

Testing for Pernicious Anaemia

As soon as the Pernicious Anaemia Society was formed it became obvious that some, though not all patients faced a long journey in being diagnosed quickly and accurately. And the results of the members' survey showed that one-third had experienced symptoms for up to a year before diagnosis. Nearly 22% had to wait 2 years, 19% for 5 years and 4% for 10 years. Regrettably, 14% of individuals experienced symptoms for more than 10 years before arriving at their diagnosis¹. For many years we have wanted to know why many of our members had to wait so long for an accurate and quick diagnosis. And it is only recently that research papers have been published that show that one of the reasons is because the current tests used to diagnose Vitamin B₁₂ Deficiency in general and whether that deficiency is caused by Pernicious Anaemia are seriously flawed.

There are a number of different tests that are used to determine the patient's B₁₂ Status and whether that deficiency is caused by Pernicious Anaemia. Below is a brief explanation of each of these tests but it is useful to note the recently published new and updated Guidelines from the British Committee for Standards in Haematology (BCSH) for the Diagnosis and Treatment of Cobalamin (B₁₂) and Folate Disorders² which states: *"The clinical picture is the most important factor in assessing the significance of test results assessing cobalamin status since **there is no 'gold standard' test to define deficiency**"*ⁱ.

TESTS TO DETERMINE PATIENTS' B₁₂ STATUS

The Full Blood Count (or FBC)

This is the first-line test and provides doctors with a general picture of the patient's blood. It includes an assessment of the size of the patient's red blood cells which can be an indicator of alcohol abuse or, more important for us, a deficiency in vitamin B₁₂. Doctors refer to enlarged red blood cells as *megaloblasts* and the patient as having *macrocytosis* (his or her blood cells will be enlarged). The test to establish whether there is any evidence of macrocytosis is the Mean Corpuscular Volume (MCV).

It is often wrongly assumed that red blood cells will always be enlarged if there is a B₁₂ Deficiency. However, only around 60% of patients with a B₁₂ deficiency will have enlarged red blood cells (macrocytosis)^{3, 4}. Another problem with relying on the MCV to indicate a B₁₂ Deficiency is that if the patient also has an iron deficiency that deficiency will lead to a reduction in the MCV – an iron deficiency and a B₁₂ deficiency will in effect cancel out each deficiency's effect on the size of the red blood cell. Indeed until recently the UK's National Institute for Health and Care Excellence (NICE) guidance on this issue specifically stated that investigating the patient's B₁₂ status should only be carried out if there was evidence of enlarged red blood cells. Thankfully that advice was removed after we pointed out the error. What we would like it for the patient's B₁₂ status to be made part of the full blood count but, as more and more doctors become aware that B₁₂ deficiency is more common than first thought, it seems that most doctors now ask for the

ⁱ Our Emphasis

patient's B₁₂ to be investigated at the same time as the FBC is performed. There is, however, a problem with the B₁₂ test – it's not accurate.

Serum B₁₂ Test

There are two main problems with the current test used to ascertain how much B₁₂ the patient has in his or her blood.

Firstly the current threshold used to diagnose any deficiency may be far too low.

Although the threshold figures will vary from laboratory to laboratory because different laboratories use analytical machines made by different manufacturers the typical cut-off value used to define B₁₂-deficiency is around 148 pmol/L (200 pg/mL) and for a growing number of scientists this is far too low and, if patients are showing symptoms of B₁₂ deficiency yet their blood result shows their B₁₂ to be above 148 pmol/L the patient should be treated. They classify patients who have a range of B₁₂ between 148 pmol/L and 300 pmol/L as being Subclinical Cobalamin Deficient.

Secondly the machines that are currently used to evaluate B₁₂ status of patients have been shown to be inaccurate and are giving false high readings in 22%, 26% and 35% of patients⁵ (the different percentages are due to the different machines used). As the BCSH puts it:

Definitive cut-off points to define clinical and subclinical deficiency states are not possible, given the variety of methodologies used and technical issues, and local reference ranges should be established.

Red Blood Cell Folate (RBC folate) and Serum Folate

RBC measures the amount of Folic Acid in the patient's red blood cell. Serum Folate is an evaluation of the patient's folic acid in the blood (rather than the red blood cells) and the latest guidelines suggest that Serum Folate is as effective as Red Blood Cell Folate as a method of evaluating the patient's folic acid.

There are three things worth mentioning about Folic Acid and B₁₂. The first is that if the patient has been supplementing using folic acid tablets (as pregnant women are advised to do) then the increase in folic acid will prevent the red blood cells from becoming enlarged (megaloblastic) as discussed above. Therefore high folic acid levels means that one of the indicators of low B₁₂ (enlarged red blood cells) will not be present when doctors interpret the results of any full blood count.

Secondly, low level of folic acid has the surprising effect of increasing the amount of B₁₂ loss in urine. The lower the patient's folic acid, the more B₁₂ will be excreted via urine⁶. And finally, healthy levels of folic acid is needed to enable the patient to convert any cyanocobalamin (a form of injectable B₁₂) to convert to Hydroxocobalamin and then to the biologically 'active' forms of B₁₂ – Methylcobalamin and Adenosylcobalamin. The current test does not distinguish between the active and inactive forms of B₁₂ and so if a patient is deficient in folic acid he or she will not be converting the inactive forms of B₁₂ into the active forms. The result will be that the patient's B₁₂ status will show normal or high, but very little of this B₁₂ will be in the active form. *If you have been supplementing your diet using folic acid tablets you should tell your doctor this before or after you have given blood to be analysed.*

This is what the new guidelines say:

"Serum folate offers equivalent diagnostic capability to red cell folate and is the first line test of choice to assess folate status".

Ferritin (iron stores)

Iron plays an important part in the formation of healthy red blood cells and so it is useful to ascertain the patient's iron stores. Low iron can be the result of haemorrhaging following an accident or from heavy menstruation.

Serum Holotranscobalamin (HoloTC) - The 'Active' B₁₂ Test.

Following on from the above, there is now a test that is being introduced gradually in many countries that doesn't measure the total amount of B₁₂ in the patient's blood but distinguishes between the 'active B₁₂' (holotranscobalamin) and the 'inactive B₁₂' (holohaptocorrin). There are a few hospitals in the UK where this test is available but results are sometimes inconclusive though in other cases there has been a substantial difference between the patient's total serum B₁₂ results and the HoloTC result. It seems that this new test offers the potential to replace the current, inaccurate test but there does need to be more investigation into its effectiveness. Here's what the new guidelines say:

"Serum holotranscobalamin has the potential as a first line test, but an indeterminate 'grey area' may still exist".

Plasma Methylmalonic Acid (MMA)

This test is not routinely carried out though it is a good indicator of B₁₂ Deficiency. When a patient has low B₁₂ levels then their MMA will be raised. If their level of MMA is extremely high then it is inevitable that the patient will have low B₁₂. The main problem with the test is that elevated MMA is also seen in patients with small bowel bacterial growth and those with renal disease.

Plasma Total Homocysteine (tHcy)

This is another test that is a reliable indicator of low B₁₂ as it rises quite significantly early on in any deficiency. However, tHcy is also raised with kidney failure, hypothyroidism, folic acid deficiency and B₆ deficiency. One of the major drawbacks of this test is that it has to be carried out within two hours of the blood being taken and that sample must have been kept cool during that period.

TESTS FOR PERNICIOUS ANAEMIA

When a patient is identified as having a deficiency in vitamin B₁₂ the cause of that deficiency should be investigated. These are the tests used to determine whether the deficiency is caused by the patient having developed Pernicious Anaemia.

Anti-intrinsic Factor Antibody Test

This is the test used to find out if the patient has 'classic' or 'full-blown' Pernicious Anaemia. If tested positive the patient will be deemed as having auto-immune Pernicious Anaemia. However the test has proved to be notoriously unreliable (known as low sensitivity by doctors). Here's what the new guidelines say:

"...and the finding of a negative intrinsic factor antibody assay does not therefore rule out pernicious anaemia".

One important point made by the new guidelines is that if the patient has received any injections of B₁₂ the test will be severely compromised and so it is only supposed to be carried out on patients who have not received a 'recent' injection. The new guidelines do not state what timescale is involved in a 'recent' injection.

Parietal Cell Antibodies

This is another classic though flawed test for Pernicious Anaemia. Around 10% of healthy individuals will test positive for this test – it is, therefore, another low sensitivity test.

OTHER TESTS

Other tests carried out as part of the Full Blood Count include the following:

Mean Corpuscular Haemoglobin (MCH)

This measures the amount of Haemoglobin per red blood cell. A high concentration is indicative of B₁₂ Deficiency

MCHC (mean corpuscular haemoglobin concentration)

This measures the average concentration of haemoglobin in the cells.

Red blood cell distribution width (RDW)

A measure of the volume of the red blood cells

OTHERS

There are still more test results included in the full blood count but they needn't concern us here.

IMPORTANT

If you are concerned about any part of your blood results you should discuss this with your doctor. Remember, he or she will have spent many years in medical school so that he or she can interpret the test results. However, as we have seen above, some of the tests are seriously flawed.

¹ Hooper M, Hudson P, Porter F, McCaddon A; Patient journeys: diagnosis and treatment of pernicious anaemia: *British Journal of Nursing*, 2014, Vol 23, No 7, 2014

² British Committee for Standards in Haematology, 2014.

http://www.bcsghguidelines.com/documents/BCSH_Cobalamin_and_Folate_Guidelines_%282%29.docx.pdf

³ Beck W S. Neuropsychiatric consequences of cobalamin deficiency; *Advanced Institute of Medicine*, 1991; 36: 33-56

⁴ Lindenbaum J, Heaton EB et al. *Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. New England Journal of Medicine* 1988; 318(26): 1720-1728

⁵ Carmel, R. & Agrawal, Y.P. (2012) Failures of cobalamin assays in pernicious anemia. *N Engl J Med*, **367**, 385-386.

⁶ Eric Van Royen, Pierre Blockx, Fritz Molter; The influence of serum folate on urinary excretion of vitamin B₁₂; *European Journal of Nuclear Medicine*, 1.7.1978, Volume 3, Issue 3, pp 175-178