common and even that there is often a “dissociation” between the two conditions: “Patients may present to haematologists and physicians with megaloblastic anaemia or to neurologists and psychiatrists with predominantly nervous-system symptoms”. There is often no evidence of either anaemia or macrocytosis and researchers have even found “a significant inverse correlation between the degree of anaemia and the severity of neurological involvement...” However, if either set of symptoms is left untreated, the patients will generally develop the other strand (Reynolds, 2006).

Dr Ralph Green, an established authority on vitamin B12, says: “it became clear that the effects of B12 deficiency were not restricted to the hematopoietic system but were often overshadowed by neurological complications and were sometimes entirely absent. Just as folate deficiency is associated with effects beyond anemia, B12 deficiency also can be associated with nonhematological complications” (Green, 2017).

A reason for this may be that vitamin B12 is implicated in two distinct metabolic pathways in the human body: one leading to haematopoiesis (the formation of blood cells) and one to myelination (the formation of myelin sheaths around nerves) (Solomon, 2007).

Our long experience has shown that anaemia is rarely present in B12 deficiency, and that a patient may be deficient despite having what would commonly be described as a “normal” serum B12 level. In contrast, in the many cases we have encountered, neuropsychiatric and neurological signs and symptoms are far more prevalent and may be the only evidence of a deficiency state which is then confirmed through a therapeutic trial. Modern research is increasingly confirming this finding.

In the early 1980s we were a lone voice, but there is now plenty of evidence to support our view. For example, in a classic study in 1988 researchers found that 28% of 141 patients had no anaemia or macrocytosis and yet clearly had neuropsychiatric signs of B12 deficiency: “We conclude that neuropsychiatric disorders due to cobalamin deficiency occur commonly in the absence of anemia or an elevated mean cell volume and that measurements of serum methylmalonic acid and total homocysteine both before and after treatment are useful in the diagnosis of these patients” (Lindenbaum et al., 1988).

Individual case reports from all over the world confirm this view. Ralph Green writes: “Although considered an ‘old’ disease, new information is constantly accruing about B12 deficiency, the broad array of its effects, and methods for its diagnosis. B12 deficiency primarily affects the hematopoietic system, but its effects extend to other tissues and organs, most notably the nervous system. The spectrum of clinical presentations is broad so that diagnosis depends first on a high index of suspicion and then on the judicious application of appropriate testing” (Green, 2017).

This view is also reflected in the medical textbook *Harrison’s Principles of Internal Medicine* which states:

“Cobalamin [the chemical name for vitamin B12] deficiency without hematologic abnormalities is surprisingly common, especially in the elderly. The risk of a non hematologic presentation for cobalamin deficiency is increased by the folate food fortification because folate can mask the hematologic effects of B12 deficiency. Between 10 and 30% of persons over age 70 years have metabolic evidence of cobalamin deficiency, either elevated homocysteine levels, low cobalamin – TCII levels or both.

Only 10% of these patients have defective production of IF and the remainder often have atrophic gastritis and cannot release cobalamin from their food. Serum cobalamin levels may be normal or low, but serum levels of methylmalonic acid are almost invariably increased due to a deficiency of cobalamin at the tissue level. The neuropsychiatric abnormalities tend to improve and serum methylmalonic acid levels generally return to normal after treatment with cobalamin. Neurologic defects do not always reverse with cobalamin supplementation.”

(Babior & Bunn, 2005, p. 605)

Despite the increasing evidence emerging from medical research, still in recent years health authorities frequently “refuse prescriptions for vitamin B12 in patients with clinical signs of neuropathy because the patients have no haematological sign, and their plasma vitamin B12 levels are reported as ‘normal’” (Smith & Refsum, 2011). This is especially worrying because, as *Harrison’s Principles of Internal Medicine* points out: “An important clinical problem is the nonanaemic patient with neurologic or psychiatric abnormalities and a low or borderline serum cobalamin level. In such patients, it is necessary to try to establish whether there is significant cobalamin deficiency...” (Babior & Bunn, 2005)

2.1.3 Limitations of the blood tests

This brings us to discussion of other hurdles in the diagnosis of vitamin B12 deficiency. It is reasonable to assume that if the blood serum level of B12 is low, then a person has B12 deficiency. The corollary is that if the blood serum level of B12 is within a “normal” range, then a person cannot have B12 deficiency. There are two problems with this assumption: the first is the unreliability of the blood test.

In a letter defending my diagnostic method in 2012, Professor A. David Smith,¹⁷ a recognised expert on the scientific aspects of vitamin B12, described the “uncertain reliability of commercially-available assays for blood levels of B12” (the full text of this letter is provided in Box 2-1 at the end of this chapter on page 62). There is currently only one commercial assay (procedure for assessing the quantity of a substance in a sample) in widespread use in laboratories worldwide for assessing the level of serum B12. This is the chemiluminescence-based assay (Combined Binding Luminescence Assay, CBLA). However, in 2012, a leading B12 researcher Professor Ralph Carmel cast doubt on the reliability of this method in a report showing that CBLA kits gave falsely high readings with blood from patients with pernicious anaemia, with failure rates ranging from 22% to 35% (Carmel & Agrawal, 2012). This was not the first time that such difficulties had been reported. In a letter to the Editor of *Clinical Chemistry* in 2000, Professor Carmel wrote: “We wish to report a serious problem in the ... chemiluminescence assay for cobalamin ... The problem is urgent for two reasons: (a) our findings suggest that many cobalamin-deficient patients are being missed; and (b) the assay is used by increasing numbers of laboratories...” (Carmel et al., 2000).

Examples of patients who were clearly clinically B12-deficient but whose blood tests showed results within the “normal range” can be found in Devalia (2006), and examples of contradictory results produced by different test methods are shown in Hamilton et al. (2006). In 2010, the American Society for Hematology also reported a case of false normal vitamin B12 levels caused by assay error (American Society of Hematology, 2011). Again, in 2016, another study reported only 19% sensitivity of the serum B12 test (Olson et al., 2016). In Japan, where other types of automated competitive protein binding (CPB) assays are used, inconsistency between results produced by three different tests has been reported (Ihara et al., 2008). Many experts now consider that the serum B12 blood test is of “limited diagnostic value as a stand-alone marker” (Hannibal et al., 2016).

The CBLA replaced older microbiologic and radioisotope-dilution assays during the past couple of decades, and may measure more than simply B12. The CBLA test gives reliable readings when measuring test solutions of pure B12 in saline (albeit that it has an upper limit of 2000 ng/L).

¹⁷ Professor A. David Smith is Emeritus Professor of Pharmacology, Department of Pharmacology, Medical Sciences Division, University of Oxford.

However, when testing samples of human blood, it may give “normal” range readings for people who clearly have B12 deficiency symptoms, and who would get a low blood serum B12 under the previous radioisotope dilution assay to confirm their symptoms (Carmel & Agrawal, 2012). What CBLA is binding to instead of B12 is still not known, but we know that people are being told that they have a “normal” (above minimum threshold) level of B12 in their blood (and therefore a diagnosis of B12 deficiency should be excluded) when not only do they clearly have symptoms which indicate B12 deficiency, but B12-replacement therapy (loading dose of B12 by injection) often reverses those symptoms.

The second problem is that the standard B12 blood serum test (in common use) does not differentiate between the active (holotranscobalamin) and inactive forms of B12 so may give a false reading of the B12 actually available for use, described as “functional vitamin B12” (Turner & Talbot, 2009) (see Chapter 1). In the 1980s, a pioneering New York-based physician/scientist Victor Herbert (1927-2002), put forward the view (which is still held although some aspects are disputed – see Golding (2016)), that about 80% of B12 in the body is bound to haptocorrin, a storage protein, and is therefore inactive, while the remainder is bound to Transcobalamin II (this combination is called holo-transcobalamin [HoloTC]), the active fraction which enters the cells for metabolic reaction (Herbert, 1987)). If both are measured, total serum B12 may appear within the normal reference range, despite the important TC-bound fraction being lower (Green, 2017).

In addition to these risk factors, there is no international, or even national (in the UK) agreement about where the cut-off point lies for the B12 in blood below which a patient needs treatment. The cut-off point varies greatly between laboratories worldwide and there is no globally accepted reference range (Tsiminis et al., 2016). The *BMJ Best Practice* suggests the following: >200 picograms/mL¹⁸ probable deficiency, 201-350 pg/mL possible deficiency, >350 pg/mL unlikely deficiency (BMJ Best Practice, 2018d). However, as Professor Smith points out in his letter, serious B12-deficiency symptoms can manifest in patients with B12 levels across the whole normal range.

Under these circumstances, arguments about what the threshold minimum level of B12 should be in the blood become academic. It is better to diagnose B12 deficiency using a combination of signs and symptoms, which should certainly include the blood serum B12 level, but focus mainly on symptoms and family history, and on confirming the diagnosis by a trial of B12-replacement therapy.

2.1.4 Other possible B12 deficiency biomarkers

There are several other methods that can be used to determine vitamin B12 deficiency:

- **Measurement of plasma total homocysteine (tHcy)**
Levels of tHcy increase from the early stages of vitamin B12 deficiency. However, one drawback to using homocysteine levels as a biomarker of B12 deficiency is that high homocysteine levels can indicate other conditions, such as folate or vitamin B6 deficiency or other illnesses.
- **Measurement of plasma methylmalonic acid (MMA)**
Levels of MMA increase with vitamin B12 deficiency. As with tHcy, raised MMA can also be caused by other illnesses but it is more specific to vitamin B12 deficiency than raised tHcy.

¹⁸ See Table 2-1 for pg/mL and ng/L unit equivalences.

The main drawback of this test is its high cost. There is no routine national quality assessment scheme for plasma MMA assays in Britain.

- **Measurement of holotranscobalamin (holoTC)**

As explained above, holoTC is the “active” fraction of plasma B12 and may be an earlier indicator of B12 deficiency than serum B12. A new measurement, The Abbott ARCHITECT Active-B12 assay, has been developed for detecting levels of serum holotranscobalamin which claims to be a more accurate marker of B12 deficiency (NICE, 2015). The appropriateness of this test is, however, disputed (Golding, 2016).

- **Bone marrow aspiration/biopsy** is rarely used.

- **The Schilling test**

This test, introduced in 1953 to determine whether a patient was producing Intrinsic Factor, is now rarely used due to its cumbersome nature and use of a radioactive element (though this was harmless).

There are also arguments against the reliability of all these indicators (Carmel, 2011). It seems, therefore, that “Whatever screening criteria are used, a number of B12-deficient patients will be missed. Therefore, there may be a case for universal vitamin B12 screening [haematinic screening]” (Chui et al., 2001).

In summary, the British Society for Haematology recommends considering plasma tHcy and/or plasma MMA as supplementary tests (if available) and suggests use of holoTC as a more indicative routine test for vitamin B12 deficiency than serum B12 in the future (Devalia et al., 2014). Other experts also recommend a combination of tests (Green, 2017).

2.1.5 Scarcity of guidelines

While guidelines for diagnosing B12-deficiency-induced anaemia can be found in the publications of medical authorities, guidelines for diagnosing vitamin B12 deficiency in the *absence* of anaemia are distinctly lacking. The UK’s National Institute for Care and Excellence (NICE) *Clinical Knowledge Summaries*, for example, a key online reference work for GPs, have no separate entry for vitamin B12 deficiency which is only mentioned in relation to anaemia (NICE CKS, 2018a). The same applies to *NHS* online (NHS, 2016c).

The key question then becomes: how do we diagnose vitamin B12 deficiency in the absence of guidelines which take into consideration the neurological and neuropsychiatric symptoms?

2.1.6 Causes of B12 deficiency

Vitamin B12 deficiency has many causes which can be grouped under the headings of poor dietary intake, increased requirements, malabsorption, other illnesses, and some medications. There are also inborn errors of cobalamin metabolism and gene mutations affecting the B12 absorption pathway (Hannibal et al., 2016). These causes need to be explored in a patient because type and frequency of treatment will depend partly on the cause.

Dietary intake may be the cause where patients follow a vegan or vegetarian diet, or where their diet is unbalanced as in eating disorders or a poor nutritional environment.

Increased requirements occur during pregnancy and while breastfeeding, at times of stress and as a result of other illnesses. Our advice from clinical evidence is that both mother and the developing foetus require optimum amount of vitamin B12 and folic acid to prevent neural tube defects (NTDs) and other related conditions in the newborn. It is good medical practice to screen all would-be mothers three months in advance with routine haematologic screening for vitamin B12 and folic acid and commence physiological supplementation of iron, vitamin B12 and folic acid.

Malabsorption is a prime cause of deficiency. The absorption route is complex and has several steps, damage to any one of which can disrupt the process. Vitamin B12 in food is bound to protein. This bond is broken by gastric acid and enzymes in the stomach which free the vitamin. The free vitamin B12 then binds to a protein called haptocorrin (a glycoprotein formerly known as Transcobalamin I or R-binder) which is made in human saliva and parietal cells in the stomach and which protects the vitamin B12 against stomach acid. As this complex passes into the more alkaline duodenum, pancreatic enzymes destroy the haptocorrin, again freeing the vitamin B12 which now binds to a molecule produced by the parietal cells called Intrinsic Factor (IF). In the ileum, the vitamin B12-IF complex is recognised by special receptors and absorbed into the blood. In the blood it is bound to two carrier-proteins. Some is stored in the liver and the remainder available for immediate use.

Disruption of this process can be caused by:

- chronic gastrointestinal disorders such as Crohn's disease;
- autoimmune reactions such as the production of anti-IF antibodies (as is the case in pernicious anaemia);
- atrophic gastritis (an inflammatory condition) and gastrointestinal surgery;
- lack of pancreatic proteases;
- long-term use of some medications such as antacids, proton-pump inhibitors, diabetic medications and oral contraceptives;
- tapeworm and other infections;
- alcohol abuse and heavy smoking.

(More detailed descriptions are found in Briani et al. (2013)).

Other illnesses Our advice is that the incidence of B12 deficiency is substantially increased in patients with other diseases thought to be immunological in origin, including Graves' Disease, myxoedema, thyroiditis, idiopathic adrenocortical insufficiency, vitiligo and hypoparathyroidism. Anti-IF antibody is usually absent from these patients.

Other causes are nitrous oxide anaesthesia (nitrous oxide inactivates B12 in the body, including brain cells) and smoke inhalation (cyanide poisoning).

2.1.7 Failure to activate B12 – failure of the entero-hepatic system

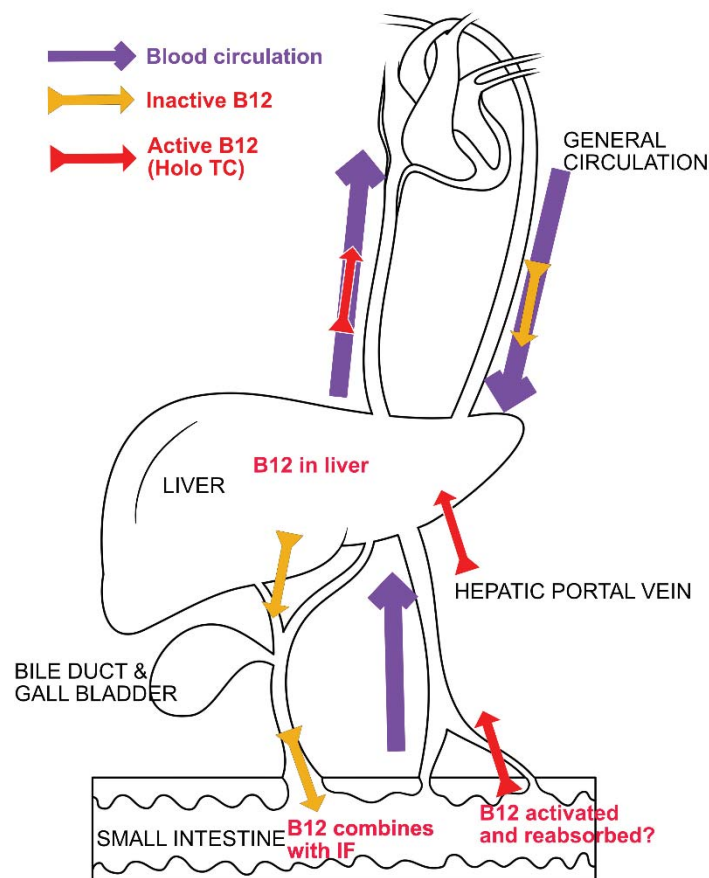
Deficiency can also arise from failure of the entero-hepatic system. As described above, the concepts of "active" and "inactive" B12 were first proposed by Herbert. However, it is not fully known how inactive B12 (thought to be stored in the liver, although this may simply be because there is a lot of blood in the liver) can be converted into active B12 and how this impacts on deficiency. One possible mechanism may be entero-hepatic circulation whereby materials carried in the blood are captured by the liver and passed into the small intestine via the bile duct. From here, they are activated with secreted intestine products (in the case of B12, IF secreted by parietal cells in the stomach) and then

reabsorbed through the intestinal wall into the bloodstream already activated. In this mechanism, the IF “passes” the B12 to Transcobalamin II.

Entero-hepatic circulation appears to be widespread and may serve a number of different functions:

- bile salts are needed in the intestine to help with the digestion of fats/lipids, and are reabsorbed and recirculated after they have done their job;
- expired or degraded haemoglobin may be secreted as bilirubin to assist digestion of fats. A higher proportion of this is not absorbed.

Figure 2-2 Entero-hepatic circulation



Graphic by Hugo Minney

Because the vitamin B12 circulates through this route frequently, if there is impaired absorption, this implies that large quantities could be lost through the gut (O'Leary & Samman, 2010). We suggest this could be one of the major causes of vitamin B12 deficiency, as the body stores are depleted.

2.2 Wide-ranging effects of B12 deficiency

2.2.1 Historical presentations

Before the Second World War, there were four principal presentations (medically recognised classifications) of B12 deficiency. The following classification is based partly on Chanarin's work (1990).

- 1) **Megaloblastic anaemia** (presence of megaloblastic bone marrow precursor cells). Requires bone marrow aspiration, presence of anti-intrinsic factor and anti-parietal cell antibodies, and typically, low serum B12/low serum folic acid/low Hb%
- 2) **Pernicious anaemia** (fatal) resulting in ineffective erythropoiesis. Anti-Intrinsic factor and/or anti-parietal cell antibodies present, low serum B12/ low folic acid and low Hb%
- 3) **Macrocytic anaemia** (macrocytic red cells MCV >100 fL appear in the peripheral blood)
- 4) **Vitamin B12 deficiency** (nutritional cause)

2.2.2 Postwar presentations

With improved nutrition in Britain and many countries after the war, a new set of five presentations were used to diagnose B12 deficiency:

- 1) **Microcytic anaemia** ((MCV <75) B12 deficiency caused by iron deficiency)
- 2) **Macrocytic vitamin B12 deficiency** (with or without anaemia or macrocytosis) subtle B12 deficiency
- 3) **Neuropsychiatric vitamin B12 deficiency** (with or without neuropsychiatric signs and symptoms)
- 4) **Vitamin B12 deficiency – APS** (autoimmune polyglandular syndrome – multisystem polyendocrine failure)
- 5) **Vitamin B12 deficiency – nutritional cause**

All of these categories may present with mild, moderate or severe signs and symptoms, and all appear to be associated with a strong family history (genetic preponderance).

In our experience at the Shinwell Medical Practice, pernicious anaemia (MCV > 97 fL; Intrinsic Factor/parietal cell antibody +ve; low B12; low folic acid) accounts for less than 10% of B12-deficiency presentations today. Note that UK laboratories are no longer routinely carrying out IF/parietal cell antibody tests.

Two more common presentations of anaemia also exist: microcytic (iron deficiency) anaemia and macrocytic (folic acid deficiency) anaemia. These two conditions are treated as follows:

2.2.2.1 *Microcytic anaemia: iron deficiency*

In most cases, iron deficiency (microcytic anaemia) can be easily diagnosed by low levels of blood iron or haemoglobin (Hb%), and easily corrected with oral iron (ferrous sulphate 200 mg TDS * 3/12; severe cases require regular iron IM injections). Because of the way the information on MCV is presented, a combination of low iron and low B12 may result in a normal “average” MCV even though the blood contains both macrocytic and microcytic red blood cells.

2.2.2.2 *Macrocytic anaemia: folic acid deficiency*

Routine haematologic screening identifies folate deficiency which is easily corrected by oral folic acid given daily for 3 to 4 months. However, due to folic acid food fortification in developed countries, folic acid deficiency is encountered much less frequently than previously. For effective DNA synthesis and maturation, both B12 and folic acid are crucial.

Folic acid supplementation can correct the megaloblastic cells in the bone marrow, but this may lead physicians to miss the neurological manifestations of the undiagnosed/untreated B12 deficiency. Hoffbrand and Provan advise that vitamin B12 deficiency should be “excluded in all patients starting

folic acid treatment at these [specified] doses as such treatment may correct the anaemia in vitamin B12 deficiency but allow neurological disease to develop” (Hoffbrand & Provan, 1997).

2.2.3 Neuropsychiatric symptoms presentation

Our experience is that 90% of patients who are diagnosed with vitamin B12 deficiency present with neuropsychiatric symptoms (percentage derived from Shinwell Medical Practice patient survey). More information is given in Chapter 8.

Neuropsychiatric symptoms include: sensory nerve symptoms, such as tingling or numbness; motor nerve symptoms, such as tremors or paralysis; brain symptoms such as “brain fog”, migraine headaches, psychoses. A huge range of presentations is possible as illustrated by the two cases below.

Case 2-1 Neuropsychiatric symptoms and alopecia

Paul Atchinson presented in March 2011 with neuropsychiatric symptoms and extensive alopecia. His B12 level was in the normal range (591 ng/L). Conventional treatment by a dermatologist had no impact. We started intensive B12 treatment. Four months later, in July 2011, his condition was much improved. By August most of his hair had grown back and by November 2011 he had completely recovered.



March 2011



9th July 2011



20th August 2011



3rd Nov 2011

Case 2-2 Lupus, rheumatoid arthritis and Raynaud's disease

Susan Laidler (born 1964) suffered joint inflammation, skin lesions, lip and mouth ulcers and poor circulation (blue fingers). In 2008 her B12 level was 674 ng/L but had dropped to 266 ng/L by March 2011. She was taking two immunosuppressant medications for the rheumatoid arthritis. The ulcers, joint inflammation and all her symptoms disappear when she receives frequent (weekly) injections of vitamin B12, but reappear when she misses an injection or two. She no longer takes the immunosuppressant medicine (of her own volition).



2.3 Our diagnostic method

2.3.1 Seven categories of deficiency

We consider that B12 deficiency can be categorised into seven categories, with four stages. It requires a clinical diagnosis by a clinician (as opposed to overreliance on a single number from a pathology laboratory), and diagnosis between these seven categories may include overlaps and multiple comorbidity.

Treatment may also be complex as absorption/transport/utilisation are complex, and regular reviews/monitoring with blood tests are essential. The proposed Protocol is enclosed as Appendix 1 to this book.

The seven categories that we have observed are:

- I. Clinically significant B12 deficiency (with moderate to severe neuropsychiatric symptoms) B12 level <200 ng/l – with other related features (gastrointestinal, haematological symptoms)
- II. Clinical B12 deficiency (with mild to moderate neuropsychiatric symptoms), a B12 level <200 ng/l with or without related features (such as gastrointestinal or haematological symptoms)
- III. Subclinical cobalamin deficiency (blood serum B12 >200 ng/L) without signs or symptoms
- IV. 'Subtle cobalamin deficiency', that is a subnormal/normal B12 level with some signs and symptoms
- V. Functional cobalamin malabsorption (unable to absorb B12 from food but able to absorb oral B12)
- VI. Transient cobalamin deficiency (condition remains reversed once corrected)
- VII. Dietary B12 deficiency due to a vegetarian or vegan diet, or poor diet (takes 10-20 years to manifest)

2.3.2 Four stages of deficiency

I.	Serum B12 concentration low; no clinical or metabolic abnormalities. Low plasma level of holotranscobalamin II
II.	Increased level of HCY and MMA and low holotranscobalamin II – low B12 level. Damaged metabolism. Neuropsychiatric signs and symptoms with mild haematological changes without anaemia
III.	The plasma and cell stores of B12 become depleted. Serum B12 is low with metabolic abnormalities
IV.	<p>Clinical signs become recognisable (Addisonian criteria)</p> <ul style="list-style-type: none"> a. Macro Ovalocytosis b. Elevated MCV or Erythrocytosis c. Lowered haemoglobin d. Patients presenting with the classical features of pernicious anaemia (PA)/vitamin B12 deficiency would therefore be expected to have progressed through stages I, II and III over several years. Some vegans and patients with malabsorption of food cobalamin may also progress through these stages sometimes over many years, but others may not progress beyond stage I or II. These considerations imply that there are many more individuals in stages I, II, and III of vitamin B12 deficiency than in stage IV (PA).

Low nutritional intake of vitamin B12 may lead to negative balance and finally to functional deficiency when tissue stores of Vitamin B12 are depleted.

Early diagnosis (stages I and II) of vitamin B12 deficiency seems to be useful because irreversible neurological damage may be prevented by cobalamin supplementation at this early stage (Chandy, 2006a).

2.3.3 Our Protocol for diagnosis

Our Protocol (Appendix 1) is based on Signs (including family history and blood values for various components) and Symptoms, which enables a clinician to make a differential diagnosis with a high level of certainty. The method for using the Protocol is as follows:

2.3.3.1 Trigger symptoms

The clinician should be immediately alert if a patient presents with **tiredness, depression, hair loss, pins and needles, numbness in hands or feet, tremors and palsies, palpitations, recurrent headache or dizziness**, and B12 deficiency should be considered. The above symptoms may be marker symptoms for a wide variety of conditions, and differential diagnosis can identify the correct diagnosis.

2.3.3.2 One-minute Health Check

B12 deficiency often occurs alongside other deficiency conditions and degenerative diseases. It is important to perform blood tests in order to ensure a comprehensive diagnosis, so that treatment for co-occurring conditions can be given at the same time as B12-replacement therapy.

Order blood tests for: FBC; Serum vitamin B12; folic acid; TSH; U+Es; LFT; Serum ferritin; Glucose; 8-9am fasting cortisol; vitamin D. This will confirm or exclude the most common conditions found alongside vitamin B12 deficiency. Other appropriate diagnostic tests at this point include parathyroid, pituitary, adrenal and ovarian hormone tests.

Ask the patient to score using the **One Minute Health Check – B12 Deficiency Signs and Symptoms** (Table 2-1 One Minute Health Check on page 58). The patient circles their actual symptom(s) in each group and scores severity of the most severe from 1-10 (where 0=no symptom (leave blank), 5=symptom affects daily life to a moderate extent, all the way up to 10 where the symptom is present all the time and severe and debilitating).

Table 2-1 One Minute Health Check

A quick score will reveal if B12 deficiency, underactive thyroid or iron deficiency anaemia are possible diagnoses, and if the physician should order further tests and commence treatment.

Signs and Symptoms	Score 1-10	Signs and Symptoms	Score 1-10
Energy/ haemopoetic		Cardiovascular/respiratory	
Weariness, lethargy, tiredness, fatigue, faints		Shortness of breath, wheeziness	
Sleepy, tired in the afternoon		Palpitations, chest pain	
Nervous system		Pallor, lemon yellow complexion	
Tremor, foot drop		Bruising, vasculitis	
¥ Loss of balance (ataxia), seizures, falls		Gastro-Intestinal (GI)	
¥ Tingling or numbness in hands and/or feet, burning sensation		Sore tongue, bleeding gums	
Restless leg syndrome		Red beefy tongue	
Facial palsy		Cracking the angles of mouth	
Spastic movements, crampy pain in limbs		Metallic taste, unusual taste, loss of appetite, loss of weight	
¥ Stiffness of limbs, muscle wasting		Gastric symptoms- acidity, heartburn	
¥ Weakness or loss of sensation in limbs, shooting pain in back/ limbs, paralysis		Intermittent diarrhoea, IBS	
Migrainous headache		Skin, hair, nail, skeletal	
Psychiatric		Premature greying	
Irritable, snappy, disturbed sleep		Alopecia, unexplained hair loss	
Confused, memory disturbance/ forgetful, foggy		Joint inflammation, swelling, pain	
Tension headaches		Dry skin, brittle nails	
* Mental slowness, mood swings, anxiety/ panic attacks, depression		Genito-Urinary (GU)	
* Psychosis, hallucinations, delusion		Heavy painful periods, irregular periods, infertility and frequent miscarriages	
Eye, Ear, Throat		Polycystic ovarian disease	
Blurred vision/ double vision/ drooping of eyelid (lid lag), orbital pain		Loss of libido	
Dizziness, tinnitus		Shooting pain from groin to perineum	
Difficulty swallowing, persistent cough		Incontinence	
Immune System		Personal and Family History	
Prone to recurrent URTI, UTI, Respiratory infections		Family history of B12 deficiency (Pernicious Anaemia), underactive thyroid, diabetes, vitiligo, depression	
Other auto-immune conditions		Vegetarian, vegan, poor diet	
Hypoadrenalism, myxoedema/ underactive thyroid		Alcoholism, smoking	

* PHQ9 Patient Health Questionnaire to be completed

¥ Neurological examination and appropriate referral if indicated

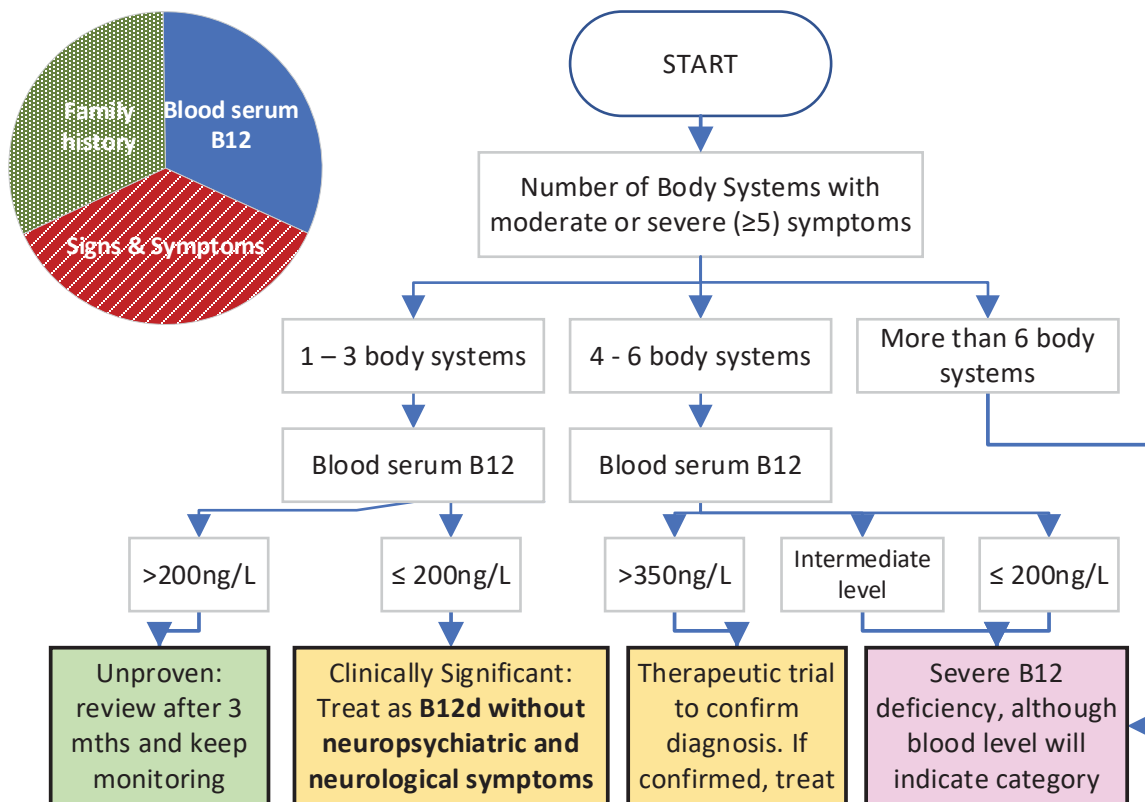
The physician should also order routine blood tests, including serum B12, in the following cases:

- ME, CFS, fibromyalgia, hypoadrenalism, MS-like presentation;
- Children born to B12-deficient mothers, presenting with behavioural problems, learning disability, dyspraxia, dyslexia and autistic spectrum disorders.

Before making a provisional diagnosis of B12 deficiency, exclude all other possible diagnoses, with appropriate blood tests as clinically indicated.

Refer to the Decision Tree below for diagnosis and treatment and await blood test results if appropriate. Note that for patients with severe signs and symptoms, treatment may need to be initiated *without waiting* for the results of blood tests.

Figure 2-3 Decision Tree



2.3.3.3 Blood serum B12 - once blood results are available:

Blood tests are categorised as follows when combined with signs and symptoms indicative of B12 deficiency:

Blood serum B12 nanograms per litre (ng/L)	Blood serum B12 nanomole/millilitre (nmol/ml)	Classification
< 200 ng/L	< 148 nmol/ml	Clinically significant/ severe B12 deficiency
200-350 ng/L	148 – 259 nmol/ml	Moderate deficiency
>350 ng/L	> 259 nmol/mL	“Subtle” (subnormal/low normal blood serum B12 but with signs and symptoms)

Note: picograms per millilitre (pg/ml) are the same as nanograms per litre (ng/L) (100 picograms/millilitre = 0.1 nanograms/millilitre), so the numbers are the same for the same classifications of condition.

Results of other blood tests: many conditions are commonly found alongside vitamin B12 deficiency, and should be treated in the normal manner at the same time as administering B12-replacement therapy. See also the hypoadrenalism (Addison’s disease or adrenal insufficiency) treatment protocol in Appendix 2.

2.3.4 Therapeutic trial

In addition to these classifications, patients can be assigned to a therapeutic trial (to confirm a suspected diagnosis) or prophylaxis (where the clinician has evidence to suggest this is needed to prevent symptoms developing or getting worse). For example, if the patient is diagnosed as having moderate or subtle deficiency (>180ng/L or >200ng/L with signs and symptoms, other autoimmune condition or family history) then they should be clinically reviewed every 4 weeks until the clinician reaches a clinical decision whether to commence treatment – even when the B12 level does not drop below 180-200ng/L. A deterioration of condition demonstrated by signs and symptoms is sufficient to commence a **therapeutic trial**.

2.3.5 Prophylaxis of vitamin B12 deficiency

In the following instances, B12-replacement therapy should be instituted as a prophylactic measure (to prevent further deterioration or even development of symptoms) regardless of blood serum B12 concentration: Prophylaxis is expected to continue for life.

- 1- **Specific medical history** renal imbalance, diabetes, >65 years old, or following GI surgery, Crohn’s colitis, early onset dementia
- 2- **Moderate/subtle B12 deficiency with mild signs and symptoms**
- 3- **Moderate/subtle B12 deficiency with severe signs and symptoms:** patient presenting with strong family history, presence of other autoimmune conditions, major signs and symptoms which could become irreversible if treatment is not commenced urgently e.g. optic neuritis/neuropathy, sudden onset blindness, Subacute Combined Degeneration (SACD), ME, CFS, MS-like presentation, single limb paralysis, sudden loss of muscle mass (Motor Neurone Disease-like presentation), non-

epileptic seizures, dysphagia, Bell's Palsy/Ramsey Hunt syndrome, Parkinson's like presentation, dementia, total alopecia, migrainous headache, temporal arteritis, recurrent miscarriages, dysfunctional uterine bleeding, or psychosis.

2.3.6 Other actions to take

- If clinical depression is suspected – complete PHQ9 and treat/refer as appropriate
- Neurological manifestation – neurological examination and refer to neurologist for further investigation

Provisional diagnosis of any other condition – refer to appropriate speciality.

Box 2-1 Document: Letter from Professor A. D. Smith

Comments on Allegations in Annex A (ES/C1-701040697) regarding Dr J. Chandy

**By Professor A. David Smith,
Emeritus Professor of Pharmacology, University of Oxford**

29 November 2013

I have been asked to comment on Allegations 1, a to f, excluding d. I have done this after reading the relevant sections of Dr Tidy's report of 6 October 2013.

I note that Dr Chandy is a very experienced GP who over the years has developed a particular interest in pernicious anaemia and vitamin B12 deficiency. My comments will be confined to scientific aspects of these conditions since I am not able to comment upon the specific claims about particular patients as I am not medically qualified. One area of my research since 1995 has been in the field of B vitamins and in particular B12 (cobalamin) in human health. I have become a recognised expert in this field and, for example, have been invited to write editorials in medical journals on B12 and to serve on an expert panel of the National Institutes of Health in Washington. I therefore consider myself qualified to make the comments below.

The allegations concern the use of vitamin B12 replacement therapy in patients who were thought to be B12 deficient. It should be recognised that severe B12 deficiency, such as pernicious anaemia, is a fatal disease. It is first necessary to give some scientific background before I can comment on the allegations.

The scientific and clinical challenges in this field can be divided into four categories:

1. The uncertain reliability of commercially-available assays for blood levels of B12
2. The introduction of more specific assays for the functional status of B12
3. The uncertainty about where the cut-off value lies for these assays in order to initiate treatment with B12
4. The variable response of patients to B12 treatment

1. The uncertain reliability of commercially-available assays for blood levels of B12

Three main methods have been used to measure the concentrations of B12 in body fluids: the radioisotope dilution assay (RIDA); the chemiluminescence-based assay (CBLA); and the microbiological assay. Only the first two methods have been used commercially but the microbiological method is widely considered to be the gold-standard and is used in research laboratories, including my own. There have been serious problems with both commercial assays. In the late 1970's it was found that the RIDA method sometimes gave falsely high values and so failed to identify B12 deficiency. The method has since dropped out of use, partly for this reason but mainly because of the need to use radioactive isotopes. The CBLA methods are used world-wide from kits supplied by several manufacturers. In the early 2000's occasional reports began to appear that the CBLA method was giving falsely high results and so missing B12 deficiency. This situation came to a dramatic conclusion in 2012 when one of the leading B12 researchers (Professor Ralph Cannel from New York) published a report in the *New England Journal of Medicine* showing that all 3 manufacturers' CBLA kits gave falsely high readings with blood from patients with pernicious anaemia, with failure

rates ranging from 22 to 35%. The manufacturers have as yet not responded satisfactorily and this leaves the clinician with a major problem: how is he to assess the B12 status of his patients who he suspects are deficient?

2. The introduction of more specific assays for the functional status of B12

It has been known for more than 30 years that two blood markers exist that reflect the body's functional status of B12: homocysteine and methylmalonic acid. Methods for measuring these markers are available, but only that for homocysteine is widely available. While the levels of these markers are raised in B12 deficiency, they are not widely used since homocysteine is not specific to B12 and methylmalonic acid assays are expensive and still only found in research laboratories. So, at present, these markers are mainly used in research settings and in the UK they are not available on the NHS. More recently, an assay method for so-called 'active-B12' has been introduced and is commercially available. Active-B12 is the form bound to a protein called transcobalamin and it is this complex which is taken up into cells of the body. Active-B12 only comprises about 20% of the total amount of B12 in the blood. In time, the Active-B12 method may become the method of choice for determining a patient's B12 status, but at present the expert advice is that more research is needed to establish its validity.

3. The uncertainty about where the cut-off value lies for these assays, in order to initiate treatment with B12

A clinician needs to know a value for the B12 level in blood below which he can say that a patient needs treatment with B12. The original cut-off value and still the most widely used internationally, was 148 pmol/L, which is equivalent to 200 pg/mL (the conversion factor from pg/mL to pmol/L is 0.738). This cut-off value identifies most patients with pernicious anaemia and at this level the characteristic haematological signs are usually found, which has led to *the view that the haematological signs are a requirement for a diagnosis*. However, it is now well-documented that patients can have pernicious anaemia without the typical haematological signs, but will show neurological and/or psychiatric signs. In a classic paper in 1988, Lindenbaum et al. found that 28% of 141 patients had no anaemia or macrocytosis and yet clearly had neuropsychiatric signs of deficiency. Since then, there have been many reports that stated that haematological signs should not be a requirement for a diagnosis of B12 deficiency. Thus, the cut-off of 200 pg/mL, originally defined on haematological grounds, has to be reconsidered in case patients with higher blood levels will suffer neurological harm. In an authoritative review, Herrmann and Obeid (2012) found that a high proportion of patients defined as B12 deficient by functional criteria had serum B12 levels above 200pg/mL They concluded "*No single parameter can be used to diagnose cobalamin deficiency. Total serum cobalamin is neither sensitive nor is it specific for cobalamin deficiency. This might explain why many deficient subjects would be overlooked by utilizing total cobalamin as status marker.*" The BMJ Best Practice Guidelines for B12 deficiency (2013) propose the following cut-off values:
< 200 pg/mL probable deficiency
201-350 pg/mL possible deficiency
> 350 pg/mL unlikely deficiency

In a recent editorial in *Journal of Internal Medicine* I reviewed the evidence that people with B12 levels above 200 pg/mL and up to about 500 pg/mL were at risk of a variety of harmful outcomes. In particular, in a paper in *Neurology* in 2008 we showed that B12 levels across the whole normal range were associated with atrophy of the brain, the atrophy being the more rapid as the levels fell towards the traditional cut-off of 200 pg/mL.

4. The variable response of patients to B12 treatment

It is well known in clinical practice that patients with B12 deficiency show widely varied responses to

the same treatment doses (see the web site of the Pernicious Anaemia Society). One of the early reports in the medical literature was by Tudhop et al. (1967) who stated: *“It is impossible to foretell, from clinical and haematological examination at the time of diagnosing pernicious anaemia, which patients will have a prolonged elevation of serum vitamin B12 of 1-2 years, and which will relapse quickly in 3-4 months following an injection of 500 pg. of hydroxocobalamin..They concluded: “Variation between patients makes it impossible to anticipate the duration of effect of a single injection of one of these drugs in any patient.”*

Comments on the specific allegations

- A. I cannot comment upon allegation ‘1f’ since this is clearly a matter of how a child is defined in the NHS.
- B. The other allegations, Ta,b,c and e’, all relate to the prescribing of vitamin B12 to treat symptoms consistent with B12 deficiency.
- C. Dr Tidy (page 335) considers that Dr Chandy should not have prescribed B12 to patients whose blood level was in the normal range, i.e. above 200 pg/mL. In my background material above I hope I have made it clear that there is no consensus in the field about what is an appropriate cut-off value for prescribing. Furthermore, the validity of the results of current methods for B12 is open to question and so a clinician has to use his/her judgement in making a decision about prescribing, according to the nature and severity of the symptoms and signs.
- D. Dr Tidy (page 336) expresses the opinion that Dr Chandy should have referred three patients with neurological symptoms for further investigation by specialists before he started treating them. In my view, this would have risked harming the patients because some of the neurological consequences of B12 deficiency are irreversible and it is good practice to treat the patient as soon as possible. I found statements to support this view in the medical literature, including the following:
 - a. “Empirical treatment, to assess any clinical response and to prevent neurological damage, may be pragmatically justifiable as the dangers of treatment are not as devastating as those of not treating”(Devalia, BMJ 2006).
 - b. “A therapeutic trial of cobalamin will prevent delay in treatment and adverse clinical consequences.” Hamilton & Blackmore BMJ, 2006)
 - c. “... our experience further supports the fact that when the diagnosis of B12 deficiency is suspected on the basis of clinical findings and additional tests, supplementation treatment should be administered even if the assayed level of the vitamin is not low..(Scarpa et al. Blood Transfusion 2012.)
 - d. “B12 assays may be vulnerable to interference resulting in normal values despite severe cobalamin deficiency. Where there is discordance between the clinical features of neuropathy - parasthesiae, loss of joint position sense, or megaloblastic anaemia and a “normal” B12 result, clinicians are advised to request storage of serum for further testing and are advised to treat the patient with B12 replacement therapy... .Treatment with B12 should not be delayed to avoid progression of neurological damage.” (UK NEQUAS, 2013)
- E. Dr Tidy (page 337) says that in his opinion a ‘rigorous clinical trial would be indicated’. In my view, this would not at all have been in the interests of Dr Chandy’s patients. The nature of their symptoms were such that to randomise them to a placebo tablet would have been unethical.

Furthermore, the great variability in the responses of patients with B12 deficiency to replacement therapy would have made such a trial scientifically invalid unless it was done on a very large scale, way outside the scope of a GP practice.

- F. My overall conclusion with regard to the prescribing of vitamin B12 by Dr Chandy is that he has put the welfare of his patients first and has treated them as best he could with regard to timing and the dose used in the context of the considerable uncertainties about the scientific basis for treatment decisions.

