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CONSENSUS

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Consensus of the Brazilian Association of Nutrology on diagnosis, prophylaxis, and treatment of vitamin B12 deficiency

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Abstract

Introduction: Vitamin B12 deficiency is quite prevalent in all age groups and all regions of the country, however, there is a need for standardization of clinical management recommendations. **Objective:** It was to achieve a consensus on the diagnosis, prophylaxis, and treatment of vitamin B12 deficiency. **Methods:** An integrative review of the scientific literature was carried out in Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases and on governmental and regulatory websites. **Results:** According to the literary search carried out by the authors of this study, 84 scientific works were selected. Based on these findings, in the timeline from 1999 to 2022, randomized controlled clinical studies represented the majority, and prospective and retrospective studies with significant sample sizes were elucidated in this consensus on the vitamin B12 administration for all patient populations. The basis of this scientific evidence was determined by appropriate clinical trials, with additional endpoints including patient clinical symptoms and biochemical parameters in addition to serum and marginal B12 level. **Conclusions:** Based on the scientific literature, the recommendations of the Brazilian Association of Nutrology for diagnosis, prophylaxis, and treatment of vitamin B12 deficiency were presented.

Keywords: Vitamin B12. Deficiency. Treatments. Routes of administration.

Definitions and physiology

General Introduction to Vitamins

Vitamins are organic molecules, micronutrients, that act in various physiological processes in the human body. Thus, they are essential for proper growth, development, and reproduction. However, the synthesis by the body is not enough to satisfy the physiological needs, so they must be ingested via diet in adequate amounts **[1]**.

Vitamins are usually divided into two groups according to their solubility. The watersoluble (water-soluble) is easily excreted by the body and little stored, so intake should be daily. They are vitamins C (ascorbic acid) and vitamins of B complex. The soluble vitamins in lipids (liposoluble), in turn, are only four: vitamins A, D, E, and K. The latter can be stored in the body's tissues. fat and depend on the concomitant intake of lipids to be adequately absorbed via diet **[2]**.

Complex B vitamins

The B-complex vitamins are part of the water-soluble group. They are widely distributed in food and play numerous roles as coenzymes in metabolic processes. They are vitamins B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B8 (choline), B9 (folic acid) and B12 (cobalamin). In this consensus, we are particularly interested in cobalamin. Inositol and PABA (para-

aminobenzoic acid) are also part of the group of B vitamins [3].

Types of Cobalamins

Cobalamins are molecules characterized by the presence of a central cobalt bonded to 1 tetrapyrrolic ring, 1 lower axial linker (being 5,6-dimethyl benzimidazole in the active form of the vitamin), and 1 upper axial linker. According to the upper axial linker, cobalamin may be named adenosylcobalamin (deoxyadenosine linkage), methylcobalamin (methyl linkage), hydroxocobalamin (hydroxyl linkage), or cyanocobalamin (cyanide linkage). Cyanocobalamin does not exist naturally, being exclusively industrially synthesized as a pharmaceutical agent. The other forms are synthesized in nature by microorganisms, mainly bacteria belonging to the microbiota of animals [4].

Sources and Absorption

Foods of animal origin are sources of cobalamin, in different concentrations. Those of plant origin, in turn, have insignificant amounts of the vitamin. Furthermore, its bioavailability is variable, as well as its stability after cooking and processing processes. Some mammals, such as ruminants, can absorb the vitamin synthesized by their intestinal microbiota. However, in humans, cobalamin synthesis occurs mainly in the large intestine, while its absorption site is found in the small intestine, so the absorption of the vitamin synthesized by the microbiota is insignificant, making the dietary acquisition of the nutrient necessary [1].

In general, the main sources of vitamin B12 are meat, mainly viscera, fish, seafood, and dairy products. It can also be found in certain varieties of seaweed, whose incorporation of the vitamin occurs by symbiotic association with nutrient-synthesizing bacteria. Other fortified industrialized products, such as cereals and vegetable milk, can be important dietary sources [1].

Table 1 shows some examples of cobalamin sources and their respective amounts, according to the Brazilian Food Composition Table (TBCA) [2].

Table 1. Sources of cobalamin and their respective amounts.

FOOD (raw, without adding any other ingredients)	***AMOUNT OF VITAMIN B12 (mcg/100g)***
beef liver	58.34
Chicken heart	8.00
Beef (skirt steak)	5.58
Beef (filet mignon)	3.63
Beef (duckling)	3.02
skinless chicken breast	0.20
whole cow's milk	0.37
mozzarella cheese	2.45
Minas cheese	1.82
Ricotta cheese	0.32
whole natural yogurt	0.44
chicken egg yolk	2.02
whole chicken egg	0.87
octopus meat	20.00
tuna meat	8.19
sardine fillet	7.26
Freshwater fish fillet (average of 7 species)	3.44

After ingestion, cobalamin must be released from proteins bound to the molecule. Hydrolysis, which occurs in the stomach in the presence of hydrochloric acid and proteolytic enzymes, releases the vitamin in its active form. Still in the stomach, it binds to the haptocorrin protein, produced by the salivary glands, preventing its degradation in an acid medium [3,4].

In the duodenum, degradation of haptocorrin and pH change allows the binding of cobalamin to a specific protein called intrinsic factor (IF), produced and secreted by the parietal cells of the gastric mucosa. This binding allows the complex to be recognized and taken up by ileal enterocytes. After endocytosis, cleavage of the B12-IF complex occurs, releasing vitamin B12 into the bloodstream, where it is transported, bound to the transcobalamin II transporter protein, to the liver. Transport to other tissues by plasma depends on binding to another globulin, transcobalamin I [3,4].

Epidemiology of B12 deficiency

Importance of Vitamin B12 for Health

Once absorbed, vitamin B12 is used as a cofactor for enzymes involved in the synthesis of deoxyribonucleic acid (DNA), fatty acids, and myelin. Vitamin B12 deficiency can lead to hematological and neurological symptoms. The excess is stored in the liver, decreasing the probability of deficiency and preventing the establishment of pernicious anemia. However, in cases where vitamin B12 cannot be absorbed, for example, due to dietary insufficiency, malabsorption, or lack of intrinsic factors, hepatic stores are depleted and deficiency occurs [5,6].

Fully Recognized Functions

Vitamin B12 plays an important role in cellular metabolism, especially in DNA synthesis, methylation, and mitochondrial metabolism, as well as playing

important roles in the blood and cardiovascular system [7], regulation of the immune system, and antiviral activity [8,9]. Furthermore, it is an essential nutrient with markedly important functions in the musculoskeletal-gut-brain axis, such as the maintenance of skeletal muscle and neurobehavioral parameters [10] and modulation of the intestinal microbiota [4,11,12].

In this sense, vitamin B12 acts as a cofactor for three main reactions in the body, such as the conversion of methylmalonic acid into succinyl coenzyme A (succinyl-CoA), the conversion of homocysteine into methionine, and the conversion of 5-methyltetrahydrofolate into tetrahydrofolate [13,14]. The reaction involving folate is essential for a series of methyl transfer reactions, being indirectly involved in the synthesis of nucleotides [15]. It has two active forms in the body, adenosylcobalamin (AdCbl) and methylcobalamin (MeCbl), which are formed through two distinct metabolic cascades [16]. AdCbl is the major form stored in cells, particularly in mitochondria, and is a cofactor for the enzyme methyl malonyl-CoA mutase, which catalyzes the isomerization of methyl malonyl-CoA to succinyl-CoA, an essential component of the tricarboxylic acid pathway of carbohydrate metabolism. MeCbl, on the other hand, is found in the cytosol but is also predominantly found in blood and other body fluids [15,17,18].

More Recent Functions That Literature Has Shown

Evidence shows that transcobalamins can suppress systemic inflammation by modulating certain cytokines (ie, interleukin-6), growth factors, and other substrates with antiinflammatory properties under normal physiological conditions. Vitamin B12 can be considered an endogenous negative regulator of nuclear transcription factor-jB (NFjB) through the regulation of nitric oxide, which plays a key role in regulating the immune response to infection [19,20]. Vitamin B12 may contribute to improving the immune response by increasing CD8, T cells, and natural killer T cells [8,9].

In addition, this vitamin has antioxidant properties by favoring the increase in the cytosolic bioavailability of glutathione and is recognized for modulating the diversity of the intestinal microbiota11. Furthermore, adaptive immunity has been associated with low levels of vitamin B12 involving viral infection [21,22].

Also, a meta-analysis of observational studies (n=21,837 persons aged 12 to 90 years) revealed a significant inverse association between dietary intake of vitamin B12 and/or vitamin B12 supplementation and the risk of depression in women [23]. However, there are still controversies regarding the efficacy of vitamin B12 used in supplementation. Some scientists claim that the natural forms of methylcobalamin and adenosylcobalamin may have greater vitamin B12 activity than the synthetic form of cyanocobalamin [5,19].

The assessment of parameters that determine deficiency or subclinical levels of vitamin B12 deficiency may be relevant in the treatment of patients affected by COVID-19 or with persistent symptoms of the disease, given the important functions of this vitamin in the muscleintestine-brain axis. The consumption of a healthy diet containing sources of vitamin B12 and, mainly, supplementation with methylcobalamin and cyanocobalamin, are promising alternatives as adjuvants in the treatment of COVID-19, especially in patients with B12 deficiency or risk of deficiency. Data from randomized clinical trials and meta-analyses have indicated that vitamin B12 in the forms of methylcobalamin and cyanocobalamin can increase serum levels of vitamin B12, resulting in decreased serum concentrations of methylmalonic acid and homocysteine and decreased pain intensity, memory loss, and impaired concentration [24].

Disability Risk Groups

Table 2 presents the main risk groups for vitamin B12 deficiency [4].

Table 2. Risk groups for vitamin B12 deficiency.

✓ Vegetarians and vegans;
✓ 60 years or older;
✓ Pregnant women;
✓ Bariatric patients;
✓ Users of drugs that reduce the acidity produced by the stomach;
✓ Users of medication for diabetes, such as metformin;
✓ Patients with Crohn's disease, ulcerative colitis, celiac disease, or irritable bowel syndrome;
✓ Women with a history of infertility and miscarriage.
✓ Immunosuppressed;
✓ Myelopathy;
✓ Multiple sclerosis;

Prevalence of Deficiency

Table 3 shows the prevalence of vitamin B12 deficiency in Brazil and worldwide.

Table 3. Prevalence of vitamin B12 deficiency globally and in Brazil.

Global Deficiency	Brazil Deficiency
(serum level: <148 pmol (200 ng/L) per liter, and marginal level: 148–221 pmol per liter)	(serum level: <148 pmol (200 ng/L) per liter, and marginal level: 148–221 pmol per liter)
❖ **Denmark, United Kingdom, Turkey, Australia, South India, and Jordan [4,5,25,26]** - Serum and marginal levels below the global average.	❖ 14.2% of Brazilian children aged up to 5 years. ❖ The proportion of children with B12 deficiency is higher in the poorest families (18.85%) and who are black (16.7%) or brown (15.2%) **[27]**.
❖ **USA [5,28,29]** • **Serum level:** - 3% of people aged between 20 and 39 years old, 4% of people aged between 40 and 59 years old, and 6% of people aged 60 years and over **[3]**. • **Marginal level:** - 15% of people aged 20-59 and more than 20% of people aged 60 and over.	❖ Higher prevalence of disability in the different age groups in the North (28.5%), then in the Southeast (14%), in the Midwest (12%), in the Northeast (11.7%), and in the South (9.6%) **[30]**.
❖ **South America, Africa, and Asia [31]** - Prevalence exceeds 40% in different subpopulations (children, young adults, women of childbearing age, pregnant women, and the elderly).	

The clinical condition of vitamin B12 deficiency

Causes

Vitamin B12 is one of the B-complex vitamins and its role in cellular metabolism is closely related to that of folate, another B-complex vitamin. much has been discovered about vitamin B12 deficiency. The main dietary sources of vitamin B12 are animal products because they obtain it through microbial symbiosis. Subsequent release of vitamin B12 from food for intestinal absorption is complex and requires intact functioning of the stomach, pancreas, and ileum. Inadequate intake, especially among children, women of reproductive age, and the elderly, as well as any pathophysiological changes in the stomach, pancreas, and intestine, resulting in disturbances in the absorption of vitamin B12, increasing the risk of deficiency **[4,32]**.

In better-income countries, the main risk factor for the development of vitamin B12 deficiency is pernicious anemia, an autoimmune disease that causes the lack of production of intrinsic factors by gastric parietal cells, necessary for the intestinal absorption of B12 and which eventually leads to the development of anemia and/or severe neurological symptoms. In addition, important risk factors are gastrointestinal surgeries, such as gastric bypass or removal of the terminal ileum, and inflammatory bowel diseases that compromise the absorption of B12. However, in low-income countries, B12 deficiency is largely due to a low intake of B12-rich animal foods, but possibly also to gastrointestinal infections and infestations and hostmicrobiota interactions. Additionally, dietary restrictions for cultural, philosophical, and other reasons such as vegetarianism, increase the risk of cobalamin deficiency and it should be noted that, contrary to common belief, lacto-ovo vegetarians are not exempt from the risk of vitamin B124 deficiency **[33]**.

It is considered relevant to care for babies born to vegetarian or vegan mothers, notably those fed exclusively with breast milk, due to the risk of cobalamin deficiency and the potentially serious deleterious effect at a neurological level **[34]**. On the other hand, the level of vitamin B12 decreases with age and therefore the prevalence of vitamin B12 deficiency increases with age. Studies have shown that the prevalence of vitamin B12 deficiency among the elderly can vary between 5% and 40%, depending on the definition of deficiency used. The chronic use of acid secretion reducers, such as proton pump inhibitors, due to insufficient hydrochloric acid and low pepsin activity, important for the release of vitamin B12 from the food matrix in the stomach, or the use of metformin, due to mechanisms unknown, also increase the risk of cobalamin deficiency **[35,36]**.

Clinical Manifestations

The effects of vitamin B12 deficiency are mainly hematologic and nervous systems. The classic manifestations of vitamin B12 deficiency were initially identified by pernicious anemia. Since then, the spectrum has changed considerably, starting with the recognition of neurological manifestations that usually predominate and can occur in the absence of hematological complications **[37]**, both of which require several years for their development. Vitamin B12 deficiency impairs hematopoiesis due to the key role of vitamin B12 in DNA synthesis. Macrocytic anemia with mean corpuscular volume $\geq$ 100 fL is characteristic of pernicious anemia. However, this characteristic is not always present at diagnosis, as almost 30% of patients do not have macrocytosis, but normocytic anemia. This usually occurs in the case of concomitant iron deficiency

and/or other diseases that cause microcytosis. On the other hand, macrocytosis is usually the first presentation of the hematological alteration for months or years before the onset of anemia. While anemic patients may have symptoms related to the anemia itself, such as weakness, reduced mental concentration, headache, palpitations, or, rarely, cardiac chest pain, patients with non-anemic macrocytosis may only have neurological symptoms. Other important hematological manifestations are thrombosis related to hyperhomocysteinemia and bone marrow failure with pancytopenia **[38]**. Pernicious anemia can affect people of all age groups, but its incidence increases with age, and conservative estimates indicate that it affects 2-3% of individuals over 65 years of age **[4]**.

Regarding neurological manifestations, vitamin B12 plays a role in DNA synthesis of myelin-producing oligodendrocytes and myelin synthesis, thus its deficiency results in demyelination of peripheral and central neurons, which is generally considered the mechanism underlying classic myeloneuropathy. B12 deficiency. Symptoms differ in severity, ranging from mild manifestations to life-threatening disorders **[39]**. In children, adequate B12 levels are crucial for normal neurodevelopment. The first postnatal months are the most dynamic and vulnerable period of brain development. The child may present pallor, psychomotor regression, hypotonia, and lethargy. Computed tomography of the brain may reveal brain atrophy with delayed myelination. The entire picture can be reversible if diagnosed and corrected in time **[40]**. For adults, affected people may present symptoms such as dysesthesia, disturbance in proprioception, paresthesia, numbness in the limbs, and difficulties in activities of daily living, such as writing or buttoning clothes. Vitamin B12 deficiency appears to be particularly common in the elderly primarily due to poor absorption. It may be responsible for neuropsychiatric disorders in this age group, among which, in addition to the above symptoms, may include depression, gait ataxia, falls, optic nerve atrophy, urinary and/or fecal incontinence, autonomic dysfunction, psychosis, and cognitive disorders **[39,41]**.

When To Suspect And Investigate?

Two groups of patients should be considered for diagnostic tests for vitamin B12 deficiency. The first comprises those with clinical evidence of vitamin B12 deficiency, including macrocytic anemia and/or neurological symptoms. The other, which comprises a much larger group of patients, with nonspecific symptoms and often without anemia, represents a greater challenge. This group includes the elderly, individuals with a B12-limited diet such as vegans, notably pregnant and lactating women on a vegan diet and their babies, women with impaired fertility, patients with gastrointestinal diseases, and patients with uncharacteristic and unexplained hematological or neurological symptoms. Such patients should be evaluated and, if vitamin B12 deficiency is present, it must be diagnosed and treated as soon as possible because, once B12-related neurological damage occurs, it may not be completely reversed after treatment **[4,40]**.

Diagnosis of B12 Deficiency

The diagnosis of vitamin B12 deficiency is based on the presence of hematological alterations and the dosages of serum cobalamin, transcobalamin, and metabolites methylmalonic acid (MMA) and homocysteine (Hcys).

Hematological Changes

The lack of vitamin B12 impairs DNA synthesis and causes an imbalance in the maturation of hematopoietic cells in the bone marrow (BM), cells with a high proliferative rate. The erythrocyte generated by this precursor cell will be macrocytic, and the granulocytic cells will have large nuclei generating hypersegmented neutrophils. The megakaryocytic lineage is also affected and, in the most severe cases, thrombocytopenia can be observed. The diagnosis of B12 deficiency should be considered in the presence of macrocytosis with increased Mean Corpuscular Volume (MCV), even before a reduction in hemoglobin is observed. The most pronounced increase in MCV is observed in Pernicious Anemia **[38]**.

Serum Cobalamin Dosage

The most appropriate method for measuring serum vitamin B12 is the competitive immunoassay by electrochemiluminescence, an automated method available in routine laboratories, with better reproducibility than the old microbiological methods or based on radioisotopes **[42]**. The assay detects residual labeled cobalamin, which competes with the cobalamin present in the patient's sample, in the presence of Intrinsic Factor added to the system **[43,44]**. The method of detecting residual cobalamin varies with the manufacturer in the kit and can be done by electrochemiluminescence (Roche®), chemiluminescence (Siemens®, Abbott®, Beckman Coulter®, VITROS®), fluorescence (Siemens® and Abbott®), or even by colorimetric methods **[42]**. Reference values vary with the reagent kit used, are provided by the manufacturer, and must be observed in

the diagnostic decision [44].

The definition of vitamin B12 deficiency, as well as borderline levels, is less clear and there are controversies in the literature. According to the World Health Organization (WHO), the lower limit of normality would be 203 pico grams per mL, but the recommendation is to observe the reference values of each kit used, or in the determination in the clinical laboratory itself [44].

Transcobalamin Dosage

Cobalamin circulates in plasma bound to two transport proteins: haptocorrin, which carries 70 to 90% of circulating vitamin B12, and transcobalamin, which transports the remaining 10 to 30%, forming the complex called holotranscobalamin. Vitamin B12 bound to haptocorrin is not made available to cells, and only that contained in holo transcobalamin is released through binding to its specific receptor. Thus, the dosage of holotranscobalamin better reflects the concentration of active vitamin B12, capable of penetrating the cell and participating in metabolic processes in the cytoplasm and mitochondria [45].

The measurement of transcobalamin can be done by enzyme assay (ELISA) or by electro or chemiluminescence, using a specific antibody. For the measurement of holotranscobalamin, a specific antibody with a high affinity for transcobalamin linked to vitamin B12 is used, which is more sensitive in detecting vitamin B12 deficiency than the measurement of cobalamin and transcobalamin itself. However, it is not widely available in clinical laboratories [45,46]. The reference values depend on the method and those provided by the manufacturer should be observed [44].

Transcobalamin measurement is useful in the evaluation of patients with Pernicious Anemia, as the presence of high anti-IF antibody titers in these patients interferes with the cobalamin measurement, causing falsely elevated levels, but does not interfere with the transcobalamin assay [44,47]. Transcobalamin is not affected by the drop in cobalamin levels observed during pregnancy and can be used to diagnose true deficiency in this condition, since serum cobalamin decreases as pregnancy progresses [48,49]. The transcobalamin level does not it is not affected by renal function, or by age [50]. An increase in transcobalamin is observed in patients with chronic myeloid leukemia and after treatment with B12 [49]. However, there is controversy in the literature about whether the transcobalamin dosage is more sensitive than that of cobalamin itself, as there are discrepancies between the types of populations analyzed [49].

Metabolic Markers: Methylmalonic acid (MMA) and Homocysteine (Hcys)

Patients with borderline cobalamin levels should be evaluated with additional tests to determine their metabolites as secondary markers: methylmalonic acid (MMA) and serum homocysteine (Hcys), which mirror the intracellular activity of cobalamin [4,38]. Adenocobalamin is a coenzyme of mitochondrial methyl malonyl-CoA mutase, while methylcobalamin is a coenzyme of methionine synthase in the cytoplasm51. In mitochondria, Adenocobalamin is a coenzyme of methyl malonyl-CoA mutase for energy generation, generating MMA, which accumulates in case of cobalamin deficiency [4,44]. The accumulation of methyl malonyl-CoA can lead to neural degeneration, causing the characteristic neurological symptoms of cobalamin deficiency [51,52]. MMA is a sensitive and specific marker, however, as it is excreted by the kidney, it may be elevated in patients with renal failure. It will also be elevated in individuals with inborn errors of metabolism. It is a costly test with limited availability [4,44].

In the cytoplasm, methylcobalamin is a cofactor for enzymes in the methionine cycle, in carbon transfer reactions through methylation and demethylation. Methionine, an amino acid ingested in the diet, is transformed into homocysteine, which allows the methylation of important compounds in several metabolic pathways. Homocysteine is again transformed into methionine, by methionine synthetase, in the presence of methylcobalamin, which is its cofactor. For this reason, cobalamin deficiency leads to the accumulation of homocysteine [4,38]. The measurement of serum Hcys is standardized and available in routine clinical laboratories and is performed by high-performance liquid chromatography (HPLC). The measurement should be performed while fasting, for better accuracy [43,44]. Hyperhomocysteinemia is also associated with an increased incidence of arterial and venous thrombosis [4,38].

Increased serum Hcys suggests B12 deficiency, but it can also accumulate in folate deficiency, renal or thyroid failure, and may also vary with age and sex. Thus, MMA is more specific for the diagnosis of B12 deficiency than Hcys [4,38].

Diagnostic Script

Patients at high risk of vitamin B12 deficiency should be evaluated initially with a complete blood count and serum vitamin B12 measurement. In patients with high MCV, even in the absence of anemia, it is important to promptly diagnose vitamin B12 deficiency to avoid neurological sequelae that may not be reversed even

after adequate treatment [53]. The definition of normal values for serum vitamin B12 was done by consensus, indicating that values above 300 pg/mL are considered normal. Values between 200 and 300 pg/mL are considered borderline and require investigation of other markers, transcobalamin, MMA, and Hcys. Levels below 200 pg/mL define vitamin B12 deficiency and have a sensitivity of 90 to 95%, however, a specificity of less than 80% [38,53,54].

Transcobalamin measurement is useful in the evaluation of patients with Pernicious Anemia, as the presence of high anti-IF antibody titers in these patients interferes with the cobalamin measurement, causing falsely elevated levels, but does not interfere with the transcobalamin assay [44,47]. It is challenging to establish normal ranges, especially in specific populations such as the elderly, pregnant women, and ethnic groups. Acquired conditions such as human immunodeficiency virus (HIV) infection, anticonvulsant use, pregnancy, myeloma, and contraceptive use can cause spurious reductions in serum cobalamin in the absence of actual deficiency.

On the other hand, an increase in vitamin B12 can be observed, masking the deficiency in conditions such as alcoholic liver disease, cancer, myeloproliferative diseases, nitrous oxide abuse, and renal failure [4,53]. About 30% of the elderly have levels below 200 pg/ mL without evidence of deficiency or metabolic alteration suggestive of cobalamin deficiency [54]. Holotranscobalamin is a better indicator of the intracellular status of B12, as it represents the active form of B12 in circulation, but there is still a lack of definition of intervals and sensitivity and specificity in the diagnosis of vitamin B12 deficiency [54,55].

If the level of vitamin B12 serum level is borderline, it is recommended to carry out secondary tests that help define B12 deficiency: reduction in the transporter protein, transcobalamin, and increase in accumulated metabolites, MMA and Hcys [4,38,43,44,52,56]. A study with vegetarians in India showed that the combination of B12 dosage, with transcobalamin and homocysteine, increases the diagnostic capacity of subclinical vitamin B12 deficiency [57]. As Hcys will be elevated in folic acid deficiency, which is also associated with macrocytosis, the dosage of folic acid together with that of B12 is useful in differentiating these two conditions [43,44]. MMA is also elevated in renal failure, hypovolemia, and in patients with neurological alterations without evidence of hematological impairment [4].

Interpretation of Results

There is no consensus with internationally defined criteria for the diagnosis of vitamin B12 deficiency. In this scenario, the B12 dosage alone is not enough to define the diagnosis. In patients with borderline levels, or with clinical or laboratory evidence, especially those with conditions that predispose to vitamin B12 deficiency, it will be useful to use the other biomarkers to corroborate the correct diagnosis of the deficiency and allow for prompt treatment. It should be remembered that neurological alterations are precocious and sometimes irreversible, and the delay in diagnosis and adequate treatment can cause sequelae [44].

Diagnosis can be quite difficult in patients who have received partial treatment but who remain disabled [53]. As there are no international standards, each laboratory calibrates and defines its normal range, which must be observed by the requesting physician. This makes it more difficult to compare data in studies that use different methods [44].

Cut-off levels depend on the method and kit used for the dosage, and the limits presented by the manufacturer of the kit used for the dosage should always be considered, and not the values observed in the literature. For vitamin B12, the lower limit of normal ranges from 118 to 221 pmol/L. For transcobalamin, it ranges from 37 to 50 pmol/L [44]. Reference values for MMA and Hcys vary with the methodology and are not standardized [53]. The upper limit levels of markers whose concentration increases in the presence of vitamin B12 deficiency also depend on the methodology and kit used. The upper limit of normality for Hcys ranges from 8 to 13.6 mol/L, and for MMA ranges from 271 to 800 nmol/L [43]. Values close to these detection limits should be considered a probable or subclinical diagnosis [44,58].

The metabolic changes typical of pregnancy make it difficult to diagnose vitamin B12 deficiency during pregnancy, and therefore its prevalence is not well known. There is an increase in MMA during pregnancy, which may indicate a higher metabolic consumption of intracellular vitamin B12 [59]. The parameters are unreliable for mild deficiency, although moderate deficiency is detectable, and population screening for vitamin B12 deficiency in pregnant women is still not recommended, not even for newborns [34,60,61].

Drug Treatment

Drug treatment, whether parenteral (intramuscular or subcutaneous), oral, sublingual, or intranasal, should be started after clinical and/or laboratory evaluation [62]. The chosen route of administration should take into account the underlying disease, the severity of the disability, a greater possibility of treatment adherence, and costs. Vitamin B12 is available in the following

pharmacotherapeutic forms: cyanocobalamin (CN-Cbl), hydroxocobalamin (OH-Cbl), methylcobalamin (methyl-Cbl), and adenosylcobalamin (adenosyl-Cbl). Each of these formulations has different bindings to proteins and receptors that facilitate the production of intracellular cobalamin, which is the biologically active form of vitamin [63]. Regardless of the pharmacological form of B12 used, all of them end up being metabolized until they reach the molecular form of cobalamin within the cell cytoplasm to thus exercise their biological function [63].

There are variations in doses and treatment times described in the literature and Table 4 describes some treatment options [62].

Table 4. Vitamin B12 treatment options.*

Congenital	Acquired
Cobalamin-binding intrinsic factor deficiency (CBLIF) - Treatment: • 1 mg IM OH-CbI/CN-Cbl daily in severe pancytopenias until resolution and then can be spaced according to serum parameter; • Eventually, patients stabilized with 1 mg injections of CN-Cbl or OHCbl twice a year.	**Inpatients without neurological involvement:** • Administer 1 mg IM OH-Cbl 3 times a week for 2 weeks then maintenance 1 mg IM OH-Cbl every 3 months; • Hospitalized patients with neurological involvement, apply 1 mg IM of OH-Cbl on alternate days until clinical improvement and after maintenance with a dose of 1 mg IM OH-Cbl every 2 months; • 50 µg low-dose CN-Cbl in asymptomatic borderline cases.
Imerslund-Grasbeck Syndrome (IGS) • Application of 1 mg IM OH-CbI daily for 10 days and then once a month for life OR Apply 1 mg IM OH-CbI/CN-CbI daily in severe pancytopenia until resolution and then space applications according to metabolic parameters; • Eventually, patients stabilize on 1 mg CN-Cbl or OH-Cbl twice-yearly intramuscular injections with careful monitoring.	**British Columbia Medical Association** • For pernicious anemia or cobalamin malabsorption linked to food malabsorption, administer -1 mg per day orally of CN-Cbl; • In most other cases, an oral dose of 250 µg/day can be used. ❖ **Reserve parenteral administration for patients with neurological symptoms:** • IM 1 mg IM CN-Cbl for 1–5 days, followed by 1–2 mg CN-Cbl orally; • Ensure serum vitamin B12 is normalized after 4 to 6 months.
Cobalamin Deficiency • Apply 1 mg IM OH-CbI daily or an initial dose of 0.5–1 mg IMOH-Cbl or CN-Cbl daily for 4–8 weeks, then the maintenance of 0.5–1 mg OH-Cbl weekly.	**Netherlands** • Dutch Organization of General Practitioners Viewpoint 2014: Treat when cobalamin dosage is below 148 pmol/L and clinical symptoms with 11 mg oral Cbl daily; • Dutch Healthcare Institute Pharmacotherapeutic Compass: IM/SC loading dose of 10 injections of 1 mg OH-Cbl at ≥ 3-day intervals, maintenance dose 1 mg once; • Every 2 months or 300 ug/month, for lifelong supplementation if the underlying cause is not removed; • In case of obvious neurological disorders: 1 mg intramuscularly once or twice a week for 2 years.
Transcobalamin II deficiency • IM administration of 1 mg OH-Cbl or CN-Cbl weekly for life.	**France** **Recommendations from the CARE B12 research group:** **Pernicious anemia:** • Give 1 mg oral CN-Cbl daily for life OR 1 mg IMCN-Cbl daily for 1 week, then once a week for 1 month, then monthly for life OR 1–2 mg IM daily for at least 2–3 months in cases of moderate to severe neurological manifestations; • In food-related cobalamin malabsorption, Crohn's disease, malabsorption or dietary deficiency: 1 mg oral CN-Cbl daily for 1 month then 125 µg-1 mg CN-Cbl daily until a cause is corrected or 1 mg IM CNCbl daily for 1 week then once weekly for 1 month and then spaced for 1–3 months until the cause is corrected 1 mg per day orally for 1-3 months in cases of severe neurological manifestations.
Haptocorrin Deficiency Treatment not indicated	**Australia** **Royal Children's Hospital (Melbourne), Immigrant Health Service—varying treatment regimes** • For infants with clinical deficiency: 250 µg–1 mg IM OH-Cbl (preferred) or CN-Cbl every other day for 1–2 weeks, then 250 µg IM weekly, switch to oral when a child is well and serum levels are normal; • For older children with mild disease, give 1 mg orally daily; • Subclinical or dietary deficiency cases, 50–200 µg orally daily.

* Table adapted from Elangovan & Baruteau [62].

Considering that vitamin B12 is a nutrient present in food, replacement through the oral route seems to be, at first sight, the most indicated. In fact, offering high levels of oral vitamin B12 even in cases of inborn error (Imerslund-grasbexk syndrome) leads to correction of the serum level of vitamin B12 in patients who do not adhere to intramuscular treatment, reinforcing the possibility of using this via. Some advantages can be recognized, such as convenience, lower cost and the habit of using oral drugs present in the population **[64]**. However, there are disadvantages, such as in patients with malabsorption or use of medications that reduce absorption, such as metformin and proton pump inhibitors **[65]**.

In fact, considering the most common situations that indicate supplementation, it can be seen that most patients would have contraindications to oral use due to the difficulty of absorption. The studies by Bolaman et al and Sanz-Cuesta et al showed that high doses of oral vitamin B12 (1,000 to 2,000 μg daily) are necessary so that approximately 0.5-4% of this dose is absorbed by passive diffusion, through a mechanism that does not require intrinsic factor or functional ileus, leading, with this strategy, to an increase in the serum level of B12, even in patients with altered absorptive pathway **[66,67]**.

Replacement via IM is the most traditional choice for treating vitamin B 12 deficiency, in cases of inadequate dietary intake, pernicious anemia, gastrectomy, ileal resection or other malabsorption syndrome **[68]**. Its advantages are the effectiveness of the dose and the fact that it does not undergo changes in the effect of passing through the intestinal absorptive pathways. However, the presence of pain, relatively higher cost, need for application in health services by a qualified professional, possibility of bleeding and infections are observed, and such inconveniences make it difficult and reduce adherence to treatment **[62,68]**.

There is the possibility, not much used, of intranasal use, which leads to adequate absorption and does not suffer the effect of passage through the gastrointestinal tract. However, there is difficulty in accessing the market and it can still cause allergies and rhinitis. The sublingual route will be discussed in more detail below. Therapeutic management based on B12 replacement remains quite heterogeneous across countries, as shown in Table 4.

New treatment guidelines, with different schedules and doses, are being studied. Conventional intramuscular replacement and the oral route have always been the preferred routes used, however, currently, data show that sublingual administration seems to be better tolerated by patients than painful intramuscular injections, in addition to being more efficient and effective than the oral route **[62,69-71]**. This is particularly true for pediatric patients **[68, 72]** and also for elderly patients and patients using medications that reduce B12 absorption **[65,70]**. Randomized studies with a large number of patients and additional outcomes, such as clinical symptoms and other biochemical parameters, in addition to plasma levels of isolated B12, have been carried out in order to validate the new therapeutic options **[62,73]**. In severe cases, it seems that the use of IM replacement for the acute phase is the most used and for the maintenance phase the use of oral, intranasal and especially the sublingual route are indicated. The sublingual route has been highlighted as the most promising, especially because it is very comfortable to use and with greater and faster absorption, raising the serum level of B12 with great effectiveness **[73]**.

Sublingual Methylcobalamin

Vitamin B12 was traditionally administered by intramuscular injections. However, the sublingual (SL) route is equally effective and is increasingly being considered for supplementation. The SL method allows direct absorption of B12 under the tongue, avoiding intestinal absorption. In addition, it has several advantages, for example, it is less costly, results in high patient satisfaction, does not require a hospital visit, is not painful, and does not result in injection-related injuries.

In a prospective study with individuals with vitamin B12 deficiency, sublingual and oral administration of 500 μg of cobalamin was equally effective in correcting their concentrations. Much of the increase was already achieved in the first week and all subjects were in the preclinical stage of the deficiency, that is, they did not have symptoms. About 50% were vegetarians and this explains the reduced level of vitamin B12 **[71]**.

Bensky and others. compared the effectiveness of SL and IM administration of B12 in terms of normalizing serum cyanocobalamin levels. In their study, 3451 patients received VB12 SL (1000 μg/day for 6 months) and 830 patients received 1000 μg/dose of VB12 IM (alternate days for 2 weeks, followed by once a week for 4 weeks). Post-treatment values differed significantly between the two groups ($p<0.001$). The authors concluded that the SL route should be the first-line treatment choice in patients with VB12 deficiency **[69]**.

A retrospective study included 129 patients with Vitamin B12 deficiency (serum level $\leq$ 200 pg/mL) aged between 5 and 18 years. They were divided into three

treatment groups intramuscular cyanocobalamin, sublingual cyanocobalamin, and SL methylcobalamin. After vitamin B12 therapy, serum levels increased significantly in all patients (Table 5). Cyanocobalamin and methylcobalamin SL was found to be as effective as IM cyanocobalamin for children with vitamin 12 deficiency in correcting the serum vitamin level and hematological abnormalities. The retrospective design is the most limiting factor of the study. Therefore, methylmalonic acid and homocysteine levels were not investigated. Furthermore, the short follow-up period and the relatively small sample size are other limitations of the study. This study concludes that cyanocobalamin and methylcobalamin SL is as effective as cyanocobalamin IM in correcting serum B12 levels and hematological abnormalities in children with deficiency. However, SL formulations, which are cheaper, safer, painless, and practical, have been used less than IM formulations for decades. The results suggest that SL formulations can be used as a first-line treatment in children with B12 deficiency **[68]**.

Table 5. Tukey's post hoc analysis of groups.

		Varying levels of Serum B12 Before and after treatment
Group cyanocobalamin *IM (n = 47)	Mean ± SD	454.5 ± 118.4
Group cyanocobalamin *SL (n = 43)		346.2 ± 108.3
P		< 0.001
Group cyanocobalamin IM (n = 47)	Mean ± SD	454.5 ± 118.4
Group methylcobalamin SL (n = 39)		418.8 ± 67.6
P		0.032
Group cyanocobalamin SL (n = 43)	Mean ± SD	346.2 ± 108.3
Group methylcobalamin SL (n = 39)		418.8 ± 67.6
P		< 0.001

* IM: Intramuscular; SL: Sublingual

Vitamin B12 deficiency is a preventable cause of growth and developmental delays in children, and it is the most common cause of childhood megaloblastic anemia, and the cause is often nutritional. The study included 158 patients with serum vitamin B12 deficiency (serum vitamin B12 level <300 ng/L) aged 0 to 3 years. According to the modalities of vitamin B12 treatment, patients were divided into three groups: oral cyanocobalamin (group 1), sublingual methylcobalamin (group 2), and intramuscular cyanocobalamin (group 3). Mean values of vitamin B12 levels increased to over 300 ng/L in all three groups. Sublingual methylcobalamin was found to be as effective as oral and intramuscular cyanocobalamin in improving vitamin B12 levels in children aged 0 to 3 years **[74]**.

Studies correlating total homocysteine (tHcy) concentrations with arteriosclerosis have become a subject of interest among health professionals and the public. Several commercial preparations of vitamin B complexes have been marketed as supplements designed to reduce elevated tHcy levels. Among these preparations are those that have been specifically designed for sublingual administration. In that study, 41 subjects, aged 50 to 80 years with total serum tHcy concentrations greater than 11 μmol/L, were treated with a six-week regimen of the vitamin B complex. Each B complex consisted of 1000 μg of vitamin B 12 (as methylcobalamin), 400 μg of folate (as folic acid), and 5 mg of vitamin B6 (as pyridoxine). A statistically significant reduction in tHcy values was observed in both groups, which received the vitamins orally or sublingually, after completion of the 6-week protocol. There was no statistically significant difference in serum concentrations of tHcy between groups, confirming the idea that there is no difference in efficacy between the two methods of administration of vitamin complexes **[75]**.

The frequency of recurrent aphthous stomatitis (RAS), the most common lesion of the oral mucosa seen in primary care, is up to 25% in the general population. A randomized, doubleblind, placebo-controlled trial was performed with primary care patients. A sublingual dose of 1000 mcg of vitamin B12 was used in patients in the intervention group for 6 months. In total, 58 patients with RAS participated in the study: 31 were included in the intervention group and 27 were included in the control group. The duration of relapses, the number of ulcers, and the level of pain were significantly reduced ($p<0.05$) at 5 and 6 months of vitamin B12 treatment, regardless of baseline levels of vitamin B12 in the blood. Vitamin B12 treatment, which is simple, inexpensive, and low-risk, appears to be effective for RAS patients regardless of serum vitamin B12 level **[76]**.

Fibromyalgia (FM) as a prototypical nociplastic pain condition presents a difficult therapeutic situation in

many cases. Given the promising data on the effect of vitamin B12 on improving pain and cognitive functions, this study aimed to determine the effectiveness of vitamin B12 on the severity of symptoms and the psychological profile of patients with FM. This study showed that a short course of sublingual vitamin B12, 1000 mcg per day, significantly improves FM severity and anxiety score. Vitamin B12 has a strong potential to be considered at least as an adjuvant therapy for FM. Preclinical and clinical studies have shown that vitamin B12 is an essential micronutrient involved in preserving the balance of inhibitory and excitatory pain neurotransmitters, moderating inflammation and, consequently, in several behavioral processes, including sleep, learning, memory, and sense of purpose. pain. Based on this, vitamin B12 could be an adjunctive therapy in FM patients suffering from nociplastic pain and other core symptoms such as fatigue, sleep, and cognitive disturbances. This prospective study was the first to attempt to clarify the effect of vitamin B12 in FM patients concerning symptom severity and psychological profiles. Considering the promising effects of vitamin B12 in improving disease severity and anxiety in FM patients, as well as the absence of any side effects at the studied dosage, we postulate that vitamin B12 has a strong potential to be considered, at least, as adjuvant therapy of FM **[77]**.

Still, a randomized clinical study developed by the authors Parry-Strong et al. 2016 compared a single intramuscular injection of hydroxocobalamin with a 3-month course of sublingual supplements of 1 mg/day of methylcobalamin on serum vitamin B12 concentrations in participants with type 2 diabetes treated with metformin. Participants on metformin treatment with vitamin B12 concentrations below 220 pmol/L were recruited. A total of 34 participants were randomized; 19 for the tablet and 15 for the injection. The mean (SD) age, duration of diabetes, and duration of metformin use were 64.2 (7.3) years, 13.7 (6.4) years, and 11.6 (5.0) years, respectively. After 3 months, mean (SD) vitamin B12 was 372.1 (103.3) pmol/L in the pill group (n=19) compared to 251.7 (106.8) pmol/L in the injection group (n=15), ANCOVA estimated difference -119.4 (95% CI -191.2 to -47.6), p=0.002. After 6 months, the mean (SD) serum B12 was 258.8 (58.7) pmol/L in the pill group (n=17) and 241.9 (40.1) pmol/L in the injection group (n=17) =15). The decrease in serum vitamin B12 level in patients with type 2 diabetes treated with metformin can be corrected through treatment with hydroxocobalamin injections or sublingual methylcobalamin supplements **[78]**.

Furthermore, vegetarians and vegans are more vulnerable to vitamin B12 deficiency with serious risks of megaloblastic anemia, cognitive decline, neuropathy, and depression. Based on this, a 12-week randomized controlled clinical study designed by authors Del Bo' et al. 2019 evaluated the ability of two different sublingual dosages of vitamin B12 (350 μg/week vs 2000 μg/week) to improve cyanocobalamin (vitamin B12) nutritional status in marginally deficient vegans and vegetarians. A total of 40 subjects with marginal vitamin B12 deficiency were enrolled and randomly divided into two groups: test group Ld (low dose, 350 μg/week) and control group Hd (high dose, 2000 μg/week) vitamin B12 supplementation. Blood samples were collected at baseline and after 15, 30, 60, and 90 days of the intervention for the determination of vitamin B12, related metabolic markers, and blood cell count. There was a significant effect of time ($p<0.0001$) and of the time × treatment interaction ($p= 0.012$) on the serum concentration of vitamin B12, which increased after 90 days of supplementation (Ld and Hd) compared to baseline. Both supplements increased ($p<0.0001$, effect of time) levels of holotranscobalamin, succinic acid, methionine, and well-being parameter, while decreasing ($p< 0.0001$, effect of time) levels of methylmalonic acid, homocysteine, and folate compared to baseline. Therefore, both supplements were able to restore adequate serum vitamin B12 concentrations and improve levels of related metabolic blood markers in subjects with marginal deficiency. The results support the use of a sublingual dose of 50 μg/day (350 μg/week) of cobalamin, rather than 2000 μg/week (provided as a single dose), to achieve a state of vitamin B12 nutritional adequacy in this target population **[79]**.

Added to this, a study carried out by the authors Gharibpoor et al. 2022 determined the effectiveness of a daily dose of 1000 mcg of oral vitamin B12 on the severity of symptoms and the psychological profile of patients with fibromyalgia (FM). Diagnoses were confirmed by an American College of Rheumatology (ACR)-based rheumatologist. Patients were instructed to take a daily dose of 1000mcg of vitamin B12 for fifty days. Outcome measures, including the Revised Fibromyalgia Impact Questionnaire (FIQR), Hospital Anxiety and Depression Scale (HADS), 12-item short-form health questionnaire (SF-12), and Visual Analogue Pain Scale (pain- VAS) were completed by patients before and after treatment. Of the 30 eligible patients, 28 patients completed the study protocol. The patients were female with a mean age of 47.50 ± 8.47 years. FIQR scores in all domains significantly improved after treatment (total FIQR: 49.8 ± 21.86 vs 40.00 ± 18.36, p-value < 0.01; function: 13.17 ± 7.33 vs 10 .30 ± 5.84,

p-value: 0.01; overall: 10.32 ± 6.22 vs 8.25 ± 6.22, p-value: 0.03; symptoms: 26.30 ± 10.39 vs 21 .44 ± 8.58, p-value < 0.01). Vitamin B12 also improved anxiety scores from 9.33 ± 4.30 to 7.70 ± 3.60, pvalue: of 0.01. Depression, pain-VAS, and SF-12 did not improve after treatment. Analysis of the generalized estimating equations (GEE) showed that the improvement in the total FIQR score is not confounded by the improvement in patients' anxiety and baseline characteristics. This study showed that a short course of sublingual vitamin B12, 1000 mcg per day, significantly improves FM severity and anxiety score **[80]**.

Also, folic acid and vitamin B12, alone or in combination, have been used to reduce homocysteine (Hcy) levels in dialysis patients. Therefore, a clinical study carried out by the authors Naseri et al. 2016 evaluated the effectiveness of high doses of oral folate and vitamin B12 in reducing plasma Hcy levels after a 12-week treatment. A total of 32 dialysis patients aged 10-324 months were screened for hyperhomocysteinemia and included in this study. Then, cases with hyperhomocysteinemia received oral folate 10 mg/day with sublingual methylcobalamin 1 mg/day for 12 weeks. In the pre-and post-intervention phases, plasma Hcy concentration, serum folate, and vitamin B12 levels were measured. A total of 18 (56.2%) patients had hyperhomocysteinemia. Vitamin B12 and folate levels were normal or elevated in all cases. Two patients were lost due to transplantation or irregular medication consumption. Plasma Hcy levels were reduced in all and reached normal values at 50%. A statistically significant difference was found between early Hcy levels and post-intervention levels (95% CI, 5.1-8.9, p=0.0001). Therefore, oral folate 10 mg/day in combination with sublingual vitamin B12 1 mg/day may be considered a favorable treatment for hyperhomocysteinemia in dialysis patients **[81]**.

A randomized, double-blind, placebo-controlled clinical trial developed by Carrozzo et al. 2009 looked at administering a sublingual tablet taken by participants every day before bed for 6 months. The test group received sublingual vitamin B12 tablets (1000 mcg of vitamin B12), while the control group took a placebo of the same shape, size, color, and taste. A total of 58 participants with recurrent aphthous stomatitis (RAS) were recruited, with 31 allocated to the intervention group and 27 to the control group. The duration of relapses, the number of ulcers, and the level of pain were reduced significantly (p<0.05) at 5 and 6 months of vitamin B12 treatment, regardless of the initial levels of vitamin B12 in the blood. During the last month of treatment, a significant number of participants in the intervention group achieved "no aphthous ulcers" a total of 74.1% vs 32.0%, p<0.01. Thus, vitamin B12 treatment, which is simple, inexpensive, and low-risk, seems to be effective for RAS patients, regardless of serum vitamin B12 level **[82]**.

Besides, breast cancer patients receiving endocrine therapy with aromatase inhibitors (AIs) often experience musculoskeletal and joint-related side effects. Thus, the authors Campbell et al. 2018 in phase II clinical study evaluated the effect of sublingual vitamin B12 supplements on musculoskeletal symptoms, such as pain and arthralgias induced by AIs, and correlated the response with serum and inflammatory biomarkers in 41 participants. Participants diagnosed with invasive breast cancer (stages I-III) and with significant musculoskeletal symptoms associated with AIs were included. Only patients with a mean pain score ≥ 4, as assessed by the Brief Pain Inventory-Short Form (BPI-SF) questionnaire, were included in the study. Participants received 2,500 mcg of sublingual vitamin B12 daily for 90 days. Mean pain scores improved by 34% (p<0.0001) at 3 months compared to baseline. Furthermore, a 23% improvement in pain was observed (p=0.0003). Analysis of the FACT-ES score results showed improvement on all scales. A decrease in pain score was correlated with an increase in serum B12 levels **[83]**.

Finally, a study published in the Lancet by the authors Delpre, Stark, and Niv, in 1999, showed that the efficacy of sublingual cobalamin replacement therapy was studied in 18 people with cobalamin deficiency. The administration was effective and convenient, and adherence was high **[84]**.

Consensus recommendations

Considering that:

a) Vitamin B12 fulfills a large number of functions in human metabolism;
b) The risk groups for nutritional B12 deficiency are wide, including patients with low intake, intrinsic factor production deficiency, anatomical alterations, malabsorption diseases, gastric acidification problems, elderly, and vegetarians, among others;
c) Deficiency can occur not only due to insufficient intake but also due to absorption problems, due to genetic or acquired diseases;
d) The prevalence of B12 deficiency is high in all age groups and throughout the national territory;
e) The manifestations of the deficiency can be varied, involving practically the whole organism, and are more common in the hematological and neuropsychomotor systems;

f) The onset of symptoms often happens abruptly in untreated patients;
g) Neurological symptoms often appear even in the absence of hematological changes;
h) The diagnosis of B12 deficiency is eminently clinical and can be confirmed by laboratory tests;
i) Drug treatment is possible and effective in reversing the condition of the deficiency;
j) The absorption of B12 from food and supplements in the digestive tract depends on its anatomical and functional integrity and, frequently, there are damages in these functions, obliging the treatment to be done through other routes;

It is recommended that

a) The possibility of diagnosing B12 deficiency is always considered in all patients treated;
b) Prophylactic supplementation should be performed in all patients belonging to risk groups, regardless of laboratory tests;
c) For the diagnosis of B12 deficiency in the office, clinical and epidemiological data are almost always sufficient, however, confirmation should be done through serum B12 dosage. Values above 300 pg/mL should be considered normal; B12 values between 200 and 300 pg/mL should be considered borderline and require investigation of other markers, such as transcobalamin, methylmalonic acid, and homocysteine; levels below 200 pg/mL should be considered as indicative of unequivocal deficiency;
d) Patients with installed B12 deficiency or those with an indication for prophylactic supplementation should be treated as quickly as possible and through an individualized therapeutic scheme that guarantees the reversal of the condition;
e) The use of the oral route is indicated only for those patients in whom there is no problem related to absorption in the digestive tract and in which there is no therapeutic urgency;
f) The parenteral and sublingual routes guarantee the passage of B12 directly to the circulatory route and, for this reason, are the preferred routes;
g) Both the parenteral and sublingual routes are effective in correcting and maintaining the nutritional status of vitamin B12, even in patients who have problems related to absorption in the digestive tract;
h) Considering the risks related to the parenteral route and the patient's comfort, and taking into account the efficiency found in the literature, the present consensus considers that the sublingual route may be the choice in most cases.

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