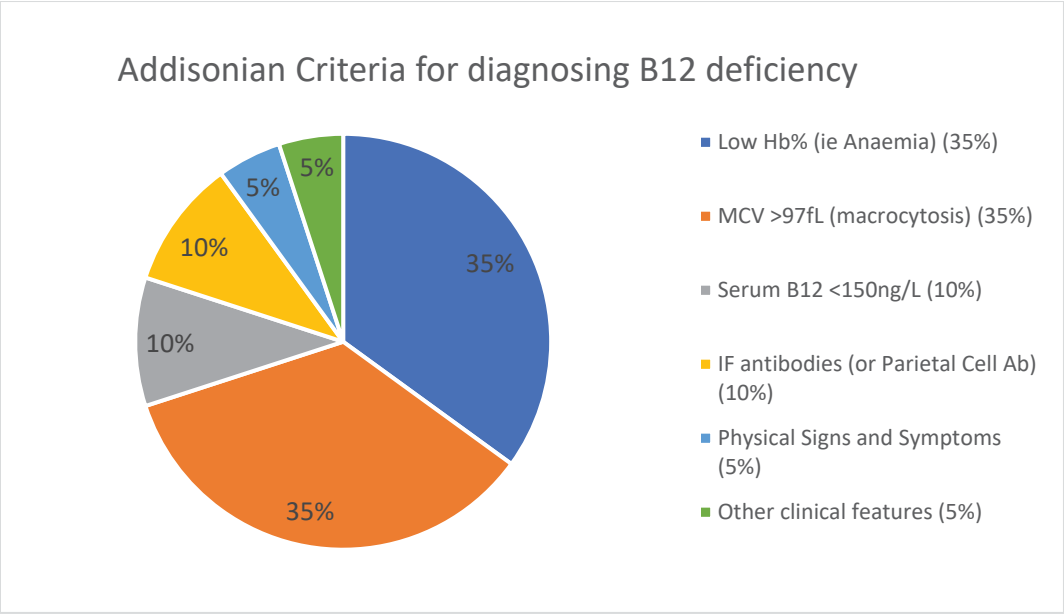
Chapter 4 Megaloblastic anaemia – not the only way to diagnose B12 deficiency

Remember, dear brothers and sisters, that few of you were wise in the world's eyes or powerful or wealthy when God called you. Instead, God chose things the world considers foolish in order to shame those who think they are wise. And he chose things that are powerless to shame those who are powerful. God chose things despised by the world, things counted as nothing at all, and used them to bring to nothing what the world considers important. As a result, no one can ever boast in the presence of God.

1 Corinthians 1:26-29

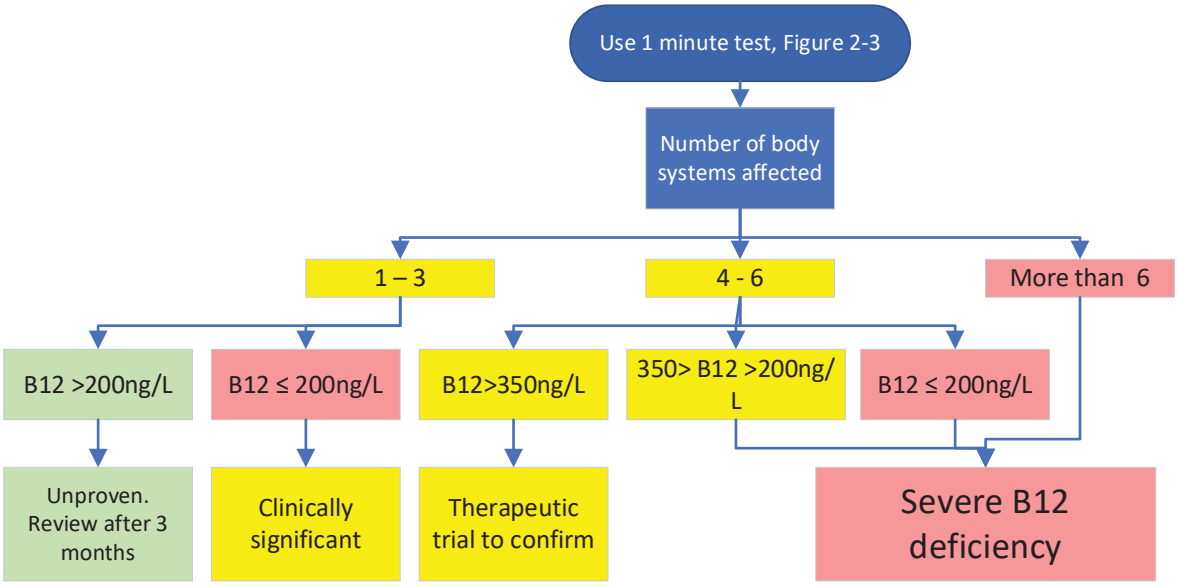
Figure 4-1 The Addisonian Criteria for diagnosing megaloblastic anaemia



Optional additional tests

- Schilling test (radioactive materials used to determine whether B12 can be absorbed – not usually permitted because of radioactivity)
- uMMA (urinary Methyl-Malonic Acid)
- Plasma homocysteine
- Transcobalamin II estimate (sometimes called "active B12 test")
- Bone marrow examination

Figure 4-2 Chandy criteria for diagnosing vitamin B12 deficiency



4.1 B12 deficiency and haematopoiesis (formation of blood cells)

As explained in Chapter 2, vitamin B12 is essential for multiple body systems which explains the many varied symptoms of deficiency. In this chapter we consider the effect of vitamin B12 deficiency on the blood where it manifests as megaloblastic anaemia. This is a blood cell maturation disorder which results from impaired DNA synthesis. In this condition, red blood cells (RBCs) are enlarged (macrocytic) and other blood cell changes occur. The impairment of RBC formation reduces the oxygen-carrying capacity of the blood and leads to the typical symptoms of anaemia, including fatigue, weakness and other acute disorders.

Megaloblastic anaemia can result from any cause of vitamin B12 deficiency. It can also result from folate deficiency or some other disorders, but B12 deficiency is the leading cause. Where it results from autoimmune attack on stomach cells (which leads to lack of secretion of Intrinsic Factor – IF – necessary for the absorption of vitamin B12) it is known as "pernicious anaemia" (PA). This illness has historically been the condition most associated with vitamin B12 deficiency, to the extent that the two conditions have been viewed as almost synonymous. This is so much so that in classical medicine, vitamin B12 deficiency is diagnosed through the symptoms of PA (see Chapter 2). What we wish to emphasise in this book, however, is that today vitamin B12 deficiency presents far more frequently with neurological or neuropsychiatric symptoms *without* anaemia and that PA is relatively rare.

In this chapter we go further and propose a new view of PA, suggesting that rather than being a cause of B12 deficiency, PA is an end-stage manifestation of this condition and can be prevented by early recognition of the neurological/neuropsychiatric symptoms. We investigate this issue and also draw attention to other aspects of the relationship of PA to B12 deficiency which are not usually well communicated to GPs.

4.2 What is megaloblastic anaemia?

There are many types of anaemia. In general, anaemia is defined as too few or abnormal RBCs (also known as erythrocytes), or too little haemoglobin (the pigment in red blood cells which carries oxygen). An analysis by Kassebaum et al. showed that in 2010 mild, moderate and severe anaemia had a worldwide prevalence of 32.9% which indicates that it is a serious health problem (Kassebaum et al., 2014). Anaemia results in insufficient oxygen delivery to the tissues, causing symptoms such as fatigue, frequent headaches, shortness of breath, pale skin, loss of appetite and mood changes. The symptoms vary according to the type of anaemia.

The BMJ Best Practice (BMJ Best Practice, 2018c), quoting World Health Organisation data, states that anaemia is defined as a haemoglobin (Hb) level <12 grams per decilitre (g/dL) in females and <14 g/dL in males, or as a Hb level <12.5 g/dL in adults. The BMJ also says that it is the most common haematologic disorder seen in general medical practice.

Anaemias can be classified in different ways. Under one classification system, the most common is iron-deficiency anaemia, with other important groups being vitamin-deficiency anaemia (including vitamin B12 and/or folic acid deficiency anaemia), aplastic anaemia, haemolytic anaemia, sickle cell anaemia and anaemia caused by other diseases (American Society of Hematology, 2018).

When organised by the functional defect in red cell production, there are three main classes of anaemia: marrow production defects (hypoproliferation); red cell maturation defects (ineffective

erythropoeisis); and decreased red cell survival (blood loss/hemolysis). Megaloblastic anaemia comes in the second category in this list.

In megaloblastic anaemia, the deficiency of vitamin B12 or folate, which leads to a lack of the components required for DNA synthesis, means that RBCs do not form properly in the bone marrow (Green, 2017). They appear as malformed immature cells (known as megaloblasts) seen both in the bone marrow and in the blood. The malformations include the retention of a nucleus (healthy RBCs shed their nucleus as they mature) and an oval shape rather than a doughnut-shape which results in a cell size larger than normal (macrocytic = mean corpuscular volume (MCV) > 97fL). The nucleus in these cells is more immature than the cytoplasm, producing nuclear/cytoplasmic asynchrony (Aslinia et al., 2006). The cells may also have a shorter life than normal RBCs (120 days) which means there are fewer of them than in a healthy person because the cells are released before they have matured enough to divide. The incomplete DNA synthesis affects all cells but is most pronounced in the RBCs. Other changes in the blood include hypersegmented neutrophils (with up to six lobes) and a reduction in the number of granulocytes (white cells) and platelets (Hoffbrand & Provan, 1997).

Harrison's Principles of Internal Medicine (2008) explains:

> *"The megaloblastic anaemias are a group of disorders characterised by a macrocytic anaemia and distinctive morphological abnormalities of the developing haemopoietic cells in the bone marrow. In severe cases the anaemia may be associated with leucopenia and thrombocytopenia. Megaloblastic anaemia arises because of the inhibition of DNA synthesis in the bone marrow, usually due to deficiency of one or other of two water-soluble B vitamins, vitamin B12 (B12 cobalamin) or folate. B12 deficiency may also cause a severe neuropathy but whether this occurs with folate deficiency is controversial. In a minority of cases, megaloblastic anaemia arises because of a disturbance of DNA synthesis due to a drug or a congenital or acquired biochemical defect that causes a disturbance of B12 or folate metabolism or affects DNA synthesis independent of B12 or folate" (Hoffbrand, 2008).*

4.2.1 Effect of macrocytosis – enlarged Red Blood Cells

Macrocytic cells are defined as cells with a Mean Corpuscular Volume (MCV) [21] greater than 80-95 femtolitres (fl) in adults, which is detected in a blood count (Hoffbrand & Provan, 1997). They are found relatively frequently and do not always indicate illness, unless there are other signs and symptoms (Aslinia et al., 2006).

RBCs carry oxygen from the lungs to the rest of the body (wherever it is needed), and carbon dioxide back to the lungs. They do this through the haemoglobin molecule, which is what gives blood its red colour. RBCs need to be able to absorb and release oxygen and carbon dioxide at a fast rate in response to activity. A healthy RBC has a doughnut shape – thin in the middle and thick around the outside, which gives it a greater surface area-to-volume ratio than a sphere or ball shape. This means that the cytoplasm, containing the haemoglobin which transports oxygen, is as close as possible to the cell membrane at all times. In order to get this shape, the cell has lost its nucleus. In

[21] MCV (fl) = [Hematocrit (per cent) x 10]/[TBC count (10^6/µL)] (Aslinia et al., 2006)

humans, the healthy RBC has an average MCV around 90 femtolitres (fL). In megaloblastic anaemia, the diameter of the RBC is still 6 – 8 µm, but the MCV can be up to 150 fL.

If the RBCs are either too large or too small or differently shaped, neither oxygen nor carbon dioxide can be effectively transported. This leads to lack of energy and even muscle pains. How many B12-deficiency sufferers have experienced these?

4.2.2 Causes of megaloblastic anaemia

The vitamin B12 deficiency which leads to megaloblastic anaemia can have many causes. These include any condition which disrupts the B12-absorption pathway, such as intestinal diseases, atrophic gastritis (chronic inflammation of the stomach which increases with age and reduces the production of acid and enzymes needed for digestion as well as of intrinsic factor), gastric surgery, pancreatic insufficiency, bacterial overgrowth, medications which inhibit production of stomach acid (e.g. antacids and proton-pump inhibitors), or autoimmune attack on stomach cells (PA). Other factors leading to deficiency include low dietary intake and genetic predisposition to deficiency. PA has been assumed to be the most common cause in elderly patients, but researchers have shown that food malabsorption rather than PA may be the leading cause: "If an image of an elderly patient with pernicious anaemia is the first thing that comes to mind when you think of B12 deficiency, take note: That image could obfuscate a more common case of B12 deficiency—one caused by food-B12 malabsorption" (Andres et al., 2007).

Megaloblastic anaemia can also be caused by folate deficiency and some other disorders, but vitamin B12 deficiency is the most common cause (Rosenblatt & Fowler, 2006). Folate/folic acid deficiency can have a dietary cause or can be induced by vitamin B12 deficiency because of the interaction of the two vitamins (see Chart 5-1 on page 113, and Chart 5-2). Other possible causes of megaloblastic anaemia are drug damage, congenital defects and myelodysplasia (a type of cancer) (Babior & Bunn, 2005, pp. 604-605).

4.2.3 Pernicious anaemia

As stated above, when the cause is specifically autoimmune attack on stomach cells, or on cells which secrete Intrinsic Factor (IF – essential for B12 absorption), the illness is called "pernicious anaemia" (PA). An autoimmune condition is where the body's defence mechanism, intended to destroy foreign cells and so prevent infection or disease, turns on the body's own cells and destroys them. Note that atrophic gastritis can also be an autoimmune condition (Minalyan et al., 2017).

The autoimmune attack in PA is caused by several types of antibodies which affect production of gastric acid necessary for separating cobalamin from food, and production of IF, which is needed for cobalamin absorption. (For further information on the B12-absorption route in the human body, see Chapter 1.) In PA, the production of gastric juice is much reduced and contains no or little hydrochloric acid (a condition known as achlorhydria) (Chanarin, 2000).

Anti-gastric parietal cell (GPC) antibodies attack principally the gastric enzyme H^+/K^+ -ATPase proton pump which regulates hydrochloric acid secretion in the stomach. Two types of anti-IF antibodies are known. Type I IF^+ blocks the binding of vitamin B12 to IF (thus preventing the formation of the B12/IF complex) while Type II IF^+ binds to the B12/IF complex, preventing it being absorbed across the intestinal wall (Andrès & Serraj, 2012). Since both hydrochloric acid and IF are required for the

proper digestion and absorption of B12, people with this condition are usually unable to absorb B12 in the quantities needed to maintain health.

The UK Pernicious Anaemia Society (PAS) explains: "Parietal cells are found in the lining of the stomach. As well as producing Intrinsic Factor, parietal cells also produce hydrochloric acid. Intrinsic Factor is essential for B12 absorption whilst hydrochloric acid allows B12 to be released from food. Parietal Cells may fail due to infection (from *Helicobacter pylori*, for example) or because the body produces antibodies that kill off the parietal cells – parietal cell antibodies" (PAS, 2018c).

It is thought of as a disease of the elderly: the average patient presentation is around 60 years old. However, it is seen in other ages, and even in children - typical PA symptoms can be seen in children under 10 years old (juvenile PA).

PA develops gradually as the body's stores of B12 are used up. Typical common symptoms include shortness of breath, extreme fatigue, lack of coordination, brain fogs, brittle nails and dry skin. Many other symptoms are also seen in increasing degrees of severity. The effects of this debilitating condition on patients have been described in detail by Martyn Hooper, Chairman of the Pernicious Anaemia Society, in his book *What you need to know about Pernicious Anaemia and Vitamin B12 Deficiency* (Hooper, 2015).

4.3 Delays in diagnosis via the "Addisonian criteria"

PA is traditionally diagnosed through the Addisonian criteria described in Chapter 2. Although well established, these criteria have a number of serious drawbacks and delays in diagnosis are common as demonstrated in a survey of PA patient experience of diagnosis and treatment in the UK conducted between 2010 and 2012 (Hooper et al., 2014). A total of 889 patients registered with the PAS completed an online survey or postal questionnaire. The results showed that: one-third of patients experienced symptoms for up to one year before diagnosis and 14% waited more than 10 years for a diagnosis! Neurological features – which should have triggered prompt diagnosis - were highly prevalent, the most common being memory loss and poor concentration. Other findings were (PAS, 2018a):

- 44% were initially wrongly diagnosed as having some other problem
- 22% had to wait 2 years for a correct diagnosis
- 19% for 5 years
- 4% for 10 years
- 14% waited 10 years or more.

In answer to the frequent question "Why did it take so long for me to be diagnosed" in its Patients' FAQ, the PAS says:

> *"There are three main reasons. Firstly, doctors don't specifically look for B12 deficiency and all too often believe that the symptoms patients complain of are associated with other diseases. Where doctors actively look for B12 deficiency they diagnose many more cases of B12 deficiency than doctors who aren't actively looking for the deficiency.*

Secondly there are problems associated with the current test used to measure the amount of B12 in the patient's blood. [See Chapter 2 and below.]

Thirdly, the test used to find out if the B12 deficiency is caused by Pernicious Anaemia also appears to be thoroughly flawed [See explanation below.]" (PAS, 2018b).

On the face of it, PA appears easy to diagnose and confirm. The small intestine requires Intrinsic IF to absorb B12, and this is produced by parietal cells (gastric cells) in the stomach wall. Where IF is not being produced, it is because the parietal cells are damaged and it should be easy to detect either IF antibodies (IF^{+ve}), or parietal cell antibodies (PC^{+ve}).

The challenge, as with so many tests, is the number of false negatives (when the result of the test indicates that there is no problem, or excludes a diagnosis). Unconfirmed reports indicate that perhaps only one-third of people with obvious symptoms of PA actually get a positive result for IF antibodies (IF^{+ve}), so we believe that many people fail to get a diagnosis because of a "false negative" (i.e. IF^{-ve}), and therefore do not get the treatment they need.

4.3.1 Drawbacks of the Addisonian Method

4.3.1.1 Macrocytosis – not a specific marker

The normal diagnostic tools are a Complete Blood Count (CBC) and a peripheral blood smear. The most common haematological signs of megaloblastic anaemia are the presence of macroovalocytes and hypersegmented neutrophils on peripheral blood smears (Andrès & Serraj, 2012). However, there are many other causes of macrocytosis which can occur without anaemia. So macrocytosis alone is far from being a sufficient indicator of B12 deficiency. Macrocytic anaemias are grouped into megaloblastic and non-megaloblastic types. If macrocytic cells are found, further tests are needed to determine the cause (Nagao & Hirokawa, 2017).

Other signs in the blood can help to confirm the diagnosis, such as neutropenia (abnormally low levels of neutrophils), thrombocytopenia (an abnormally low level of platelets), pancytopenia (low blood counts for both red and white cells and platelets), intramedullary haemolytic component (see, for example, Khalil et al. (2012)) and pseudothrombotic microangiopathy[22] (Andrès & Serraj, 2012). A case of pseudothrombotic microangiopathy due to severe vitamin B12 deficiency, for example, is described in Veit (2017).

4.3.1.2 Serum B12 blood test not reliable as a stand-alone marker

As described in Chapter 2, the method for measuring serum B12 in common use is not completely reliable although it can be a used as an indicator in conjunction with other markers. Another drawback of the test is that it measures all B12 in the blood, rather than just the bioactive B12 which is the significant portion. In addition, there is no agreed national or international standard reference range for B12 levels. Finally, it is our experience that many patients whose B12 blood level is "normal" nevertheless manifest clinical symptoms of deficiency, suggesting that requirement differs

[22] Pseudothrombotic angiopathy is anemia, thrombocytopenia (abnormally low platelet level), and schistocytosis (circulating red blood cell fragments) caused by vitamin B12 deficiency.

by person (see, for example, case showing that "normal cobalamin serum levels do not rule out a cobalamin deficiency" in Roessler and Wolff (2017)).

4.3.1.3 IF⁺ and GPC⁺ antibodies – tests not totally reliable

Antibodies present in the blood usually indicate the presence of a pathogen (a disease-causing organism that should not be there). The body creates antibodies to attack the pathogen, and antibodies are very specific – they only attack exactly what they are designed to attack. Because the antibodies are so specific, if they can be detected, they are considered a reliable indication of whether the pathogen is present, or has been present recently.

The presence of antibodies (Ab^+) to either parietal cells or IF (Parietal Cell Ab+ (PC^+) or IF Ab+ (IF^+)) shows that vitamin B12 deficiency is probably due to absorption issues. This is a useful diagnostic criterion to confirm vitamin B12 deficiency.

However, the results for tests for antibodies are not totally reliable. For IF^+, a negative result (lack of the presence of antibodies) is true for everyone who does not have the condition (it is *specific*), but *sensitivity* (the percentage of people who are actually suffering from the condition but reported falsely as "negative") is reported to be only about 37-50% (Andrès & Serraj, 2012) or 40-60% (Devalia et al., 2014) so a "negative IFAB assay does not therefore rule out pernicious anaemia [vitamin B12 deficiency]" (Devalia et al., 2014). The equivalent figures for anti-GPC antibodies are higher, at 81.5% and 90.3% (Andrès & Serraj, 2012) but this test is less useful because it also shows 10% positivity in healthy individuals. Therefore, "a positive GPC antibody test is not definitive for pernicious anaemia" (Khan et al. (2009) cited in Devalia et al. (2014))".

A common reason why doctors do not diagnose B12 deficiency is because B12 deficiency is progressing down a different route, e.g. neurological pathology. It is also possible that a diagnosis may be refused even though anti-GPC antibodies are present, because the doctor was looking for anti-IF antibodies.

We note that the British Society for Haematology (BSH) **Guidelines for the diagnosis and treatment of cobalamin and folate disorders** begin by saying that the clinical picture should be "the most important factor in assessing the significance of test results assessing cobalamin status because there is no 'gold standard' test to define deficiency" (Devalia et al., 2014). The Guidelines advise (quoting Carmel et al. (1996)): "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". They also state: "Oval macrocytes, hypersegmented neutrophils and circulating megaloblasts in the blood film and megaloblastic change in the bone marrow 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)."

4.3.2 Landmark case did not show macrocytosis or IF-antibody

The patient whose condition initially started me on my voyage of discovery of the widespread effects of vitamin B12 deficiency (Glenise Mason, Case 5-7, also described in the Introduction) is an illustration of the above points. Her blood test did not show either macrocytosis or anti-IF antibody; in fact, it showed *micro*cytosis (RBCs smaller than normal - indicative of iron-deficiency anaemia). It was her pallor, neuropsychiatric and other symptoms that alerted me to the possibility of B12

deficiency. Because there were no classical signs of B12 deficiency, the laboratory was reluctant to do a B12 blood test. After some considerable persuasion, they finally agreed to do the test which showed her B12 level to be very low at only 185ng/L. I therefore diagnosed B12 deficiency and instituted B12-replacement therapy by injection. Her B12 level rose, the *micro*cytosis resolved and her haemoglobin level normalised.

From that time (the early 1980s), realising the inadequacy of the classical guidelines, I began to develop other means of diagnosing vitamin B12 deficiency. With experience, I became able to diagnose B12 deficiency at an early stage of its development, through neurological and neuropsychiatric signs and symptoms. These were subsequently confirmed either by a serum B12 blood test alone, or (where the serum B12 level was above the level considered by the NHS as "normal") by a therapeutic trial.

As a result of this approach, from then onwards I encountered no cases of PA. I continued to order full blood count tests for patients whom I suspected had vitamin B12 deficiency but these regularly did not show macrocytosis. Nevertheless, their B12 level on presentation had been low as demonstrated subsequently either by a serum B12 test or by their favourable response to a therapeutic trial. For a while, I also ordered anti-IF antibody tests because these were required at the time but as these *always* proved negative (because I was identifying cases of B12 deficiency before the autoimmune reaction had developed), I eventually abandoned them as unnecessary. (The anti-IF antibody test is expensive so this also resulted in some saving to the NHS.)

As there are generally no effective guidelines for diagnosing vitamin B12 deficiency without anaemia, we developed our own which are given in the **Protocol for excluding B12 deficiency (Megaloblastic anaemia/pernicious anaemia) from adult and child patient presentation** (provided in full in Appendix 1)**.**

Our method for diagnosing vitamin B12 deficiency, including megaloblastic anaemia, shown in Figure 4-2 at the head of this chapter, is straightforward and accords much more attention to family and dietary history and signs and symptoms than the Addisonian method. Of course, we also consider blood test results but, because of their unreliability described above, we do not depend solely on these.

In our method, the presenting signs and symptoms are first assessed using the **One-minute health check** (part of our Protocol in Appendix 1, see page 56 and page 271). If B12 deficiency is suspected, blood tests are ordered for the full blood count, serum vitamin B12 and folic acid, TSH, U+Es, LFT, serum ferritin, glucose, early morning cortisol and vitamin D to confirm or exclude the most common conditions found alongside vitamin B12 deficiency. Figure 4-2 summarises how decisions on treatment are made using our system. The decisions are fully described in our Protocol. The two main categories of presentation that we observed at the start were: (1) a small number of people with the typical haematological signs of B12 deficiency anaemia but no neurological effects; and (2) patients with neurological signs and symptoms but no macrocytosis.

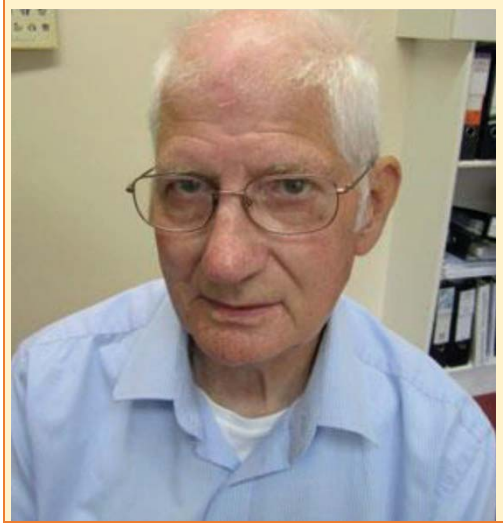
It is useful to determine the cause of B12 deficiency if found, and to establish whether this is a problem of genetics, low dietary intake or malabsorption (including PA). The presence of GPC antibodies and/or IF antibodies will help in this, with the provisos stated above.

4.3.3 Pernicious anaemia – is it preventable?

Ever since the cure for PA was discovered in the 1920s it has, to our knowledge, tended to be assumed that PA is the cause of vitamin B12 deficiency, in other words that PA develops first (from origins which are not well understood). Careful observation in clinical practice has suggested to us, that far from being the beginning of the cycle, PA may be the *end-stage* of a process of gradually increasing vitamin B12 deficiency.

It is well known that atrophic gastritis, for example, which is prevalent in the elderly, can lead to vitamin B12 deficiency because of reduced release of cobalamin from food (Andrès et al., 2004). The ensuing B12 deficiency produces further gastrointestinal disorders, such as IBS/diverticulosis, unexplained diarrhoea, Crohn's colitis, mouth ulcers, bleeding gums (see, for example, Case 4-1 and Case 4-2). These may weaken the digestive system to the point at which the autoimmune condition of PA develops.

Case 4-1 Gastrointestinal symptoms and speech difficulties



This patient, John Derek Marlow, aged 74, presented with difficulty swallowing, incoherent speech and colitis. Tests indicated a B12 level of 155 ng/L. After six months of B12 treatment (loading dose, followed by monthly injections) he was transformed. He reported that his throat felt clear; he no longer choked on fish nor had swallowing difficulties. His speech was lucid. His hair was also growing back! At that stage, however, his colitis had not improved – in many patients colitis is one of the first gastrointestinal symptoms to improve.

We also observed the frequent co-occurrence of autoimmune conditions in our B12-deficient patients. We would therefore like to suggest that B12 deficiency itself is in some way contributing to the onset of autoimmune illness (see Chapter 7). It is documented elsewhere that PA/vitamin B12 deficiency often occurs in patients with Autoimmune Polyglandular Syndrome (APS) (Zulfiqar & Andrès, 2017). Vitamin B12 deficiency usually develops over a number of years; damage to the digestive system as it progresses may exacerbate the deficiency by preventing further absorption of B12. In our view, there is a possibility that these cumulative disorders may be contributing to the development of PA and APS.

The link between B12 deficiency and the immune system is also demonstrated by a case in my Practice where a patient sadly died of pneumonia during the period of the PCT embargo when her B12 injections were stopped and her immune system was thereby compromised. Her twin sister had the same condition but survived because she was resumed on B12 treatment sooner.

This makes us consider that PA/vitamin B12 deficiency appears to be preventable. In other words, there is strong clinical evidence that PA, which is an autoimmune illness, is a *result* as well as an

accentuating cause, of vitamin B12 deficiency. I was led to this conclusion by the fact that from the moment I developed an effective system for diagnosing vitamin B12 early deficiency, I had no more cases of PA/end-stage vitamin B12 deficiency *whatsoever* in my Practice for the three ensuing decades that I worked there as a GP. This is, in my view, overwhelming evidence that, if diagnosed and treated soon enough, B12 deficiency will not progress to the PA stage. What I am suggesting is that the autoimmune attack on parietal cells of the stomach and IF-secreting cells in PA is a **result** of the deterioration in body systems resulting from B12 deficiency.

I therefore propose that B12 deficiency is progressive, and easily treated in the early stages, preventing progression.

Case 4-2 Difficulty swallowing, weight loss, fatigue and depression

Beverley Winfield, born 1968, suffered from difficulty swallowing (glossopharyngiditis). Her blood serum B12 level was recorded as 224 ng/L. If she had been treated from the start, the demyelination of the glossopharyngeal nerve could have been prevented. She was referred back and forth to various specialties to find the cause of her swallowing difficulties, loss of weight, fatigue and depression. One hospital had suggested that she might have throat cancer. She accidentally came to see me in November 2006. I diagnosed B12 deficiency although her B12 blood level recorded as 446 ng/L. I commenced her on an intensive course of vitamin B12-replacement therapy and soon she was able to swallow without difficulty and to join her husband and children for tea. The transformation in her appearance can be seen by comparing the two photographs.

4.4 The importance of early diagnosis

4.4.1 Early: simple B12 deficiency

In my view, the difference between 'simple', or immediately treatable, B12 deficiency and End-Stage/Potentially Fatal Pernicious Anaemia is substantial and identification of B12 deficiency early is of crucial importance. We can prevent fatal/pernicious anaemia (the common pre-war period presentation – see Chapter 2) if we treat proactively and methodically when a patient first presents with vitamin B12 deficiency. The following example illustrates:

Example

- For example, a person (male/female/child), possibly non-meat-eater/vegetarian/vegan, presents to their GP with classic neuropsychiatric signs and symptoms and/or an autoimmune disorder.
- A blood test confirms vitamin B12 deficiency (blood serum B12 less than 200 ng/L (pg/ml) or between 201-350 ng/L (pg/ml) (classed as 'subtle B12 deficiency' (Babior & Bunn, 2005) and (BMJ Best Practice, 2018d)).

- Prompt vitamin B12-replacement therapy prevents progression to lifelong PA, or to any other end-stage presentation, such as Multiple Sclerosis-like presentations.

4.4.2 B12 deficiency end-stage presentation

PA/fatal anaemia results when vitamin B12-replacement therapy is delayed or totally missed. If a patient is not diagnosed promptly, perhaps due to a "mixed bag" of symptoms, or has not sought medical advice from the outset, then the body will deteriorate further. Without sufficient vitamin B12 the body is not able to sustain itself as optimally as it could. As explained earlier (see Chapter 1 or Chapter 2) this is because B12 is vital for all bodily functions and cell processes to work effectively and efficiently. In layman's terms, the body weakens overall; the person becomes excessively fatigued and unable to cope with standard daily tasks and sometimes generally to deal with life. In addition to an increase in classic neuropsychiatric signs and symptoms, the delay may result in a multi-system polyendocrine syndrome (APS) where the body attacks its own cells (see Chapter 7). This is an extremely serious condition. There could be demyelination of the nerves which progresses from the simple inflammation which mostly only occurs in Stage One.

The following will occur:

- IF antibody will be positive.
- Parietal cell antibody may be positive. (The parietal cell secretes IF but if the parietal cell is damaged then the IF will not be secreted to carry the B12 throughout the body.)
- Gastric cells will atrophy.
- With poor stomach acid production, food will not be digested by the stomach and B12 will not be released from food.

The solution is to commence vitamin B12-replacement therapy urgently and to screen for accompanying autoimmune polyglandular disorders. If such disorders are found, appropriate hormone-replacement treatment, for example with physiological doses of hydrocortisone or levothyroxine will be needed. The B12/cortisol combination reverses the autoimmune tendency and speeds up remyelination of the nerves. (Note that standard treatment for MS-like presentations in hospital is to give intravenous B12 with physiological doses of cortisol (a steroid hormone), but patients are then sent home and there is no follow-up. So after about a month their symptoms return.)

To conclude: ***pernicious anaemia/fatal anaemia*** will still exist if a person's vitamin B12, folic acid, haemoglobin or ferritin levels are extremely low. In these cases, the patients may need a blood transfusion. But it ***should not still exist if we treat proactively with haematinic screening and replace as per the deficiency.***

4.5 Vitamin B12 interactions with folate

4.5.1 The "methyl-folate trap"

As stated above, megaloblastic anaemia can be caused by either folate[23] deficiency or vitamin B12 deficiency. The effects on blood cells are the same so from the point of view of haematological signs,

[23] "Folate" is the term used to describe natural sources of this vitamin. "Folic acid" describes the synthetic form. They are both also known as vitamin B9.

the two conditions are identical. The reasons for this as so far understood are a result of the interaction of the two vitamins. The folate cycle (a series of chemical reactions involving folate in the body) is responsible for producing thymidine, a constituent of DNA and needed for the healthy formation of RBCs. It follows that if there is insufficient supply of folate/folic acid, then RBC formation will be impaired. A deficiency of folate/folic acid can be caused by poor diet (such as diets lacking in green leafy vegetables or cow's milk), or by alcoholism, some gastrointestinal diseases, increased requirements as in pregnancy, severe blood loss and use of some medications (Nagao & Hirokawa, 2017). Most importantly, folate deficiency can also be, and often is, caused by vitamin B12 deficiency: B12 deficiency leads to megaloblastic anaemia because it impairs the folate cycle. Direct folic acid deficiency is less common in developed countries today because of food fortification with folic acid which was introduced to prevent the severe birth defects discovered to be caused by folate deficiency in pregnancy. (However, some consider folate supplementation without B12 supplementation to have drawbacks (Smith et al., 2008)).

The biochemistry of folate has been investigated by many researchers since the isolation of folic acid from spinach in 1941. In the 1950s and 1960s the role of folate compounds in single-carbon unit transfer in amino acid conversions, including homocysteine to methionine and in purine and pyrimidine synthesis was elucidated (Hoffbrand & Weir, 2001). One chemical reaction has been shown to be especially important in DNA synthesis: thymidylate synthesis in which deoxyuridine monophosphate (dUMP) is methylated by the folate compound 5, 10 methylene tetrahydrofolate (THF) to thymidine monophosphate (dTMP).

That a lack of B12 could lead to folic acid deficiency was first proposed in the early 1960s when it was noticed that most patients suffering from megaloblastic anaemia had deficiencies of either vitamin B12 or folic acid or both (Herbert & Zalusky, 1962). This provoked investigation of the interaction between the two vitamins and led to formulation of the hypothesis that vitamin B12 deficiency leads to folate being trapped in cells in an unusable form.

The biochemistry of the interaction between B12 and folic acid is complex and beyond the scope of this book to describe in detail but explanations are provided in, for example, Shane (2008) and Green (2017), among others. What follows is a simplification to provide some general understanding of the processes involved.

A vital step in the folate cycle is the conversion of the folate compound 5 methyl-tetrahydrofolate (MTHF) to 5, 10 methylene THF. In vitamin B12 deficiency, this conversion does not occur, or occurs at a lower rate. The folate gets "trapped" in the MTHF compound and is not available for other reactions, including thymidine synthesis. It is trapped because the conversion cannot take place without a cobalamin (vitamin B12)-dependent enzyme, methionine synthetase (MS). In the normal folate cycle, this enzyme takes a methyl group (consisting of carbon with 3 hydrogen atoms, CH_3) from MTHF, thereby converting it to THF from which it can be recycled to 5, 10 methylene THF.

This is known as the "methyl-folate trap" hypothesis. The trap occurs at the point where the folate cycle intersects with the methionine cycle. When the cycles function normally, the cobalamin molecule takes the methyl group and forms methylcobalamin. It then donates the methyl group to homocysteine which is thereby converted to methionine. This frees the THF which becomes available for other reactions. In summary: "Impairment of methionine synthetase activity in vitamin

B_{12} deficiency results in the accumulation of methyltetrahydrofolate which can neither be utilized for other reactions nor demethylated to provide free tetrahydrofolate" (Bender, 2003).

Until relatively recently, the hypothesis had never been tested in human beings but the presentation of a rare case (Smulders et al., 2006) provided an opportunity to demonstrate most features of the methyl-folate trap and thereby provide evidence for it.

4.5.2 Masking of B12 deficiency

Conversely, there is concern that high doses of synthetic folic acid can temporarily correct megaloblastic anaemia and mask vitamin B12 deficiency, leading to an undiagnosed progression of neurological damage to an irreversible stage. This concern first arose in the 1950s when it was seen that patients treated with the newly-discovered folic acid, on the assumption that it would cure pernicious anaemia, developed a rapid worsening of their neurological symptoms after an initial improvement (Reynolds, 2006).

The fact that large doses of folic acid can correct megaloblastic anaemia implies that the methyl-folate trap can be overriden. One possible mechanism for this is that high-dose folic acid overcomes the block "through dihydrofolate reductase (DHFR) reduction to tetrahydrofolate" (Green, 2017 Figure 3). Varela-Moreiras et al. (2009) explain:

> *"Since folic acid (pteroylglutamic acid, the synthetic form of folate) is reduced directly to tetrahydrofolate, it escapes the metabolic block caused by insufficient cobalamin. Thus, folic acid treatment corrects the megaloblastic anemia caused by cobalamin deficiency. As a result, the hematological marker of the deficiency (anemia) is corrected, and the clinical sign of the deficiency is masked. The resulting delay in diagnosis of the deficiency can lead to irreversible neurological damage."*

It is not clear whether the same effect would occur in a vitamin B12-deficient patient with a high natural folate intake (such as in a vegetarian diet). The issue seems mainly to relate to administration of folic acid, which can be given in much higher doses than a normal diet would provide and which is more readily bioavailable than dietary folate.

The potential masking of B12 deficiency can in our view be easily avoided through meticulous family history-taking and observance of signs and symptoms as described above which would obviate a misdiagnosis.

4.5.3 Hyperhomocysteinaemia

The trapping of folate because of cobalamin deficiency has another important consequence: it means that another vital biochemical reaction, the methionine cycle, cannot complete. In a healthy person, following conversion of homocysteine to methionine, the latter is converted in further steps to S-adenosyl methionine (SAMe) which is important for the production of neurotransmitters and for DNA methylation. In this reaction, SAMe gives off its methyl group and thus becomes homocysteine, starting the whole cycle again.

If this cycle is impaired, levels of homocysteine in the blood rise, creating the condition known as hyperhomocysteinaemia. "Homocysteine, otherwise a normal amino acid, is both vasculotoxic [poisons or damages the heart and vascular/circulatory system] and neurotoxic [poisons or damages

the nerve system] when elevated to 17 or more µmol/l [in the blood serum]" (Herbert, 2002). At vasculotoxic levels, there is a high risk of heart attacks, thrombotic strokes and peripheral venous occlusion. High levels of homocysteine also have adverse effects on pregnancy (see Chapter 5 of this book and Refsum (2001)). Herbert further explains:

> *"Three publications have suggested folic acid deficiency is the cause of hyperhomocysteinaemia in the elderly. However, if analysed appropriately, all subjects – folic-acid deficient patients with hyperhomocysteinaemia – also have B12 deficiency. I [Victor Herbert] strongly suggest that it is the B12 deficiency, rather than the folate deficiency, that is causing the hyperhomocysteinaemia. The B12 deficiency may also be causing the folic acid deficiency in these patients. In summary, the low serum folates are secondary to malabsorption due to gut B12 deficiency, and the low red cell folates are secondary to malabsorption because B12 is necessary both to get folate into red cells and to keep it there (by polyglutamating it)." (Victor Herbert, answer to discussion on Carrazana (2002) in Herbert ed.* ***Vitamin B12 deficiency****, (p.26)).*

Researchers have found that the majority of cases of hyperhomocysteinaemia result from low levels of vitamin B12 and/or folic acid. Some have suggested that hyperhomocysteinaemia may be a direct result of severe gastric damage as in atrophic gastritis (Santarelli et al., 2004). Others have also found a link between atrophic gastritis and coronary heart disease via hyperhomocysteinaemia (Senmaru et al., 2012).

4.5.4 Vitamin B12-replacement therapy for hyperhomocysteinaemia

When vitamin B12 supplement (B12 replacement) is given to a patient who is deficient in B12 and in folate, tests show that the folate levels in the blood increase to non-deficient (normal) levels (Chandy, 2006a) even without folate supplementation. Vitamin B12 enables the utilisation of folate which is in many foods (vegetarian and non-vegetarian), raising the levels in blood serum and other body tissues. Even the erythropoietic process (development and maturation of RBCs) is enhanced. The risks of hyperhomocysteinaemia are therefore reduced.

4.6 Risk of other conditions and associated autoimmune disease

In addition to the neurological and neuropsychiatric conditions that are described elsewhere in this book, patients with vitamin B12-deficiency anaemia may develop other complications. Investigations should include endoscopy to determine whether the patient suffers from atrophic gastritis or other conditions such as gastric carcinoma or gastric polyps which are more common in this type of anaemia than in healthy people (Hoffbrand & Provan, 1997). Patients with vitamin B12 deficiency anaemia may also develop iron deficiency anaemia if the cause of their condition is chronic atrophic gastritis (Devalia et al., 2014).

Multiple autoimmune conditions are also found in patients with pernicious anaemia. The most common such conditions are (Hoffbrand & Provan, 1997):

- Graves' disease
- Myxoedema
- Thyroiditis (hypo- or hyper- thyroidism)

- Idiopathic adrenocortical insufficiency (hypoadrenalism, low cortisol)
- Vitiligo
- Hypoparathyroidism