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The diagnosis of vitamin B12 deficiency using biomarkers and algorithms

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ABSTRACT

As a cofactor for mitochondrial methylmalonyl-CoA mutase and cytosolic methionine synthase (MS), vitamin B12 (cobalamin, Cbl, B12) is an essential water-soluble micronutrient (MCM). Homocysteine (Hcy) and methylmalonic acid (MMA), respectively, accumulate as a result of the inactivation of MS and MCM caused by a Cbl deficiency, whether caused by dietary deficiencies or genetic faults in Cbl metabolism. The preferred blood indicators used to assess B12 status include holo-transcobalamin (holo-TC), Hcy, and MMA together with total B12 and its bioactive protein-bound form. Clinically speaking, a vitamin B12 shortage results in megaloblastic anaemia, neurological degeneration, and, if untreated, death. Subclinical vitamin B12 deficiency typically manifests without symptoms or with very mild general symptoms that are frequently misdiagnosed as unrelated illnesses. It is typically defined as a total serum B12 level of less than 200 pmol/L. The diagnostic significance of blood vitamin B12 as a standalone marker has now been demonstrated by numerous investigations. Vitamin B12 deficiency is not necessarily indicated by low serum levels, and the presence of normal or even high serum vitamin B12 levels has been linked to severe functional deficiencies of the micronutrient. This review covers the methods used to diagnosis B12 insufficiency, the utility and limitations of existing biomarkers of vitamin B12 status in newborn screening, infant and adult diagnostics, and atypical discoveries of vitamin B12 status in a variety of human illnesses.

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Keywords: vitamin B12, cobalamin, homocysteine, methylmalonic acid, holo-transcobalamin, diagnostic algorithm, functional deficiency of B12.

INTRODUCTION

Vitamin B12 Deficiency

All bodily cells need vitamin B12, a water-soluble micronutrient with the chemical name "cobalamin" (B12 = Cbl). B12 must be obtained from diet and a complicated intracellular pathway because humans cannot make it. This ensures that the vitamin is processed and delivered to its intended locations (Hannibal et al., 2009). A global public health concern is vitamin B12 insufficiency brought on by poor consumption and malabsorption. According to estimates, between 15 and 20% of older Americans are B12 deficient (Allen, 2009). Around 10% of the senior male population in Germany and 26% of the old female population have insufficient levels of vitamin B12 (Hartmann, 2008; Grober et al., 2013). Over 650 million people in India, or about 75% of the population, are B12 deficient (Antony, 2001; Refsum et al., 2001), a condition that is only partially attributable to a significant majority of the population eating a vegetarian diet.

A complex illness, vitamin B12 deficiency is brought on by inadequate intake (nutritional deficiency) as well as genetic or acquired abnormalities that interfere with B12 absorption, processing, and trafficking pathways (functional deficiency). Methionine synthase, a cytosolic enzyme, catalyses the production of methionine from homocysteine using methylcobalamin (MeCbl) as a cofactor (MS). This process produces tetrahydrofolate (THF), which is required for the de novo production of nucleic acids and is produced from N5-methyl-tetrahydrofolate (N5-CH3-THF). The anaplerotic reaction that produces increased demands for the Krebs cycle and the precursor to heme biosynthesis succinyl-CoA, conducted by mitochondrial methylmalonyl-CoA synthase (MCM), requires adenosylcobalamin (AdoCbl).Homocysteine (Hcy) and methylmalonic acid (MMA), which enter the bloodstream and cause hyperhomocystinemia and methylmalonicacidemia, are accumulated due to a lack of B12 and genetic abnormalities that affect its cellular processing and trafficking.

Adults should consume 2.4 g of vitamin B12 day, which is the amount found in a typical western diet (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of

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Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline, 1998). The main causes of vitamin B12 insufficiency around the world include malabsorption brought on by ageing, inadequate nutrition, and acquired abnormalities in vitamin B12 metabolism. Rarely do vitamin B12 metabolism inborn mistakes occur.When mothers have low amounts of the micronutrient during pregnancy and in their babies, vitamin B12 insufficiency is sometimes not properly identified (Wheeler, 2008; Sarafoglou et al., 2011). It is highly advised to ensure adequate vitamin B12 intake before becoming pregnant, while pregnant, and after giving birth (BjrkeMonsen et al., 2001; Rasmussen et al., 2001; Bjrke-Monsen et al., 2008; Hinton et al., 2010; Dayaldasani et al., 2014). Other groups at risk for vitamin B12 insufficiency include the elderly, vegetarians and vegans, those who have undergone bariatric surgery, and people with gastrointestinal conditions that result in ileal resections more than 20 cm (Majumder et al., 2013; Kwon et al., 2014). (Battat et al., 2014). A cobalamin deficiency may be temporarily induced by some medications, including metformin (Greibe et al., 2013b; Aroda et al., 2016) and proton-pump inhibitors (Howden, 2000; Wilhelm et al., 2013), but this condition may be reversible upon completion of treatment and/or with oral vitamin B12 supplementation.

In this article, we go through three elements of determining a person's B12 status: The importance of metabolites as indicators of vitamin B12 insufficiency in newborns and adults, the application of algorithms to predict subclinical and clinical B12 deficiency, and the diagnosis of vitamin B12 deficiency in special groups are all covered in the following three sections.

SERUM BIOMARKERS OF VITAMIN B12 DEFICIENCY: STRENGTHS AND LIMITATIONS

Total Serum Vitamin B12: The measurement of total serum vitamin B12 is the most direct evaluation and possibly the best initial test to detect vitamin B12 status. There are many clinical chemistry laboratories that offer this assay. Most clinical chemistry labs throughout the world employ ranges for normal (>250 pmol/L), low (150-249 pmol/L), and acute deficiency (149 pmol/L) vitamin B12 (Clarke et al., 2003; Selhub et al., 2008; Mirkazemi et al., 2012). The fact that this biomarker measures total circulation vitamin B12, of which 80% is linked to haptocorrin and not accessible for cellular absorption, is one of its limitations. This assay's inaccuracy to represent cellular vitamin B12 status is another drawback. The

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levels of blood B12 may not necessarily reflect the condition of cellular B12, according to the findings of investigations comparing serum and cellular vitamin B12 (Carmel, 2000; Solomon, 2005; Devalia et al., 2014; Lysne et al., 2016). Patients with inborn defects of vitamin B12 metabolism in particular may exhibit normal or low serum levels of the vitamin despite cellular deficiency. Furthermore, in elderly people with normal serum vitamin B12 levels, functional vitamin B12 insufficiency brought on by oxidative stress has been discovered (Solomon, 2015). Cyanocobalamin (CNCbl) treatment cured a functional vitamin B12 insufficiency as measured by a decrease in tHcy and MMA levels in the serum (Solomon, 2015). As a result, when used alone, total serum B12 is not a valid indicator of vitamin B12 status. However, this marker shouldn't be written off because research suggests that total serum vitamin B12 may be useful for predicting prognosis and status of diseases with abnormally high serum vitamin B12 levels (>650 pmol/L), including cancer (Arendt et al., 2016) and autoimmune lymphoproliferative syndrome (ALPS) (Bowen et al., 2012).

Homocysteine: One-carbon metabolism produces the metabolite homocysteine, which is remethylated by MeCbl-dependent MS or betaine-homocysteine methyltransferase as a component of the methionine cycle and destroyed by cystathionine -synthase (CBS) in the transsulfuration pathway. Because the availability of both vitamin B12 and folate (as N5-CH3-THF) is required for MS to convert HCy to Met, dietary deficits in either of these micronutrients result in the buildup of HCy in blood and urine. Similar to this, this metabolite is elevated as a result of inborn metabolic mistakes that affect the upstream processing and trafficking of B12 or folate, a condition collectively known as hyperhomocystinemia. The normal range of total plasma Hcy (tHcy) in human plasma is 5– 15 µmol/L (Ueland et al., 1993) and values >13 µmol/L may be regarded excessive in adults (Jacques et al., 1999). (Jacques et al., 1999). Due to the release of homocysteine that has been bonded to biological components, homocysteine levels in serum are always higher than those in plasma (Jacobsen et al., 1994). As a result, plasma rather than serum should be used to measure tHcy levels. The reporting of tHcy levels typically ignores gender and age reference intervals, despite the fact that they have been established in several research (Jacobsen et al., 1994; Rasmussen et al., 1996; van Beynum et al., 2005). Elevated Hcy has a dual biochemical basis, which limits the utility of this biomarker as a sole indicator of vitamin B12 status. This is also true for the newborn screening for the disorders cblD, cblF, and cblJ (Huemer et al., 2015), which are inborn defects of vitamin B12 metabolism.

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Methylmalonic Acid: When AdoCbl-dependent MCM in the mitochondrion is inactive, MMA rises. Vitamin B12 deficiency results in the inactivation of MCM, which causes a buildup of its substrate methylmalonyl-CoA, which circulates as free MMA. Since other vitamins of one-carbon metabolism have no effect on the process catalysed by MCM, MMA is thought to be a more accurate indicator of vitamin B12 insufficiency (Clarke et al., 2003). Serum levels of MMA are elevated, with ranges between >260 and 350 nmol/L. (Clarke et al., 2003). However, only a few illnesses, like renal failure, cause a rise in MMA (Iqbal et al., 2013). For instance, one study found that 15–30% of people with high serum levels of vitamin B12 also had excessive levels of MMA, which may indicate renal dysfunction rather than true vitamin B12 insufficiency (Clarke et al., 2003). Therefore, the usefulness of this marker in older individuals and those with suspected or diagnosed renal illness should be carefully considered. Consideration should be given to evaluating a second indicator of vitamin B12 status, such as holo-transcobalamin (holo-TC) (Iqbal et al., 2013). Another study revealed that individuals with impaired renal function may have problems with the clearance of both Hcy and MMA (Lewerin et al., 2007).

Total Serum Holo-Transcobalamin: Through the successive binding of the three protein transporters haptocorrin (HC), intrinsic factor (IF), and transcobalamin (TC), dietary B12 is transported in the digestive tract (Fedosov et al., 2007; Fedosov, 2012). Every cell in the body receives vitamin B12 attached to TC (holo-TC) after it has been absorbed in the intestine. Transcobalamin receptor (TCblR; CD320)-mediated endocytosis is how cells take up holo-TC (Quadros et al., 2009). Holo-TC is the only part of dietary vitamin B12 that is accessible for systemic distribution, hence its level in serum has been successfully used as a measure of bioactive vitamin B12 (Valente et al., 2011). (Nexo et al., 2000, 2002; Valente et al., 2011; Yetley et al., 2011). 6- 20% of the total vitamin B12 in serum is represented by holo-TC (Nexo et al., 2000, 2002; Valente et al., 2011; Yetley et al., 2011). This marker correlates well with the level of vitamin B12 in erythrocytes and is more accurate than serum B12 at determining the physiologically active fraction of vitamin B12 in circulation (Valente et al., 2011). In comparison to Hcy and MMA, holo-TC has demonstrated superior diagnostic utility for determining the vitamin B12 status of aged patients (Valente et al., 2011). Holo-TC levels in healthy individuals typically vary from 20 to 125 pmol/L. (Valente et al., 2011). The mechanisms that regulate holo-TC homeostasis in the general population and in diseases that affect vitamin B12 transport and use still require further study. For instance, patients taking

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chemotherapy, those with macrocytosis, and people with the TC polymorphism 67A>G who do not have a vitamin B12 deficit have all been linked to abnormally low levels of holo-TC (Vu et al., 1993; Wickramasinghe and Ratnayaka, 1996; Riedel et al., 2005, 2011). A sample of 218 institutionalised elderly patients revealed low sensitivity (44%) of holo-TC as a measure of vitamin B12 sufficiency (Palacios et al., 2013). It is currently unknown if and how holo-TC levels differ in people with inborn defects that impact intracellular vitamin B12 metabolism (cblA-cblJ). Therefore, more research is needed to determine the diagnostic utility of holo-TC as a first line test.

Propionylcarnitine (C3) and its Ratios with Acetylcarnitine (C3/C2) and Palmitoylcarnitine (C3/C16): Propionylcarnitine (C3), a marker of methylmalonic aciduria and propionic acidemia, accumulates in vitamin B12 insufficiency in addition to the four conventional markers mentioned above (Sarafoglou et al., 2011). A study revealed that markers C3, C3/C2, and C3/C16 show a negative connection with maternal levels of vitamin B12 in the first trimester of pregnancy and may thus have diagnostic relevance, even though vitamin B12 insufficiency is not a main test in newborn screening programmes (Dayaldasani et al., 2014). The discovery opens the door to early detection of vitamin B12 shortage or insufficiency during the first trimester of pregnancy and appropriate treatment, even thoughit calls for more research regarding the functional biomarkers Hcy and MMA.

ALGORITHMS FOR THE DIAGNOSIS OF VITAMIN B12 DEFICIENCY

The World Health Organization (WHO) states that the serum levels of the micronutrient, with the following cut-offs and definitions, are used to determine vitamin B12 status in adults: Vitamin B12 "adequacy" is defined as >221 pmol/L; "low B12" is defined as 148–221 pmol/L; and "B12 deficiency" is defined as 148 pmol/L. (de Benoist, 2008; Allen, 2009). But standalone indicators of B12 status, such serum B12, haven't been sufficient for conclusively identifying vitamin B12 deficiency (Fedosov, 2013; Palacios et al., 2013; Remacha et al., 2014; Fedosov et al., 2015). Furthermore, age effects are not taken into consideration by the WHO definition. Worldwide, algorithms that integrate at least two biomarkers have been used, and each has pros and cons (Palacios et al., 2013; Remacha et al., 2014).

According to a study done on a Swedish population, tHcy should be used as the first line test when doctors request testing for suspected vitamin B12 or folate deficiency. Only when tHcy> 9 M should additional markers be evaluated to distinguish between vitamin B12 and

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folate deficits (Schedvin et al., 2005). This strategy was successful in cutting diagnostic expenses by 30%. (Schedvin et al., 2005).

VITAMIN B12 DEFICIENCY IN INFANTS

Based on maternal cobalamin reserves during pregnancy, placental function, gestational age, and birth weight, infant cobalamin status is determined at birth. According to numerous studies (Porck et al., 1983; Miller et al., 1993; Perez-D'gregorio and Miller, 1998; Obeid et al., 2006), the cobalamin transport to the foetus is rigorously regulated by a complicated cobalamin metabolism in the placental-fetal compartment. According to studies (Luhby et al., 1958; Baker et al., 1975; Giugliani et al., 1985; Frery et al., 1992; Obeid et al., 2006), cobalamin and holo-TC levels in the placenta, cord blood, and newborn serum correlate with maternal levels but are 2-3 fold higher (Luhby et al., 2006). This gives the newborn a liver store of about (McPhee et al., 1988).

CHALLENGES IN THE DIAGNOSIS OF VITAMIN B12 DEFICIENCY

Subclinical B12 Deficiency Is Asymptomatic: Due to its much higher prevalence (up to 40% of the population in western countries) than clinical deficiency, subclinical vitamin B12 deficiency, which is defined as a total serum B12 concentration of 150-249 pmol/L (Carmel, 2012, 2013), warrants special attention in terms of diagnosis and management (Carmel, 2012, 2013). Subclinical vitamin B12 does not have an established aetiology (Carmel, 2013). The syndrome usually has non-malabsorptive origins, develops without overt clinical symptoms, and has minimal or no rise in tHcy and MMA (Carmel, 2013). Despite the fact that subclinical B12 deficiency occurs far more frequently than clinical B12 deficiency, subclinical B12 deficiency seldom progresses to clinical deficiency, and the need for B12 treatment has not yet been fully proven (Carmel, 1996, 1998, 1999, 2011, 2013). In a study of elderly Chileans who were asymptomatically B12 deficient, patients demonstrated increased function of myelinated peripheral nerves and a favourable correlation with folate status (Brito et al., 2016).

Functional B12 Deficiency: Serum Markers vs. Cellular Status of Vitamin B12: There is no correlation between serum and cellular levels of vitamin B12, according to a number of studies (Carmel, 2000; Solomon, 2005; Devalia et al., 2014; Lysne et al., 2016). This is significant for people with inborn errors of cobalamin metabolism because serum levels of

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the micronutrient are within the normal range (Watkins and Rosenblatt, 2013). In this sense, holo-TC and serum vitamin B12 should not be used as the only indicators of B12 deficiency in neonatal screening. In a very small number of metabolic centres globally, time-consuming metabolic studies are still used to identify genetic complementation or absence of function. Using functional studies on cultured fibroblasts removed from skin biopsies or molecular genetic analysis of putative genes, the diagnosis of inborn errors of vitamin B12 metabolism is carried out in newborn screenings where biochemical markers are suggestive of vitamin B12 deficiency (Watkins and Rosenblatt, 2013).

Combined B12 and Iron Deficiency: There is no correlation between serum and cellular levels of vitamin B12, according to a number of studies (Carmel, 2000; Solomon, 2005; Devalia et al., 2014; Lysne et al., 2016). This is significant for people with inborn errors of cobalamin metabolism because serum levels of the micronutrient are within the normal range (Watkins and Rosenblatt, 2013). In this sense, holo-TC and serum vitamin B12 should not be used as the only indicators of B12 deficiency in neonatal screening. In a very small number of metabolic centres globally, time-consuming metabolic studies are still used to identify genetic complementation or absence of function. Using functional studies on cultured fibroblasts removed from skin biopsies or molecular genetic analysis of putative genes, the diagnosis of inborn errors of vitamin B12 metabolism is carried out in newborn screenings where biochemicalmarkers are suggestive of vitamin B12 deficiency (Watkins and Rosenblatt, 2013).

VITAMIN B12 STATUS IN SPECIAL POPULATIONS

The Elderly: The prevalence of vitamin B12 deficiency in the elderly is demonstrated to rise with age, despite what appears to be a sufficient consumption (Allen, 2009; Miles et al., 2015). The elderly are more susceptible to gastric dysfunction, with varying degrees of gastric atrophy and achlorhydria interfering with the absorption of vitamin B12 from foods. As a result, this is most likely not a physiological alteration brought on by ageing per se (Carmel, 1997). The majority of elderly vitamin B12 insufficiency cases are accompanied with metabolic abnormalities caused by the shortage (Carmel, 1997). However, there is scant evidence from observational studies connecting this to clinical deficiencies symptoms (Miles et al., 2015).

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BariatricSurgeryandGastrointestinal Disorders:

A healthy digestive system is crucial for vitamin B12 absorption because it is responsible for I the production of intrinsic factor by parietal cells in the stomach, (ii) the dissociation of B12 from haptocorrin and binding to intrinsic factor in the duodenum's neutral environment, and (iii) the absorption of B12 in the ileum. By restricting intestinal absorption and/or reducing food intake, pernicious anaemia, an inflammatory disease that targets gastric parietal cells, and bariatric surgery increase the patient's chance of developing vitamin B12 insufficiency. Bariatric surgery may lessen the secretion of intrinsic factor in the stomach, which is typically created and released in response to food intake, in addition to reducing intake of the vitamin itself (Marcuard et al., 1989).

Neurological Disorders: It is widely known that a vitamin B12 shortage has negative effects on the development and progression of neurological deficits (Kumar, 2014). Contrarily, there is debate regarding whether vitamin B12 deficiency contributes to neurological illnesses including Parkinson's, Alzheimer's, and others. Low serum vitamin B12 levels are associated with an increased risk of neurodegenerative illness and cognitive impairment, according to a study of 43 research conducted worldwide (Moore et al., 2012). While a small fraction of the dementias looked at in some of these studies reacted well to vitamin B12 supplementation, patients with an established, pre-existing vitamin B12 deficiency did not see any benefits (Moore et al., 2012).

GENETIC DETERMINANTS OF VITAMIN B12 STATUS

In addition to inborn metabolic errors that affect the intracellular pathways of vitamin B12 trafficking and assimilation (see Froese and Gravel, 2010; Watkins and Rosenblatt, 2011 for excellent reviews on the subject), mutations in the transcobalamin gene (TCN2) (Keller et al., 2016), high levels of the soluble transcobalamin receptor (sCD320) (Hoffmann-Lucke et al., 2013), and polymorphism Falsely low levels of circulating holo-TC can result from mutations in the TCN2 gene without having an impact on other indicators of vitamin B12 deficiency (Keller et al., 2016). Increased concentrations of a heavier form of the sCD320 resulted in elevated serum levels of holo-TC and vitamin B12, albeit the causes of these differences are still unknown (Hoffmann- Lucke et al., 2013). It's possible that increased glycation or the development of higher order oligomers of sCD320 are the causes of the compound's higher apparent molecular weight (Hoffmann-Lucke et al., 2013). Strong relationships between

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plasma MMA and SNPs in acylCo-A synthetase (ACSF3) and HIBCH were found in a genome-wide analysis of 2210 healthy Irish people, with these loci accounting for 12% of the variance in MMA concentration (Molloy et al., 2016). In a novel step in the breakdown of valine, 3-hydroxyisobutyric acid-CoA is converted to 3-hydroxyisobutyric acid by HIBCH. Serum MMA concentrations can be determined independently by the presence of SNPs in HIBCH. Investigations into the chemical mechanism by which polymorphic variations of HIBCH cause increased MMA are ongoing (Molloy et al., 2016). Patients with mutations in the enzyme methylmalonatesemialdehyde dehydrogenase and the enzyme ACSF3, which has both malonyl-CoA and methylmalonyl-CoA activity, have also been shown to have transient elevations in MMA (Alfares et al., 2011; Sloan et al., 2011). Elevated MMA is also caused by mutations in the mitochondrial succinate-CoA ligase (SUCLG1) (Valayannopoulos et al., 2010).

CONCLUDING REMARKS

In order to diagnose vitamin B12 deficiency in both children and adults, at least two biomarkers must be used. Total serum vitamin B12 is ambiguous for the diagnosis of functional deficits, such as those brought on by inborn metabolic errors, among the diagnostic biomarkers currently available. Holo-TC is a useful tool for assessing vitamin B12 status because it captures the biologically active pool of the vitamin in blood, but little is known about its homeostasis. The aberrant amounts of holo-TC found under numerous unrelated illness conditions and in people with otherwise normal total serum vitamin B12 levels serve as an example of this. Although tHcy is especially helpful as a measure of vitamin B12 status in the newborn, its accuracy and specificity are debatable in adults due to its dependence on other factors, such as folate status. In people of all ages with normal renal function, methylmalonic acid remains to be the most sensitive and specific measure for vitamin B12 sufficiency. Although not a first-line test, the usage of acylcarnitines can be seen as auxiliary to diagnosis. Finding early indicators of low vitamin B12 status is difficult, which makes it difficult to diagnose the clinically and biochemically silent subclinical vitamin B12 insufficiency in a timely manner. The effects of low vitamin B12 levels in certain populations, such as the elderly, vegetarians and vegans, children, and pregnant women, also go unnoticed by patients and medical professionals. The discovery that low vitamin B12 status is linked to more pronounced metabolic indicators of vitamin B12 deficiency in the presence of high folic acid concentrations (Miller et al., 2009) emphasises the significance of

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nutrient-nutrient interactions, which have only been taken into account in the last ten years and are especially pertinent to nations that regularly fortify their food supply with folic acid.

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