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REVIEW ARTICLE

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Vitamin B12 deficiency and clinical laboratory: Lessons revisited and clarified in seven questions

M. Salinas[1,2] | E. Flores[1,3] | M. López-Garrigós[1] | C. Leiva-Salinas[4]

[1]Clinical Laboratory, Hospital Universitario de San Juan, San Juan de Alicante, Spain

[2]Department of Biochemistry and Molecular Pathology, Universidad Miguel Hernandez, Elche, Spain

[3]Department of Clinic Medicine, Universidad Miguel Hernandez, Elche, Spain

[4]Department of Clinical Radiology, University of Missouri Health, Columbia, MO, USA

Correspondence
María Salinas, Clinical Laboratory, Hospital Universitario de San Juan, San Juan de Alicante, Alicante, Spain.
Email: salinas_mar@gva.es

Abstract

The objective of this review article is to address the most frequently asked questions that pathologists and primary care physicians might face when dealing with a patient with suspicion of vitamin B12 deficiency. More specifically, the article mainly discusses the importance and prevalence of the deficit, how to recognize it, and the important role of a prompt diagnosis confirmation based on laboratory biomarkers for efficient replacement therapy.

KEYWORDS

biomarkers, clinical laboratory, diagnosis, pernicious anemia, vitamin B12

1 | INTRODUCTION

The seven questions answered in this article revolve around vitamin B12 deficiency. This review discusses the absorption and physiology of vitamin B12, the importance and prevalence of the deficit, how to early recognize it, and later focuses on the different available laboratory biomarkers to identify patients with vitamin deficiency for a prompt replacement therapy.

The complete structure of vitamin B12 was determined by Dorothy Hodgkin and her collaborators by means of X-ray crystallographic methods. These studies revealed that the vitamin was a cyanolated, cobalt-containing, amidated tetrapyrrole.[1] The cobalt is located in the center of a ring-contracted modified tetrapyrrole macrocycle, coordinated via the 4 pyrrole nitrogen atoms. The cobalamin tetrapyrrole ring, exclusive of cobalt and other sidechains, is called a *corrin*. All compounds that contain this corrin nucleus are corrinoids. Cobalamins differ in the nature of additional side groups bound to cobalt, for example, hydroxyl (Hydroxycobalamin-H-Cbl), deoxy-5′-adenosine (Deoxy-5′-adenosylcobalamin-Ado-Cbl), methyl (methylcobalamin-Me-Cbl), and cyanide (Cyanocobalamin Cn-Cbl). The term vitamin B12 is used ubiquitously to refer to the different forms of cobalamin, such as Me-Cbl and Ado-Cbl, and they are also referred to as complete corrinoids. Me-Cbl and Ado-Cbl are the active forms of vitamins used as coenzymes in the cell.[2]

Vitamin B12 is required in these coenzyme forms for the conversion of L-methylmalonyl CoA to succinyl-CoA and homocysteine to mehionine. These pathways are critical in the metabolism of branched-chain amino acids and fatty acid and in the regeneration of the methyl donor S-adenosylmethionine. Its dysfunction creates a shortage, affecting DNA synthesis and the physiological processes such as hematopoietic process of the erythrocytes.[3]

2 | HOW IS DIETARY VITAMIN B12 ABSORBED?

Dietary vitamin B12 is normally bound to proteins in food and requires release by gastric acid and pepsin in the stomach. Once vitamin B12 is free, it attaches to salivary haptocorrin (HC) that protects the vitamin from the acidic environment of the stomach while it is transported to the small intestine. In the small intestine, vitamin B12 binds to intrinsic factor (IF) produced by gastric parietal cells. In ileum, the IF-vitamin B12 complex then binds to the cubam receptor, which facilitates endocytosis into the lysosome. In the lysosome, IF is degraded and the liberated vitamin B12 is released into the cytosol and then transported to the bloodstream. The majority of vitamin B12 (80%) are bound to the HC, whereas the remainder is bound to transcobalamin (TC). Like IF, TC binds only vitamin B12. Significantly,

only TC is able to facilitate uptake into cells via the TC receptor-mediated endocytosis.[4]

3 | WHAT ARE THE KEY PHYSIOLOGICAL ROLES OF VITAMIN B12?

Intracellular vitamin B12 is metabolized into adenosylcobalamin or methylcobalamin. Inside the cells, vitamin B12 acts as a coenzyme for 2 different enzymes, methylmalonyl-CoA mutase, and methionine synthase.[5] Vitamin B12 is vital for appropriate red blood cell formation, neurological function, and DNA and RNA synthesis. Impaired DNA synthesis can cause cell arrest in the DNA replication or S phase of the cell cycle, resulting in errors in DNA replication, and/or apoptotic death.[6]

4 | WHAT ARE THE DIETARY REQUIREMENTS OF VITAMIN B12?

As described in the vitamin B12 Dietary Fact Sheet from the National Institutes of Health (NIH), the recommended daily intake of vitamin B12 ranges from 0.4 mcg in young infants to 2.4 mcg in adults; slightly higher amounts might be needed during pregnancy and lactation.[7] Total body storage of vitamin B12 ranges from 2 to 5 mg, and approximately half of it is stored in the liver. If vitamin B12 intake ceases, deficiency would usually not develop for at least 1-2 years, sometimes even longer.[8]

5 | WHAT IS THE PREVALENCE OF VITAMIN B12 DEFICIENCY?

The prevalence of vitamin B12 deficiency is likely to vary among different populations and depends on the threshold used to define deficiency. In general, vitamin B12 storage declines with age and therefore prevalence of vitamin B12 deficiency increases as subjects get older. Studies have shown that prevalence of vitamin B12 deficiency among elderly can range between 5% and 40% depending on the definition of vitamin B12 deficiency used.[9]

Approximately 6% of the western population over the age of 60 years has low plasma vitamin B12 and as many as 20% may have marginal vitamin B12 status.[10] In a 2016 series, among 3324 patients with anemia in a general practice population in the Netherlands, 249 had macrocytosis.[11] Of these, 46 had vitamin B12 deficiency (1.4% of all individuals with anemia; 18% of those with macrocytic anemia).

The groups at risk of vitamin B12 deficiency are[12]:

- Older than 65 years.
- Decreased absorption (eg, gastrectomy, bariatric surgery, Crohn's disease, celiac disease, pancreatic insufficiency, bacterial overgrowth, fish tapeworm infection).
- Autoimmune conditions, such as thyroid disease.
- On medication known to interfere with vitamin absorption, metabolism or stability: nitrous oxide, metformin, and proton pump inhibitors.

In subjects older than 65 years, there is a high prevalence of autoimmune disorders with presence of anti-parietal cell antibodies (APCA), and anti-intrinsic factor antibody (AIFA); that result in autoimmune-based gastric atrophy (ABG) with severe damage of the oxyntic gastric mucosa. The loss of parietal cells, which normally produce hydrochloric acid, as well as IF results in vitamin B12 deficit. If this co-exists with anemia and macrocytosis, it is called pernicious anemia (PA), defined as the presence of hemoglobin concentration <13 g/dL for men and <12 g/dL for women,[13] mean corpuscular volume ≥100 fL,[14] low levels of vitamin B12,[15] along with concomitant ABG and IF deficiency.

PA is a macrocytic anemia due to vitamin B12 deficiency, which, in turn, is the result of deficiency of IF, a protein that binds avidly to dietary vitamin B12 and promotes its transport to the terminal ileum for absorption.[14] The mean age of patients with PA ranges from 59 to 62 years, which challenges the common notion that PA is an exclusive disease of the elderly, and suggests that, in clinical practice, PA is probably under-diagnosed in elderly and younger patients.[16] Its prevalence is around 2% in people over 60 years and is uncommon before that age; with only 10% of cases occurring in subjects that are younger than 40 years. It appears to be more frequent in women than in men, confirmed in a survey in patients older than 60, in which the prevalence of PA was 2.7% in women and 1.4% in men.[17]

Traditionally, vitamin B12 deficiency has been associated to PA. However, subjects with low serum vitamin B12 concentrations rarely have anemia or macrocytosis.[18] In fact, early detection and treatment have led to a lower percentage of vitamin B12 deficiency patients with PA.[18]

6 | WHAT ARE THE MAIN CLINICAL MANIFESTATIONS OF VITAMIN B12 DEFICIENCY?

Vitamin B12 deficiency is silent and under-diagnosed, as its onset and progression are slow and patients may get used to their symptoms. Nevertheless, the clinical consequences of undiagnosed vitamin B12 deficiency may be serious, including wide range of neurological and mood disorders.

Vitamin B12 deficiency results in 3 main potential complications: haematological effects such as macrocytosis, hypersegmentation of neutrophils, anemia, leukopenia and thrombocytopenia, and megaloblastic changes in bone marrow, demyelinating disorder of the central nervous system that may leads to other serious and often irreversible neurological conditions, and gastric neoplastic lesions when deficit of vitamin B12 is due to ABG. Of the 3 main adverse effects of vitamin B12 deficiency, haematological complications can be reversed with treatment, as opposed to neurological symptoms and gastric cancer.

Subtle neurologic, cognitive, or psychiatric changes are one of the most commonly encountered symptoms in primary care practice. The most common neurologic findings in vitamin B12 deficiency are symmetric paresthesias or numbness and gait problems.[12] The neuropathy is typically symmetrical and often affects the legs more than the arms. The classic neurologic finding in vitamin B12 deficiency, sub-acute combined degeneration of the dorsal (posterior) and lateral columns (white matter) of the spinal cord due to demyelination, is characteristic if present, but may not occur in all cases, especially if diagnosed earlier in the course of the deficiency. In individuals with vitamin B12 deficit, neuropsychiatric symptoms can be present even in the absence of anemia or macrocytosis, and the lack of these hematologic changes cannot be used to exclude vitamin B12 deficiency.[19]

Other neurological-psychological manifestations include[20]:

- Depression or mood impairment
- Irritability
- Insomnia
- Cognitive slowing
- Forgetfulness
- Dementia
- Psychosis
- Visual disturbances, which may be associated with optic atrophy
- Peripheral sensory deficits
- Weakness, which may progress to paraplegia and incontinence if severe
- Impaired position sense
- Impaired vibration sense
- Lhermitte's sign, a shock-like sensation that radiates to the feet during neck flexion
- Ataxia or positive Romberg test
- Abnormal deep tendon reflexes
- Extrapyramidal signs (eg, dystonia, dysarthria, rigidity)
- Restless legs syndrome

Vitamin B12 deficiency is substantially a benign disorder for a large number of patients. However when deficit is due to PA patients are at increased risk for a gastric adenocarcinoma and gastric carcinoid type I.[21] Chronic hypergastrinemia in patients with PA is associated with Enterochromaffin-like (ECL) cell hyperplasia and gastric carcinoids. It has been reported that approximately 4%-5% of patients with PA develops gastric carcinoids.[22] Moreover, the role of hypochlorhydria in the development of gastric cancer has been highlighted.[23,24] Finally, ascorbic acid decreases in the case of gastric atrophy and also its protective action. A prospective study shows a gastric cancer incidence in patients with PA of 0.1% to 0.5%.[22] A study of patients with ABG during an observation period of 6.7 years, has reported an annual incidence risk of 0.14% for developing gastric cancer.[25]

In all, we should increase our awareness of this disorder, whose definite diagnosis may be preceded by reliable and non-invasive serological screening.[26]

7 | WHAT BIOMARKERS ARE AVAILABLE TO DIAGNOSE VITAMIN B12 DEFICIENCY?

Symptoms and signs of vitamin B12 deficiency are vague and very often unrecognized. Prompt diagnosis and treatment are required before neuropsychological symptoms become irreversible or permanent.

Some authors are in support of vitamin B12 deficiency screening in the elderly. However, such strategy is not generally recommended and only focuses on patients with one or more risk factors, such as gastric or small intestine resections, inflammatory bowel disease, long-term use of metformin proton pump inhibitors or histamine H2 blockers, vegans or strict vegetarians, and adults older than 75 years.[27] Although no formal recommendation for screening in asymptomatic people exist, the higher risk of occurrence in the elderly and easy and safe replacement therapy, more liberal testing and treatment is advised in the elderly.[28]

There are several serum biomarkers that might be useful in the work-up of a patient with vitamin B12 deficit. In addition to the vitamin B12 assay, holoTC level in serum, which is reduced in vitamin B12 deficiency, is considered a marker of vitamin B12 deficiency.[28] Elevated levels of total homocysteine (tHcy) and methylmalonic acid (MMA) have been proven as markers for insufficient intracellular vitamin B12.[5] Table 1 shows advantages and limitations of the different biomarkers.

Serum vitamin B12 levels are associated with symptoms of memory impairment and with objective evidence of cognitive impairment. However, the associations of cognitive impairment with holoTC and with metabolites of vitamin B12 (tHcy and MMA) are stronger than those with serum vitamin B12. This suggests that holoTC might be a more reliable indicator of intracellular vitamin B12 status than the standard vitamin B12 assay, although availability and cost would also have to prove its superiority. Despite this evidence, the request of vitamin B12 keeps increasing (Figure 1).

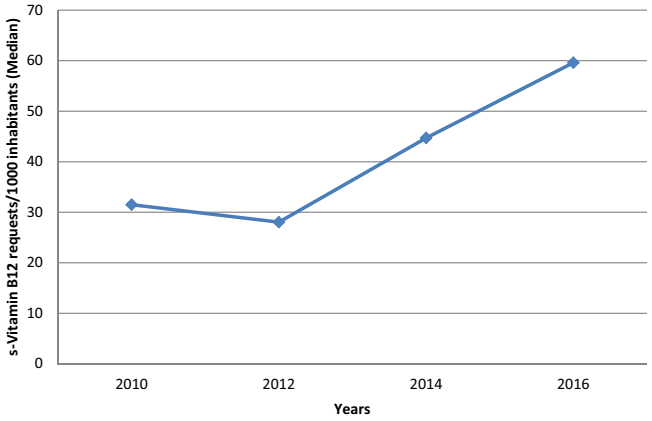

FIGURE 1 Scattered plots showing the evolution of vitamin B12 request per 1000 inhabitants in the different editions of REDCONLAB investigations. Number of annual vitamin B12 requests in primary care in Spain expressed as vitamin B12/1000 inhabitants

TABLE 1 Advantages and disadvantages of vitamin B12 deficiency biomarkers

Test	Advantages	Limitations
Vitamin B12 (serum)	Widespread[29]	Assay is not standardized[28] Not defined deficiency, that is a cut-off for deficiency[30]
Holotranscobalamin	Active vitamin B12[28] More reliable indicator of intracellular vitamin B12 status[39]	No agreement between different methods Insufficient sensitivity[33]
Methylmalonic acid Homocysteine	Insufficient vitamin B12 in the cells causes an increase in the concentration[5] More reliable indicators of intracellular vitamin B12 status[39]	More expensive Not readily available Reference intervals are not standardized[28]

Several limitations exist regarding the biomarkers to identify Vitamin B12 deficiency. Serum vitamin B12 assay is not standardized,[29] and there is no agreed upon cut-off to define deficiency. The World Health Organization suggested using 150 pmol/L (200 pg/mL) in 2008; however, total vitamin B12 levels of 156-450 pmol/L cannot rule out vitamin B12 deficiency, and some authors even consider those latter values to be too low.[30] On the other hand, plasma MMA and tHcy are more expensive, not readily available and reference intervals are not standardized either.[28]

Because the only fraction of dietary vitamin B12 bioavailable for systemic distribution is in the form of holoTC, the level of holoTC in serum has been successfully utilized as a surrogate of bioactive vitamin B12.[31] Holo-TC represents approximately 20% of total vitamin B12 present in serum. This marker is considered to be more accurate in assessing the biologically active fraction of vitamin B12 in circulation than serum vitamin B12 itself, and its level correlates with the concentration of serum vitamin B12 in erythrocytes.[31] On the other hand, the diagnostic value of holoTC—whose normal range in healthy subjects is 20-125 pmol/L[31]—has proven superior to Hcy and MMA for the assessment of vitamin B12 status in the elderly. However additional research is needed to elucidate the mechanisms that control holoTC homeostasis in the normal population and in pathologies that alter vitamin B12 transport and utilization. For example, abnormally low levels of holoTC have been documented in patients on chemotherapy, with macrocytosis, and in individuals carrying the TC polymorphism 67A>G, without any evidence of vitamin B12 deficiency.[32] Additionally, insufficient sensitivity (44%) of holoTC as a marker of vitamin B12 status was noted in a cohort of 218 institutionalized elderly patients.[33] At present, it is unknown whether holoTC levels vary in patients harboring inborn errors affecting intracellular vitamin B12 metabolism. Thus, the diagnostic value of holoTC as a first line test still needs further investigation.

APCA and AIFA should be evaluated in the patient with vitamin B12 deficit. The importance of measuring APCA in every B12 deficient patient lies in that positivity is associated with excess risk of gastric carcinoma and gastric carcinoid tumor.[22] APCA are found in 90% of patients with PA, but have low specificity and are seen in ABG without megaloblastic anemia as well as in various autoimmune disorders. AIFA are less sensitive, being found in only 60% of patients with PA, but are considered highly specific for PA.[28] In fact, once the patient is diagnosed with PA single endoscopic screening for gastric carcinoma and gastric carcinoid tumor is recommended. The presence of IF deficiency may be difficult to prove, and increasing confidence is placed on the detection of AIFA for the diagnosis of PA, which is viewed as a useful marker of the disease. Earlier studies have shown that 40%-60% of patients with PA are AIFA positive,[34] which rises to 60%-80% with increasing duration of disease,[35] which yielded for AIFA, a sensitivity and specificity of 37% and 100%, respectively, and for APCA, a sensitivity and specificity of 81% and 90%, respectively. The combined assessment of both autoantibodies increases diagnostic performance, with 73% sensitivity and 100% specificity.

Future research should study whether AIFA is present in vitamin B12 deficient patients without anemia. In fact, beyond being a specific hallmark of PA, AIFA and APCA may be interpreted as an expression of damage to the oxyntic mucosal, given the correlation between histological score of ABG and the titer of both antibodies.[25] APCA were found in 13 of the 95 people tested and AIFA indicative of PA in 3 of the 13 people.[18]

As APCA and AIFA reflect ABG, in a patient showing a positive test could be an indirect indicator to confirm vitamin B12 deficiency, solving, at least in these cases the problem of the lack of a reliable serum marker for diagnosis.

8 | WHAT IS THE TREATMENT OF VITAMIN B12 DEFICIENCY?

Vitamin B12 replacement treatment permits correction of the anemia, whereas the neurological complications may be reversed only if replacement treatment is administered early after onset.

The traditional treatment of vitamin B12 deficiency is the intramuscular injection of cyanocobalamin, generally, 1 mg/d for 1 week, followed by 1 mg/wk for 1 month, and then 1 mg every 1 or 2 months ad perpetuum.[36]

Oral administration of high-dose vitamin B12 (1 to 2 mg daily) is considered as effective as intramuscular administration for correcting anemia and neurologic symptoms.[27] However, despite many

studies suggesting oral administration of vitamin B12 to be easy, effective and less costly than intramuscular administration, debate surrounds the effectiveness of the oral route. This may help explain why it is little used by health professionals. In the United States, patients usually receive vitamin B12 injections of 1 mg/d in their first week of treatment; then weekly injections in the following month, and finally monthly injections after that.[37] In Denmark, however, patients receive injections of 1 mg cyanocobalamin per week during the first month and every 3 months after that, or 1 mg hydroxycobalamin every other month.[38] According to the protocols, a higher dosage of cyanocobalamin might be used to prevent early relapse: first patients receive IM injection of 5 mg/d cyanocobalamin for 5 days, which replenishes the vitamin B12 body stores; then stores are maintained by IM injection of 5 mg cyanocobalamin every 3 months.[26]

The percentage of vitamin B12 absorption improves with supplementation; therefore, patients older than 50 years and vegans or strict vegetarians should consume foods fortified with vitamin B12 or take vitamin B12 supplements.[27]

9 | CONCLUSION

Vitamin B12 deficiency is common and has severe and potentially irreversible clinical sequelae. There is no widespread consensus regarding a definitive single diagnostic biomarker for vitamin B12 deficiency. More studies are needed to define such standard marker, and to investigate whether positivity of APCA or AIFA could indirectly confirm the presence of the deficit.

The Clinical Laboratory plays a key role in vitamin B12 deficiency. It should design and lead active screening strategies to increase its detection before the clinical symptoms arise, identify cases of autoimmune disease, and promote prompt treatment after abnormal serological tests.

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