VITAMIN B12 DEFICIENCY: DIAGNOSTIC CHALLENGES AND LABORATORY BIOMARKERS

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Annotation. *Vitamin B12 is a B vitamin that has an important role in cellular metabolism, especially in DNA synthesis, methylation and mitochondrial metabolism. Clinical B12 deficiency with classic haematological and neurological manifestations is relatively uncommon. However, subclinical deficiency affects between 2.5% and 26% of the general population depending on the definition used, although the clinical relevance is unclear. B12 deficiency can affect individuals at all ages, but most particularly elderly individuals. Infants, children, adolescents and women of reproductive age are also at high risk of deficiency in populations where dietary intake of B12-containing animal-derived foods is restricted. Deficiency is caused by either inadequate intake, inadequate bioavailability or malabsorption. Disruption of B12 transport in the blood, or impaired cellular uptake or metabolism causes an intracellular deficiency. Diagnostic biomarkers for B12 status include decreased levels of circulating total B12 and transcobalamin-bound B12, and abnormally increased levels of homocysteine and methylmalonic acid. In this research, we will discuss vitamin B12 deficiency, various diagnostic tests, and an algorithm used in the investigation of vitamin B12 deficiency.*

***Key words**: B12- deficiency anemia, internal Castle factor, macrocytic anemia, methylmalonic acid, homocysteine.*

Vitamin B12 deficiency is not uncommon, particularly in certain patient populations such as elderly, neuropsychiatric, and psychiatric patients. Hematological changes, including macrocytic anemia, are a more well-known symptom, but are not always present or are revealed later in the disease course [4].

The classic picture of B12- deficiency anemia was described by Thomas Addison in the middle of the XIX century: glossitis with characteristic neurological manifestations on the background of anemic syndrome. In such cases, the recognition of the disease is not difficult and requires only laboratory confirmation before prescribing treatment, the timely start of which often leads to a complete recovery of the patient. A serious diagnostic problem is the most common subclinical form of vitamin B12 deficiency- without the development of anemia. Delayed therapy can lead to the development of persistent neurological abnormalities. In this regard, knowledge of the nonspecific manifestations of vitamin B12 deficiency, the causes of its occurrence, as well as informative approaches to diagnosis and effective methods of treatment of this condition is of particular importance [1,5].

The pathological process in vitamin deficiency B12 affects almost all organs and systems, the nature and severity of clinical manifestations in each case are individual and depend, in addition to the duration of existence and severity of the deficiency, on a number of concomitant factors. Moderate deficiency manifests itself with clinical manifestations of a general anemic syndrome (shortness of breath, palpitations, pallor, dizziness, etc.), Hunter's glossitis (papillary atrophy, "varnished" tongue), and then neurological disorders (distal sensory neuropathy) are added. However, such a sequence of symptoms is not necessary at all: neurological manifestations often precede the

development of anemic syndrome and abnormalities in clinical blood analysis (macrocytic anemia, pancytopenia), and Hunter's glossitis occurs in no more than 10% of cases [2].

Degenerative changes in the spinal cord occur in the demyelination of the fibers that make up the posterior and lateral cords. Without treatment, double sided peripheral neuropathy can progress to axonal degeneration and neuronal death. These changes lead to impaired proprioceptive and vibrational sensitivity and areflexia. There is an uncertain gait, awkwardness of movements, which are replaced by spastic ataxia. Damage to the peripheral nerves is manifested by impaired perception of taste and odors, atrophy of the optic nerve. In extremely severe cases, such a picture ends with the development of dementia, episodes of extensive psychosis with hallucinosis, paranoia and severe depression are possible [3]. In extremely severe cases, such a picture ends with the development of dementia, episodes of extensive psychosis with hallucinosis, paranoia and severe depression are possible. In this regard, vitamin B12 deficiency should be included in the differential diagnostic range in patients with neurological symptoms of unclear genesis, and delay in diagnosis and treatment can lead to irreversible consequences. In 20% of cases, such neurological phenomena are detected in isolation without concomitant anemia. In this regard, vitamin B12 deficiency should be included in the differential diagnostic range in patients with neurological symptoms of unclear genesis, and delay in diagnosis and treatment can lead to irreversible consequences [6].

The cause of classical B12- deficient anemia is the autoimmune destruction of the parietal cells of the stomach, which leads to the development of atrophic autoimmune gastritis with reduced production of internal Castle factor, in combination with which 99% of vitamin B12 entering the stomach (external Castle factor) is absorbed. The causes of vitamin B12 deficiency also include reduced consumption of foods rich in this vitamin and alcohol abuse.

However, in recent years, the leading etiological factor has been a decrease in the release of vitamin B12 associated with food transport proteins due to a hypo- or anacid condition, including druginduced (taking proton pump inhibitors, H2- histamine receptor blockers, antacids), in patients after gastric surgery. It has been shown that the risk of developing a B12- deficient condition is directly proportional to the dosage and duration of administration of proton pump inhibitors and H2-histamine receptor blockers, and after their withdrawal the risk is significantly reduced. The development of vitamin B12 deficiency has also been shown in patients taking metformin for a long time. The mechanisms of deficiency development in this case have not been established, however, there is a dependence on the duration of treatment and the dose of the drug. An increase in homocysteine levels against the background of a decrease in vitamin B12 concentration increases the risk of cardiovascular complications in patients with type 2 diabetes mellitus, and therefore, according to some authors, screening for vitamin B12 deficiency among patients receiving metformin is necessary. These and other, more rare causes of deficiency are presented in the table.

Despite the high frequency and potential severity of B12-deficient conditions, there are still no international consensus documents on diagnostic and treatment methods. Over time, the diagnostic arsenal changes, clinical experience accumulates, and in this regard, it seems important to familiarize doctors with modern guidelines for the management of patients with this pathology [2,4].

The clinical significance of an isolated decrease in cobalamin levels (without clinical manifestations) is questionable, at the same time, in patients with obvious clinical manifestations of vitamin B12 deficiency, cobalamin levels may remain within normal limits ((falsely normal cobalamin content). Consequently, in some cases it is justified to use additional diagnostic methods to identify functional or biochemical deficiency. Such tests include the

determination of plasma levels of homocysteine, methylmalonic acid (MMA) and serum concentrations of holotranscobalamin. Unfortunately, these studies can not be performed in all laboratories, and the lack of standard reference boundaries also complicates the situation. Thus, it is necessary to state the absence of a "gold standard" for the diagnosis of vitamin B12 deficiency. Due to the fact that cobalamin and folic acid are involved in the same biochemical processes and deficiency of both vitamins leads to the development of macrocytic anemia, their level is determined simultaneously. With a true vitamin B12 deficiency, folic acid levels tend to be normal or even elevated, but a combined deficiency is also possible. With a deficiency of vitamin B12 and /or folic acid, a clinical blood test reveals microcytic, hyporegenerative anemia in combination with the appearance of hypersegmented neutrophils. At the same time, these changes are not specific and may be absent in the early and even late stages of deficiency. Macrocytosis occurs in myelodysplastic syndrome, in patients who abuse alcohol, suffer from liver diseases, chronic obstructive pulmonary disease, hypothyroidism, hemolysis, after bleeding and splenectomy. The development of macrocytosis is also possible against the background of taking certain medications: used to treat patients infected with human immunodeficiency virus/acquired immune deficiency syndrome, anticonvulsants, folic acid antagonists, cytostatics, trimethoprim / sulfamethoxazole, biguanides, cholethyramine. Cases of false macrocytosis due to cold agglutination, hyperglycemia and severe leukocytosis have also been described. In the absence of additional clinical and laboratory phenomena, isolated macrocytosis does not require additional examinations. At the same time, in the presence of vitamin B12 deficiency, macrocytosis may be absent: in 25% of patients with neurological manifestations, the size of red blood cells does not change, i.e. the normal size of red blood cells does not exclude the presence of vitamin B12 deficiency [3,7].

Determination of serum cobalamin levels is currently the recommended initial method of examining patients with suspected vitamin B12 deficiency. In this case, the total cobalamin is determined: both the "inactive" form and the "active" form. Unfortunately, the sensitivity and specificity of the method are far from 100%, which does not allow us to consider it as the main method for making a diagnosis. In doubtful cases, it is necessary to study the substrates involved in the same metabolic processes as vitamin B12 (homocysteine, MMA), the level of which is used to judge the metabolic or biochemical deficiency of vitamin B12 [1,6].

It is believed that the level of serum total cobalamin < 148 pmol/l (200 ng/ L) has a sensitivity of 97% in the diagnosis of vitamin B12 deficiency [8]. At the same time, there is no consensus on what should be considered a threshold value. In particular, it is unclear what level should be considered a subclinical deficiency for its diagnosis and treatment in the early stages. A high titer of antibodies to Castle's internal factor can lead to falsely normal results of the analysis for the level of cobalamin, which should be taken into account when interpreting the results [2].

It is also necessary to remember the reasons for the increase in cobalamin levels: taking vitamin B12 preparations (including complex ones) orally or in injections (in such cases, the picture of the primary blood also changes: for example, on the 8th day of such injections, patients with cobalamin deficiency may experience reticulocytosis, which, with a poorly collected medical history, directs the diagnostic search along the wrong path), alcoholic liver disease, cirrhosis of the liver and acute hepatitis, renal insufficiency, chronic myeloid leukemia, acute leukemia, ischemic polycythemia and myelofibrosis, hepatocellular cancer, metastatic liver damage, alcoholic liver disease, cirrhosis of the liver and acute hepatitis, renal insufficiency, chronic myeloid leukemia, acute leukemia, ischemic polycythemia and myelofibrosis, hepatocellular carcinoma, metastatic liver damage, breast cancer and colon cancer. In such

cases, a paradoxical increase in the level of cobalamin in the blood is possible in the presence of symptoms of vitamin deficiency B12 and confirmation of biochemical (metabolic) deficiency by increasing concentrations of homocysteine and MMA. An increase in the level of cobalamin in these cases is associated with an increase in the concentration of inactive vitamin B12 (chologaptocorrin), while the level of transcobalamin II and its associated active form, mine B12, decreases without providing tissues with sufficient amounts of the latter. In such patients, in parallel with the exclusion of these diseases, trial treatment with vitamin B12 preparations may be indicated [3].

Cobalamin deficiency in the early stages, sometimes before the development of clinical manifestations, leads to an increase in the serum concentration of homocysteine, and the severity of the increase is proportional to the severity of the deficiency. However, an increase in homocysteine levels is nonspecific for vitamin B12 deficiency, it is also noted in folic acid deficiency, vitamin B6, in patients with renal insufficiency, hypothyroidism, and a hereditary predisposition to hyperhomocysteinemia is known. The reference limits for homocysteine are also not unified, although in most laboratories a concentration of > 15 mmol / l is taken as an increased level, at the same time, the need for each specific laboratory to develop its own standards, depending on the analysis method used, is recognized. When determining the level of homocysteine, especially great importance is attached to the preanalytical stage of analysis: the blood sample should be stored in a cooler, and centrifugation should be carried out within 2 hours after receiving the sample.

The plasma level of MC increases with vitamin B12 deficiency, but its increase is also noted in patients with renal insufficiency, hemaconcentration and the syndrome of excessive bacterial growth in the small intestine. Despite these limitations, a pronounced increase in MMA (> 0.75 mmol/l) is typical precisely for vitamin B12 deficiency, although different laboratories give

different upper limits of normal values (from 0.27 to 0.75 mmol/l). Despite the limits, a pronounced increase in MC (> 0.75 mmol/l) from typical precisely for vitamin B12 deficiency, although different laboratories give different upper limits of normal values (from 0.27 to 0.75 mmol/l). A falsely low level of cobalamin with a normal value of MMA and homocysteine may be observed in patients with paraproteinemia, while after treatment of this pathology, the level of cobalamin returns to normal [5].

The higher specificity of determining the level of holotranscobalamin compared with the level of cobalamin in the diagnosis of vitamin B12 deficiency has been shown in several studies evaluating the level of MMA as a reference method [10-12]. In healthy people, the level of holotranscobalamin ranges from 19-42 pmol / l (lower limit of the norm) to 134-157 pmol/l (upper limit of the norm). According to the results of a recent study, the level < 32 pmol/ l should be considered the most normal value of this indicator. Determining the level of holotranscobalamin in routine clinical practice will reduce the proportion of results that are not subject to unambiguous interpretation, especially in patients over 65 years of age. The level of cobalamin decreases physiologically during pregnancy and when taking hormonal contraceptives, however, the concentration of holotranscobalamin is not subject to such fluctuations, which makes it a more reliable tool for assessing vitamin B12 deficiency in these clinical situations [2,4]. If we consider that the determination of the concentration of holotranscobalamin is possible with the standard equipment of a modern clinical laboratory, and also does not require special conditions at the pre- analytical stage, the diagnostic advantages of this test suggest that in the near future it will be considered as a method of selection at the beginning of a diagnostic search if a deficiency is suspected vitamin B12 [4].

Autoimmune gastritis is one of the causes of an irreversible decrease in the production of CIF, which leads to the development of vitamin B12

deficiency and megaloblastic anemia. It can accompany other autoimmune diseases such as Hashimoto's thyroiditis, type 1 diabetes mellitus, vitiligo and hypocorticism. The diagnosis of autoimmune gastritis is confirmed by the presence of antibodies to CIF (AB- CIF). AB-CIF have high specificity in the diagnosis of autoimmune gastritis (low frequency of false positive results), while the sensitivity of this analysis is low (40-60%), therefore, the absence of an increase in the level of AB-CIF does not allow us to exclude the diagnosis of "autoimmune gastritis" (in this case, we can talk about "seronegative" variant by analogy with seronegative variants of other autoimmune diseases). The frequency of seropositive results increases with age, and is also higher in some ethnic groups (Latinos, African Americans). Autoimmune gastritis is a relatively rare disease, therefore, screening in the general population is unjustified. The probability of diagnosis increases in the presence of concomitant autoimmune diseases, as well as in patients with hereditary autoimmune gastritis. A high titer of AB in CIF can lead to false normal results of a study on the serum level of cobalamin. A positive test result for antibodies to gastric parietal cells is found in 10% of healthy people, i.e. the test has a low specificity, and therefore a positive result is insufficient for the diagnosis of autoimmune gastritis [1,7]. If autoimmune gastritis is suspected, esophagogastroduodenoscopy (EGDS) is indicated to exclude malignancy, at the same time, there is no need to perform a control EGDS in such patients.

Conclusions. Vitamin B12 deficiency represents an important diagnostic challenge due to the frequent absence of classical hematological manifestations. Reliance on serum cobalamin levels alone is insufficient, as normal values may coexist with functional deficiency. The combined assessment of homocysteine, methylmalonic acid, and holotranscobalamin significantly improves diagnostic accuracy, particularly in high-risk populations. Implementation of an integrated laboratory diagnostic algorithm

enables early detection and prevention of irreversible neurological complications.

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