Special Article

The role of vitamin B_{12} in viral infections: a comprehensive review of its relationship with the muscle–gut–brain axis and implications for SARS-CoV-2 infection

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This comprehensive review establishes the role of vitamin B_{12} as adjunct therapy for viral infections in the treatment and persistent symptoms of COVID-19, focusing on symptoms related to the muscle–gut–brain axis. Vitamin B_{12} can help balance immune responses to better fight viral infections. Furthermore, data from randomized clinical trials and meta-analysis indicate that vitamin B_{12} in the forms of methylcobalamin and cyanocobalamin may increase serum vitamin B_{12} levels, and resulted in decreased serum methylmalonic acid and homocysteine concentrations, and decreased pain intensity, memory loss, and impaired concentration. Among studies, there is much variation in vitamin B_{12} doses, chemical forms, supplementation time, and administration routes. Larger randomized clinical trials of vitamin B_{12} supplementation and analysis of markers such as total vitamin B_{12}, holotranscobalamin, total homocysteine and methylmalonic acid, total folic acid, and, if possible, polymorphisms and methylation of genes need to be conducted with people with and without COVID-19 or who have had COVID-19 to facilitate the proper vitamin B_{12} form to be administered in individual treatment.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), which was recognized by the World Health Organization as a pandemic in early 2020.[1] This viral infection often causes respiratory-tract infection symptoms, but it is not limited to impairing lung function, because the infection can systematically affect the organism, undermining gastrointestinal,[2] cardiovascular, and renal functions;[3] or even the nervous[4] and muscular systems.[5,6] In addition to patient recovery, a growing concern has been that many patients have presented with post-acute COVID-19, long COVID-19, or persistent post–COVID-19 symptoms, which refer to effects or symptoms of this disease that continue for weeks or months beyond the initial illness.

Long COVID-19 appears to be a multisystem disease with varying symptoms.[7,8] The main reported symptoms are shortness of breath, chest pain, headaches, neurocognitive difficulties, depression and other mental health conditions, muscle pains and weakness, gastrointestinal disorders, rashes, metabolic disruption, and thromboembolic conditions.[9–11] The symptoms reported either during long COVID-19 seem to directly involve the skeletal muscle–gut–brain axis, which refers to the mutual interaction among these 3 systems.[12–14] Even though relationships that consolidate this axis are not yet well established, there is a need to search for strategies that can strengthen it vis-à-vis this infectious disease.

Although population vaccinations are advancing in many countries, studies show that the virus has mutated, still infecting and reinfecting thousands of people, which has led to growing concern among health agencies.[15] Nutritional status, chronic diseases, and age have been identified as important variables for the outcome of COVID-19.[16] In this sense, the search for nutritional strategies that aim to reduce susceptibility to SARS-CoV-2 infection or the long-term complications of COVID-19 has been constant in several studies.[16–18]

In this scenario, vitamin B_{12} (also known as cobalamin) is a water-soluble vitamin that is part of the group of vitamins in the B complex. It has important functions in the blood and cardiovascular system,[19] also being involved with the regulation of the immune system and antiviral activity.[20,21] Furthermore, this vitamin is an essential nutrient with markedly important functions in the skeletal muscle–gut–brain axis, such as maintenance of skeletal muscle and neurobehavioral parameters[22–25] and modulation of gut microbiota.[26] Vitamin B_{12} was ranked among the top 4 substances for potential use in treatment for COVID-19, on the basis of findings from a study carried out with the help of molecular modelling and virtual screening tools, using data on US Food and Drug Administration–approved drugs.[27] Thus, vitamin B_{12} combined with a healthy diet can be an important adjuvant in treating COVID-19 and in patients treated after COVID-19 infection.

The subclinical deficiency rates of vitamin B_{12} are high in developing countries and vegetarian populations because the main source of this vitamin are animal foods.[28–30] In addition, older adults, people who have had bariatric surgery, and those are at increased risk of B_{12} deficiency, and use of some medications also is a risk factor.[31] Vitamin B_{12} deficiency leads to hematologic, neuropathologic, and cardiovascular disorders, mainly by interfering in the homocysteine (Hcy) metabolism and the methylation reactions of the organism.[32,33]

Given that vitamin B_{12} is involved in various functions in the body and is influenced by several clinical conditions known to be at risk, depending on the outcomes of COVID-19, identifying vitamin B_{12} status is necessary for patients with current COVID-19 infection and those who have had COVID-19. Considering the relationship of vitamin B_{12} with the muscle–gut–brain axis and its role in viral infections and the immune system, we aimed in this comprehensive review to provide evidence and novel insights into the role of B_{12} during treatment and persistent symptoms of COVID-19.

SEARCH STRATEGY AND SELECTION CRITERIA

An online literature search was performed in the PubMed, Scopus, Web of Science, and Cochrane Library databases; on Google and ClinicalTrials.gov; and the International Clinical Trials Registry Platform to perform a comprehensive review about the role of vitamin B_{12} in COVID-19 prognosis. The following Medical Subject Heading and free-text search terms associated with vitamin B_{12} were input: "vitamin B12" OR "cobalamin" OR "cyanocobalamin" OR "methylcobalamin" OR "adenosylcobalamin" OR "hydroxycobalamin" OR "holotranscobalamin" OR "B12 deficiency" OR "vitamin B12 metabolism," which were first used singly and then subsequently matched, in turn, with the terms associated with COVID-19 and other respiratory viral infections: "COVID" OR "COVID-19" OR "SARS-CoV-2" OR "SARS-CoV" OR "MERS" OR "respiratory infection" OR "viral infection" OR "viral disease."[34]

The third round of searches ensued with terms referring to how the virus gains entry and causes damage in organs. Thus, "ACE-2" OR "gut" OR "brain" OR "muscle" OR "muscle-gut-brain axis" OR "COVID symptoms" OR "long-COVID" OR "post COVID" OR "persistent symptoms COVID" were added, in turn, to the first round of searches.[34] The minimum eligible sample size of studies was 20 participants.

Only publications focusing on vitamin B_{12} relative to COVID-19 prognosis, other viral infections, and diseases with similar symptoms were eligible for inclusion. All searches, including title and abstract screening, were performed by 2 investigators working independently. Any discrepancies were resolved through consensus. Only articles published in English were short-listed; all articles deemed potentially eligible were retrieved for full-text review, and preprint articles were excluded.

VITAMIN B_{12}: FUNCTIONS, SOURCES, AND DEFICIENCY

The term vitamin B_{12} is generally used to describe cobalamin, which is chemically composed by a heterocyclic corrin ring made up of 4 pyrroles with cobalt at the center of the ring.[19] Vitamin B_{12} comprises many forms, including cyano-, methyl-, deoxyadenosyl-, and hydroxy-cobalamin.[35] Cyanocobalamin is the synthetic form of vitamin B_{12} and can be found in supplements and fortified foods.[19]

The biggest dietary sources of vitamin B_{12} are viscera, such as liver (26–58 μg), meat (3–10 μg), dairy foods (0.3–2.4 μg), eggs (1–2.5 μg), poultry (trace amounts to 1 μg) in 100 g wet weight.[11,26,36] Bonito fish and clam extracts contain considerable amounts of free vitamin B_{12}, 41 μg and 132 μg/100 g wet weight, respectively.[37] Gastrointestinal fermentation supports the growth of these vitamin B_{12}–synthesizing microorganisms, and this vitamin is subsequently absorbed and incorporated into animal tissues, such as those of ruminants.[19]

Vitamin B_{12} is not synthesized by plants; therefore, low serum B_{12} levels may be more prevalent among vegetarians, and especially vegans.[32] Vegans and even lacto-ovo-vegetarians with only a small intake of eggs and dairy foods may require supplemental vitamin B_{12} from fortified foods or supplements.[33] Some foods, like cheddar cheese, "veggie burgers," breakfast cereals, sunflower margarine, yeast extracts, vegetable stock, sausage mixes, and vegetable margarine are fortified with vitamin B_{12}.[38,39] The US Institute of Medicine has recommended that adults older than 51 years consume most of their vitamin B_{12} from fortified foods or supplements, bearing in mind that older adults are at higher risk of B_{12} deficiency due to the physiological reduction in intrinsic factor secretion necessary for absorbing this vitamin, as well as due to the use of drugs that can reduce the bioavailability of cobalamin.[35]

Vitamin B_{12} has also been reported to be present in lower levels in nonanimal foods, including edible algae, some mushrooms, and fermented foods such as tempeh, kimchi, miso, and tea.[40] For example, *Chlorella*,[41] *Spirulina*,[42] and *Porphyra yezoensis*, commonly known as purple laver or nori, can produce a cobalamin-like compound, also called pseudo-cobalamin, which has an inactive corrinoid.[39]

Bacterial vitamin B_{12} is synthesized by the gut-resident *Propionibacterium. Freudenreichii*, *Lactobacillus reuteri*, *L. coryniformis*, *L. plantarum*, *L. coryniformis*, *Bifidobacterium animalis*, *B. infantis*, and *B. longum*, among others, to produce adenosylcobalamin.[21] The aforementioned bacteria are recognized for having probiotic activity, which points to the importance of the relationship between vitamin B_{12} and the gut microbiota, because probiotics are defined as living microorganisms that provide benefits to the host's health when administered in adequate doses.[43]

The recommended daily allowance of vitamin B_{12} is 3–5 μg/day (2.4 μg/day for adults, 1.2 μg/day for children up to 8 years of age, and 2.6 μg/day for pregnant women and breastfeeding mothers).[35] The average, Western nonvegetarian diet will contain 5–7 μg/day of vitamin B_{12}, which is sufficient to maintain normal cobalamin homeostasis.[44]

Dietary cobalamin is released from food proteins by the action of stomach acid, where it is rapidly complexed to haptocorrin or transcobalamin I (a salivary B_{12}-transfer protein). The haptocorrin-B_{12} complex suffers proteolysis in the duodenum by pancreatic proteases. In the proximal ileum, B_{12} is released to bind to the gastric intrinsic factor, which is a B_{12}-transfer protein essential for ileal cobalamin absorption. Next, the intrinsic factor-B_{12} complex can enter mucosal cells in the distal ileum, and it again binds to transcobalamin (a serum B_{12}-transfer protein). Finally, the transcobalamin-B_{12} complex circulates in the blood and then enters target cells.[45] Most of the circulating B_{12} is bound to haptocorrin, which is unavailable for immediate delivery to cells. Between 10% and 30% of circulating B_{12} is bound to transcobalamin, forming holotranscobalamin or transcobalamin II.[46]

Specific blood-transport nonglycosylated protein is synthesized in most tissues that deliver cobalamin to cells by a receptor-mediated endocytosis. This protein binds to cobalamin with a high affinity and is encoded by a gene located on chromosome 22.[47]

Evidence shows that transcobalamins can suppress systemic inflammation by modulating certain cytokines (ie, interleukin-6), growth factors and other substrates with anti-inflammatory properties under normal physiological conditions. Vitamin B_{12} can be considered an endogenous negative regulator of nuclear transcription factor-κB (NFκB) through the regulation of nitric oxide, which plays a key role in regulating the immune response to infection.[48,49] Vitamin B_{12} contributes to improving the immune response via an increase in CD8 + T cells and natural killer T cells.[20,21] In addition, this vitamin has antioxidant properties through the reduced glutathione-sparing effect: it is capable of increasing the cytosolic bioavailability of reduced glutathione and thus can promote the synthesis of oxidized

glutathione.[48] In addition, vitamin B_{12} is recognized to modulate the ecology of the gut microbiota.[26]

Vitamin B_{12} is essential for DNA synthesis and regulation. It is involved in many important metabolic pathways, especially in the metabolism of lipids, carbohydrates, and proteins, and plays a central role in hemopoiesis. Methylcobalamin is a cofactor of 2 enzymes present in mammalian cells: methionine synthase and methylmalonyl-CoA mutase enzyme.[33] When B_{12} levels are too low in the body, the result is an increase in methylmalonic acid (MMA) and Hcy concentration due to inhibition of methylmalonyl-CoA mutase and methionine synthase, respectively.[33] The increase in Hcy causes folate sequestration and interrupts DNA synthesis. Increased MMA levels cause demyelinating defects in the nervous system and elevate propionic acid, resulting in metabolic acidosis.[50]

The symptoms of subclinical B_{12} deficiency are subtle and often not recognized. A B_{12} deficiency can remain without symptoms for a long time, leading to a chronic deficiency. For many years, B_{12} concentrations < 148 pmol/L in blood have been identified as being deficient. Nevertheless, due to the limitations of sensitivity and specificity of individual assays, 2 or more biomarkers should be used in combination to accurately diagnose vitamin B_{12} deficiency, such as direct (total B_{12} and holotranscobalamin) and functional (Hcy and MMA) biomarkers.[51]

Vitamin B_{12} deficiency occurs at all ages (but mainly in the older adult population) and in both sexes, especially in people who have a restricted diet in foods of animal origin either by choice or due to lack of financial resources to purchase these foods.[52] Deficiency is much more common in developing countries, starting in early life and persisting across the life span,[30] and can be associated with insufficient nutrition or microbial infections. Determining the prevalence of subclinical deficiency of vitamin B_{12} is challenging, however.[45]

Studies have shown a high prevalence of vitamin B_{12} deficiency in populations with different types of vegetarian diets, specifically $> 60\%$ in vegans and $> 40\%$ in lacto- or ovolactovegetarians.[53,54] Vegans have a higher prevalence of B_{12} deficiency compared with other vegetarians that depends, in part, on dietary rigidity and length of time following this lifestyle.[53,55] The lactovegetarian and ovolactovegetarian groups have intermediate vitamin B_{12} status when compared with vegans and omnivores, because although milk, dairy products, and eggs contain some amount of vitamin B_{12}, the intake of this vitamin through a lacto- or ovolactovegetarian diet is considered limited.[53,54] The use of vitamin B_{12} supplements may be necessary for these groups because the bioavailability of vitamin B_{12} from supplements is greater than that from foods.[53]

Low B_{12} concentrations in the body may be present in different pathophysiological situations, including pregnancy, old age, smoking, and comorbidities such as hypertension, diabetes mellitus, pancreatic insufficiency, autoimmune gastritis, gastrectomy or gastric bypass, diseases or resection of the ileum, bacterial overgrowth, celiac disease, inflammatory bowel disease, or uremia-related malnutrition.[22,23,56] Treatments such as antibiotics, proton pump inhibitor medications, antihyperglycemic medicines (eg, metformin), nitrous oxide anesthesia, a nonsteroidal anti-inflammatory drug, some anticonvulsants, and colchicine interfere with B_{12} absorption and metabolism.[33,45]Angiotensin-converting enzyme inhibitors have also been associated with low B_{12} levels in older adults.[57,58]

Cobalamin deficiency causes a decrease in hemoglobin levels, characterizing megaloblastic or pernicious anemia with manifestations that include skin pallor, decreased energy and exercise tolerance, fatigue, shortness of breath, and palpitations.[59] Scientific evidence indicates that not necessarily the deficiency but the low status of the B_{12} biomarker has been associated with increased total Hcy level, which is involved in increased generation of reactive oxygen species in lipid peroxidation and tissue damage to the endothelium vascular and thromboembolism.[41,51] Hyperhomocysteinemia due to low B_{12} status leads to an increased risk of several chronic diseases of aging, including cardiovascular disease and osteoporosis; however, these investigations are limited.[41,51]

The neurologic complications of B_{12} deficiency occur at a later stage of depletion than the indicators we discuss later in this article and are not specific for this deficiency. Alterations in peripheral nerves, followed by degenerative alterations of the posterior spinal cords and cortical spinal ducts, have been reported, in addition to sensory disturbances in the extremities (tingling and numbness). This deficiency was associated with severe symptoms of depression, suicidal behaviors, reduced cognition, mental fatigue, bad or depressed moods, mania, psychosis, and intense agitation.[49]

Other symptoms related to cobalamin deficiency are elevated lactic dehydrogenase levels, mechanical hemolysis, thrombocytopenia, intravascular coagulation thrombosis, low reticulocyte count, vasoconstriction, and renal and pulmonary vasculopathy.[60,61] Vitamin B_{12} deficiency may induce macrocytosis, peripheral neuropathy, ataxia, dizziness, cognitive disturbances, depression, delirium, psychosis, paralysis, muscle cramps, fibromyalgia-like symptoms, and fatigue.[22–25]

ACTION OF VITAMIN B_{12} ON THE SKELETAL MUSCLE–GUT–BRAIN AXIS

Skeletal muscle, gut, and brain are tissues with a collaborative role in regulating physiological processes, including energy homeostasis. The skeletal muscle–gut–brain

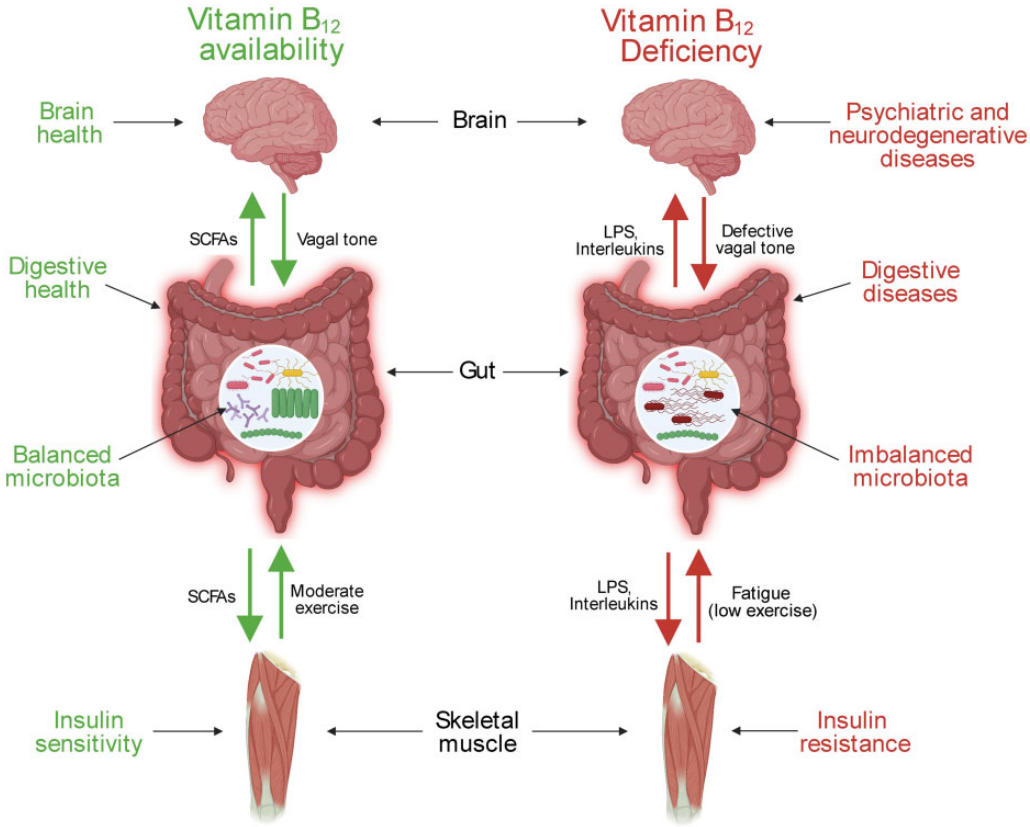

Figure 1 **Impact of vitamin B_{12} on the muscle–gut–brain axis**. *Abbreviations*: LPS, lipopolysaccharide; SCFA, short-chain fatty acid.

axis is a recently introduced concept that is supported by increasing clinical and preclinical evidence showing a close relationship between the muscle–gut and gut–brain axes (Figure 1). [12–14] The skeletal muscle–gut–brain axis is critically regulated by microbiota, a community of gut-resident microorganisms composed of $\sim 10^{14}$ cells classified in $>$ 1000 different bacterial species.[13,62] Gut microbiota composition is regulated by several factors, such as diet, drugs, stress, and exercise.[12] Homeostatic imbalance in this microbial composition, known as dysbiosis, is related to a sedentary lifestyle and some diseases like Alzheimer's disease, colitis, and obesity in humans and rodents (Figure 1).[14,63–65]

Gut microbiota modulate a wide range of functions in the host under normal conditions, including nutritional status, metabolism, and immunity, promoting physical and brain benefits (Figure 1).[14,66,67] Microbiota exert their effects through direct cross-talk with tissues other than the intestine. A bidirectional communication linking gut microbiota and muscle, as well as gut microbiota and brain, has been demonstrated.[12,13] Moderate physical activity and healthy diets promote a balanced microbiome (eg, a high Bacteroidetes to Firmicutes ratio) along with the production of neurotransmitters (eg, serotonin, dopamine), secondary bile acids, and short-chain fatty acids, including acetate, propionate, and butyrate (Figure 1).[13,66] These bacteria-produced compounds positively affect both skeletal muscle and brain, decreasing inflammation and risk of psychiatric and neurodegenerative disorders, as well as increasing insulin sensitivity and glucose control (Figure 1).[12,13] Opposite effects are observed in dysbiosis conditions (eg, a low Bacteroidetes to Firmicutes ratio), where high levels of lipopolysaccharides and pro-inflammatory cytokines (eg, interleukin-1β, interleukin-6, tumor necrosis factor α) are produced by gut bacteria (Figure 1).[12,13] Skeletal muscle and brain effects on microbiota are exerted by direct physical activity and vagal colonocyte modulation.[12,68]

The aforementioned evidence shows the close relationship among muscle, gut, and brain, supporting the concept of the muscle–gut–brain axis. Thus, its modulation can be used as a valuable strategy to develop therapies against diseases related to the brain and muscle, such as psychiatric disorders and neurodegenerative and muscular diseases.

Vitamin B_{12} is exclusively produced by archaea and bacteria[69] and is required for DNA and methionine synthesis, as well as catabolism of fatty acids and amino acids.[70] This vitamin emerges as a serious candidate to modulate the muscle–gut–brain axis, because of its ability to modulate functions at the muscular, intestinal,

and cerebral levels. Treatment with cobalamin improves skeletal muscle dysfunction in patients with hyperhomocysteinemia[71] and reduces formalin-induced muscle pain.[72] Vitamin B_{12} deficiency at the brain level is associated with affective disorders, behavior changes, psychosis, cognitive impairment or decline, and dementia (including Alzheimer's disease and vascular dementia protection).[73]

Treatment with vitamin B_{12} in rats with experimental autism improved impaired markers of this neurologic condition.[74] Modulation of the muscle–gut–brain axis by vitamin B_{12} is also possible by acting on gut microbiota. This is based on the fact that resident bacteria in the colon produce cobalamin, which regulates gene expression in gut Bacteroidetes.[26] Moreover, vitamin B_{12} protects from dysbiosis and promotes different microbial responses in a murine model of colitis.[63]

That vitamin B_{12} can act at any level of the skeletal muscle–gut–brain axis makes its use an interesting option to treat associated diseases. Thus, cobalamin may be an important agent to prevent or improve neurologic consequences of COVID-19.

VITAMIN B_{12} ANTIVIRAL ROLE

Adaptive immunity has been associated with low levels of vitamin B_{12} involving viral infection.[75] It seems plausible that severe vitamin B_{12} deficiency can also be associated with increased risk and severity of infections, because this deficiency affects the functions of phagocytes, production of interferon, replication of viruses, and maturation of T lymphocytes.[76–78] In 9 studies, researchers reported the effects of vitamin B_{12} supplementation on viral diseases (Table 1).[79–87]

In this respect, it is useful to note that most studies to date have looked at the association between vitamin B_{12} levels and the anti-inflammatory and immune-modulating properties of patients infected with HIV. First, a randomized controlled trial (RCT) showed the relationship of a continuous measure of cobalamin level to psychological distress in bereaved HIV-1(+) and HIV-1(−) patients. The mood outcomes in this study were inversely related to levels of serum vitamin B_{12}. Moreover, the lower plasma cobalamin levels also were associated with the presence of symptoms consistent with major depressive disorder.[80]

The second study was a cross-sectional study[81] including antiretroviral therapy (ART)-naïve adults. Logistic regression was used to determine factors associated with suboptimal vitamin B_{12} levels. Compared with people with normal concentrations of B_{12}, individuals with vitamin B_{12} deficiency had a longer known duration of HIV infection. In addition, participants eligible for ART (ie, those with CD4 count < 350 cells/μL) with suboptimal B_{12} had a higher mean rate of CD4 cell population decline than counterparts with normal B_{12} levels.

In the third study, researchers performed a multicenter clinical trial in asymptomatic patients with HIV with a CD4 T-cell count between 375 and 750 cells/μL at screening evaluation.[83] The authors evaluated the effect of high-dose micronutrient supplements (16 capsules/day) on measures of HIV disease progression used to guide ART initiation. The weak but significant correlation of levels of serum vitamin B_{12} levels with CD4 count observed in this study may suggest either that low B_{12} levels may predict CD4 decline, or that B_{12} and CD4 count decline in concert.

More recently, Shivakoti et al[86] conducted a secondary analysis of a random subcohort sample from a multinational randomized trial of a combination ART regimen efficacy among 1571 combination-ART–naïve adults. The researchers aimed to investigate the relationship of micronutrients and inflammation with CD4 cell recovery. The results showed that small numbers of participants (17.1%) were deficient in vitamin B_{12}. Therefore, the analysis was not powered to detect significant differences in CD4 count among participants who were B_{12} deficient and those who were not.

In the same period, Tenford et al[85] performed a case-cohort design study to evaluate the association between micronutrient deficiencies and incident tuberculosis in a diverse population with high incidence of HIV, particularly in low- and middle-income countries. The vitamin A and vitamin D levels at ART initiation were independently associated with increased risk of incident tuberculosis in the ensuing 96 weeks. The median values of vitamin B_{12} did not differ significantly between groups.

In addition, 2 different viral conditions were included in this review: hepatitis and norovirus infection. Three studies investigated the ability of vitamin B_{12} to repair tissue damage and compensate for diminished hepatic storage during viral hepatitis. In the first RCT, researchers reported the therapeutic effect of coenzyme-B_{12} in hepatitis A compared with that of hydroxycobalamin after the 1972 epidemic.[79] Admitted patients who were born in even-numbered years received hydroxycobalamin. Patients born in odd-numbered years were treated with coenzyme B. All patients had to rest during the period of abnormal serum bilirubin levels. The group treated with coenzyme B showed a tendency to more rapid normalization of aminotransferases than did the patients treated with hydroxycobalamin.

In the second randomized controlled study, researchers compared pegylated interferon-α plus

Table 1 **Main studies showing the role of B_{12} in parameters of the immune system related to viral infection**

Reference	Design	Target condition	No. of participants	Population (age)	Dose and time	Main results
Iwarson and Lindberg (1977)[79]	RCT	Acute viral hepatitis	40	2 groups of patients from the 1972 epidemic with short-incubation hepatitis A (aged 15–45 y)	1 mg HOCbl, IM, per d for 12 d, followed by 1 mg of oral HOCbl per d for 23 d	A significant return of serum aminotransferase levels to normal was observed in the group treated with coenzyme-B_{12}.
Baldewicz et al (2000)[80]	RCT	HIV	159	Bereaved HIV-1(+) and HIV-1(−) homosexual men (aged 30–40 y)	Observational study	Serum cobalamin level was inversely related to self-reported overall distress level and specifically to depression, anxiety, and confusion subscale scores, as well as to clinically rated depressed and anxious mood.
Semeere et al (2012)[81]	Cross-sectional study	HIV	204	ART-naïve adults (34.4 [SD ± 9.4] y)	3 doses of 1 mg of parenteral vitamin B_{12}[a]	HIV-infected, ART-naïve individuals had a lower mean vitamin B_{12} level (384 pg/mL) than the mean B_{12} level reported in a population of healthy university students (469 pg/mL).
Rocco et al (2013)[82]	RCT	Chronic viral hepatitis	94	Patients with chronic hepatitis, naïve to antiviral therapy (51–53 y)	5000 μg of IM vitamin B_{12}[a] for 4 wk	Vitamin B_{12} supplementation significantly improved sustained viral response rates in patients with HCV naïve to antiviral therapy.
Balfour et al (2014)[83]	Multicenter RCT	HIV	218	Asymptomatic patients with HIV (38.1 [SD ± 8.9] y).	Vitamin B_{12}[b] divided into 16 capsules of micronutrient supplements per day for 2 y.	Lower baseline levels of B_{12} (<133 pmol/L) correlated with lower baseline CD4 count ($r = 0.2$; $P = 0.007$) in multiple linear regression adjusted for sex and body mass index, as well as in unadjusted analysis ($r = 0.21$; $P = 0.02$).
Sugihara et al (2017)[84]	Prospective cohort study	Chronic viral hepatitis	90	Patients with chronic viral hepatitis and viral-induced cirrhosis (30–88 y)	Observational study	The serum vitamin B_{12} level was a significant independent predictor for overall survival in patients with chronic viral liver disease. Falsely elevated serum vitamin B_{12} levels (584.5 pg/mL) were associated with severity and prognosis in viral liver disease.

(continued)

Table 1 **Continued**

Reference	Design	Target condition	No. of participants	Population (age)	Dose and time	Main results
Tenforde et al (2017)[85]	Case-cohort study	Patients with HIV with incident TB	332	Incident TB after ART initiation in patients infected with HIV (29–41 y), CD4+ T-cell counts <300 cells/mm^3	Observational study	Using established deficiency cutoffs, only 6% of patients with TB and 9% of those without TB were deficient in vitamin B_{12} (<148 pmol/L; $P = 0.39$).
Shivakoti et al (2019)[86]	Random subcohort	HIV	270	Adults positive for HIV with CD4 count < 300 cells/mm^3 (29–41 y)	Observational study	Deficiency in vitamin B_{12} (<148 pmol/L) was associated with lower CD4 reconstitution compared with those with sufficient levels.
Williams et al (2019)[87]	Nonrandomized controlled clinical trial	Norovirus infection	52	Healthy adults exposed to norovirus (21–28 y)	Observational study	Norovirus infection elicited a time-limited inflammatory response. However, the concentrations of vitamin B_{12} did not differ over time within the inflamed group.

[a]Vitamin B_{12} form not mentioned.
[b]Dose and vitamin B_{12} form not mentioned.
Abbreviations: ART, antiretroviral therapy; HOCBL, hydroxycobalamin; HCV, hepatitis C virus; IM, intramuscularly; RCT, randomized clinical trial; TB, tuberculosis.

ribavirin (standard of care) with the standard of care plus vitamin B_{12}.[82] The results showed that, in patients with chronic hepatitis C virus infection naive to antiviral therapy, vitamin B_{12} supplementation improved the overall rate of sustained viral response to pegylated interferon-α and ribavirin by 34%. The effect seemed to be particularly pronounced in difficult-to-treat patients, namely, those infected with hepatitis C virus genotype 1 and with a high baseline viral load.

Authors of the third study analyzed the relationship between vitamin B_{12} levels and liver disease severity and long-term prognosis in patients with chronic viral hepatitis and cirrhosis.[84] Consecutive patients with chronic viral hepatitis and viral-induced cirrhosis were prospectively enrolled in this prospective cohort. The authors found that serum vitamin B_{12} levels were significantly higher in patients with cirrhosis with Child-Pugh C than in patients with chronic hepatitis or Child-Pugh A/B cirrhosis. Therefore, serum vitamin B_{12} level was a significant independent predictor for overall survival in patients with chronic viral liver disease.

Recently, Williams et al[87] evaluated associations between inflammation and micronutrient biomarkers after norovirus exposure. Serum vitamin B_{12} was analyzed using a competitive protein-binding chemiluminescence immunoassay. Vitamin B_{12} concentrations did not differ over time within the inflamed group at the end point. The authors suggested that the lower vitamin B_{12} concentrations at baseline that appeared protective of norovirus infection may have a genetic explanation, such as a single nucleotide polymorphism. Genetic variants of fucosyltransferase 2 have been associated with lower vitamin B_{12} status, and the homozygous fucosyltransferase 2 nonsecretor genotype is characterized as resistant to norovirus infection.

There is some evidence in the available literature that vitamin B_{12} is involved with nucleoprotein metabolism, and it is suggested that its administration may accelerate the repair of damaged cells and thus be responsible for the more favorable course of the illness in the group to which it was administered. Moreover, the serum vitamin B_{12} level seems to be associated with a positive prognosis in some viral conditions. However, the mechanisms behind this association are still uncertain, and more high-quality studies that investigate the underlying mechanisms of this interaction are needed.

EFFECTS OF VITAMIN B_{12} ON SYMPTOMS DURING AND AFTER COVID-19

COVID-19 affects people of all ages and sexes, but the severity of COVID-19 symptoms predominantly increases in elderly individuals, men, and people with comorbidities such as obesity, malnutrition,

hypertension, and diabetes mellitus who generally have inadequate nutritional status and inflammation.[88–91]

Patients with COVID-19 may present acute polyneuropathy such as Guillain–Barré syndrome and variants, which affect the peripheral nervous system due to an exacerbated immune response to infection or also as a postinfectious immune-mediated response.[6,92–94] The most common Guillain–Barré syndrome and variants symptoms are severe back pain and muscle weakness, and there may be long-term complications, including severe disability, pain, and fatigue.[92,94]

Some COVID-19 symptoms can persist for weeks or months after symptoms onset; this condition is called acute post–COVID-19 (from week 5 to week 12), long COVID-19 (from week 12 to week 24), or persistent post–COVID-19 symptoms (lasting >24 weeks).[8] The symptoms include gastrointestinal symptoms (eg, diarrhea, nausea and vomiting, abdominal pain); neurologic manifestations (eg, concentration impairment, anxiety and depression symptoms, headache, migraine, dementia, stroke, obsessive-compulsive disorder, anorexia, apathy, executive deficits, vertigo, memory or cognition loss, hallucinations, sleep disturbances, posttraumatic stress disorder, loss of taste (ageusia) or of smell (anosmia); neuromuscular disorders (eg, fatigue); and muscular disorders (eg, muscle weakness, myalgia).[9,95–98]

Various vitamin B_{12} deficiency symptoms are similar to those found in patients with COVID-19 and post–COVID-19.[22–25] Studies tested vitamin B_{12} supplementation to alleviate some of the symptoms of various diseases that are also present in COVID-19 (Table 2).[23,99–112] Two RCTs[99,110] and 5 meta-analyses[23,100–102,109] reported benefits of vitamin B_{12} supplementation in methylcobalamin (0.5–1 mg orally or local injection for 2 weeks to 1 year) and cyanocobalamin (2000 mg orally or 1–1000 mg via intramuscular route for 90 days and 4 months) forms. The benefits were mainly in analgesic action and attenuation of neurologic symptoms.

Other meta-analyses[107,111,112] and RCTs[106,108] did not indicate significant results in relieving pain, fatigue, or neurologic symptoms by supplementation with these vitamin B_{12} forms. However, the authors of these meta-analyses listed some limitations that open the way for carrying out more RCTs to answer questions: a small number of available trials and high heterogeneity between included studies[107]; a long time difference between the start of vitamin B_{12} supplementation and the appearance of measurable benefits; heterogeneity in the included studies; only 1 high-quality RCT evaluated the effects on fatigue, making it impossible to estimate the meta-analysis[111]; small variations in combined estimates in sensitivity and subgroup analysis; and a low number of studies for subgroup analyses.[112]

A meta-analysis of observational studies ($n=21$ 837 people 12–90 years old) revealed a significant inverse association between dietary intake of vitamin B_{12} and/or vitamin B_{12} supplementation and the risk of depression in women.[10] The most used vitamin B_{12} forms in the included studies were methylcobalamin and cyanocobalamin. There is still controversy regarding the effectiveness of vitamin B_{12} used in supplementation; some scientists claim that natural methylcobalamin and adenosylcobalamin forms may have greater vitamin B_{12} activity than the synthetic cyanocobalamin form.[48,113] Cyanocobalamin must be broken down to cobalamin and cyanide must be converted to the active forms of B_{12} in the human body, whereas genetic single nucleotide polymorphisms can interfere in the metabolism and conversion to intracellular active forms of vitamin B_{12}.[113]

Nevertheless, Obeid et al[114] proposed that cyanocobalamin supplementation (the most stable and inexpensive form) is as effective as the cobalamin coenzyme forms methylcobalamin and adenosylcobalamin. Methylmalonic aciduria and homocystinuria type C protein convert cyanocobalamin into the active methylcobalamin and adenosylcobalamin forms in cells, as long as there is no remethylation disorder cblC in methylmalonic aciduria and homocystinuria type C protein, cblF and cblJ in the protein integral membrane of lysosomal cobalamin transport-escort protein LMBD1 and ATP-binding cassette subfamily D member 4—required for lysosomal release of transport of cobalamin.[115,116] The guidelines for diagnosis and management of the cobalamin-related remethylation disorders recommend treatment with parenteral hydroxylcobalamin in suspected cases of remethylation disorder, the incidence of serious complications, and for significant improvement in patient survival.[115]

Thus, vitamin B_{12} can be used in adjunct treatment of mild to severe COVID-19 symptoms, because of its analgesic function and role in neuromuscular disorders (Figure 2). The appropriate choice of the chemical form of vitamin B_{12}, the dose, and treatment time will depend on individual factors such as the type of vitamin B_{12} deficiency, age, preexisting diseases, type of methylation gene, and medication used.

Many protocols performed in hospitals in Brazil that receive patients affected by COVID-19 recommend supplementation or intramuscular injection of vitamin B_{12} in cases where a deficiency or subclinical deficiency of vitamin B_{12} is identified. This clinical practice may be related to the increase in Hcy in patients with severe COVID-19.[117] Pharmacological treatment with B_{12} usually implements high doses (1000–2000 μg/day) for an average of 1–3 months.[22,34,44,118] Vitamin B_{12} therapy reduces oxidative damage and inflammation levels,

Table 2 **Effects of vitamin B_{12} treatment on symptoms related to COVID-19 prognosis**

Reference	Design	Target condition	No. of participants	Population (age)	Dose and time	Main results
Mauro et al (2000)[99]	Randomized, double-blind, placebo-controlled trial	Low back pain	60	Patients with a proven medical history for back pain, without vitamin B_{12} deficiency (18–65 y)	1000 mg daily of cyanocobalamin IM for 2 wk	Alleviating the low back pain and related functional disability, decreasing the consumption of paracetamol
Sun et al (2005)[100]	Meta-analysis of RCTs	DN	231	Patients with diabetic PN, without vitamin B_{12} deficiency (53–56 y)	0.5 mg of methylcobalamin injection 3 times per week for 4 wk; or 0.5–500 mg of oral methylcobalamin 3 times a day for 4–16 wk	Improved somatic symptoms, such as pain and paresthesia. In 3 studies, methylcobalamin therapy improved autonomic symptoms (peripheral neurophysiology, oral dryness, and dysuria).
Vidal-Alaball et al (2005)[101]; Butler et al (2006)[102]; and Wang et al (2018)[23]	Meta-analysis of RCTs	Oral vs IM vitamin B_{12} to treat vitamin B_{12} deficiency	153	Patients with megaloblastic anemia, (16–86 y)	1000 μg daily of oral vitamin B_{12}[a]; 2000 μg daily of oral cyanocobalamin, 1000 μg daily IM cyanocobalamin or vitamin B_{12}[a] for 90 d and 4 mo	In both times: All doses increased serum vitamin B_{12} levels. Dose of 1000 μg (oral and IM) improved cognitive function (ie, loss of memory, impaired concentration), sensory neuropathy, and vibration sense. Doses of 2000 μg (orally) and 1000 μg (IM) decreased serum methylmalonic acid and serum homocysteine concentrations; improved or clearing of paresthesia, ataxia, or memory loss.
Talaei et al (2009)[103]	RCT, single-blind	DN	100	Patients with diabetes (duration $>$ 3 years; 18–53 y old) with DN, and without vitamin B_{12} deficiency	2 mg IM vitamin B_{12}[a] twice weekly for 3 mo	Decrease in pain and paresthesia scores, and tingling sensation; no changes in vibration, position, pinprick, and nerve conduction parameters

(continued)

Table 2 **Continued**

Reference	Design	Target condition	No. of participants	Population (age)	Dose and time	Main results
Volkov et al (2009)[104]	RCT, double-blind	RAS	58	Patients with RAS and without vitamin B_{12} deficiency (22.54–42.67 y)	1000 μg daily of sublingual vitamin B_{12}[a] for 6 mo	Decreases in pain level, number of ulcers, and duration of outbreaks at 5 and 6 mo of treatment, regardless of initial vitamin B_{12} levels in the blood.
Syed et al (2013)[105]	RCT	Major depressive disorder	73	Patients with depression and low to normal B_{12} levels (24.28–51.06 y)	1000 μg IM vitamin B_{12}[a] every week in addition to the antidepressants (imipramine 100–250 mg/d and fluoxetine 20–40 mg/d) during the 6 wk	Improved depressive symptoms
Taneja et al (2013)[106]	RCT, double-blind	Diarrhea and acute lower respiratory tract infections	1000	North Indian children with or without B_{12} deficiency (6–30 mo)	1.8 μg of oral vitamin B_{12}[a] for 6 mo	The supplementation significantly improved vitamin B_{12} status, but vitamin B_{12} administration did not reduce the incidence of diarrhea or lower respiratory infections.
Almeida et al (2015)[107]	Meta-analysis of RCTs	Major depressive episodes	1695	Patients with major depression with or without B_{12} deficiency (16–85 y)	0.1–0.5 mg daily of oral vitamin B_{12}[a] for 52 wk to 7 y, or 1 mg local injection of cyanocobalamin for 4 wk	Vitamin B_{12} did not decrease the severity of depressive symptoms.
Scholten et al (2018)[108]	RCT, double-blind	Severe fatigue	95	Patients with irritable bowel syndrome or inflammatory bowel disease, and normal vitamin B_{12} blood levels (18–65 y)	1000 μg daily of oral vitamin B_{12}[a] for 8 wk	Increased vitamin B_{12} blood levels, but not improved fatigue, quality of life, or depressive or anxiety symptoms.
Wang et al (2018)[109]	Meta-analysis of RCTs	Herpetic neuralgia	383	Patients with postherpetic neuralgia and with or without B_{12} deficiency (47.01–74.70 y)	1 mg daily of methylcobalamin, local injection, for 2–4 wk	Improved the quality of life, decreased pain intensity and analgesics use
Didangelos et al (2021)[110]	Randomized, double-blind, placebo-controlled trial	Neurophysiological parameters, pseudomotor function, life quality, and level of pain	90	Patients with DN, metformin use, and low vitamin B_{12} levels (53.4–71.8 y)	1000 μg daily of oral methylcobalamin for 1 y	Increased plasma B_{12} levels and improved all neurophysiological parameters, pseudomotor function, pain score, and quality of life, but it did not improve cardiovascular autonomic reflex tests and MNSI.

(continued)

Table 2 **Continued**

Reference	Design	Target condition	No. of participants	Population (age)	Dose and time	Main results
Markun et al (2021)[111]	Meta-analysis of RCTs	Cognitive function, depressive symptoms, and fatigue	6276	Patients with or without mild cognitive impairment, with or without vitamin B_{12} deficiency (66–82 y)	0.1–1 mg daily of oral cyanocobalamin or methylcobalamin; or 1 mg IM cyanocobalamin once or twice weekly up to 2 y	Vitamin B_{12} supplementation did not improve cognitive function and depressive symptoms, and idiopathic fatigue analysis was not possible.
Stein et al (2021)[112]	Meta-analysis of RCTs	PN	2948	Patients with PN and lowered plasma vitamin B_{12} level (33–86 y)	0.75–2 mg daily of oral methylcobalamin for 28–168 d	The presence of PN was associated with lowered B_{12} levels. B_{12} treatment showed a nonsignificant association with symptom improvement (eg, numbness, paresthesia, pain, and/or dysesthesia), perhaps due to the low number of studies included in the meta-analysis (n = 4).

[a]Vitamin B_{12} form not mentioned.

Abbreviations: DN, diabetic neuropathy; IM, intramuscularly; MNSI, Michigan Neuropathy Screening Instrument Examination; PN, peripheral neuropathy; RAS, recurrent aphthous stomatitis; RCT, randomized controlled trial.

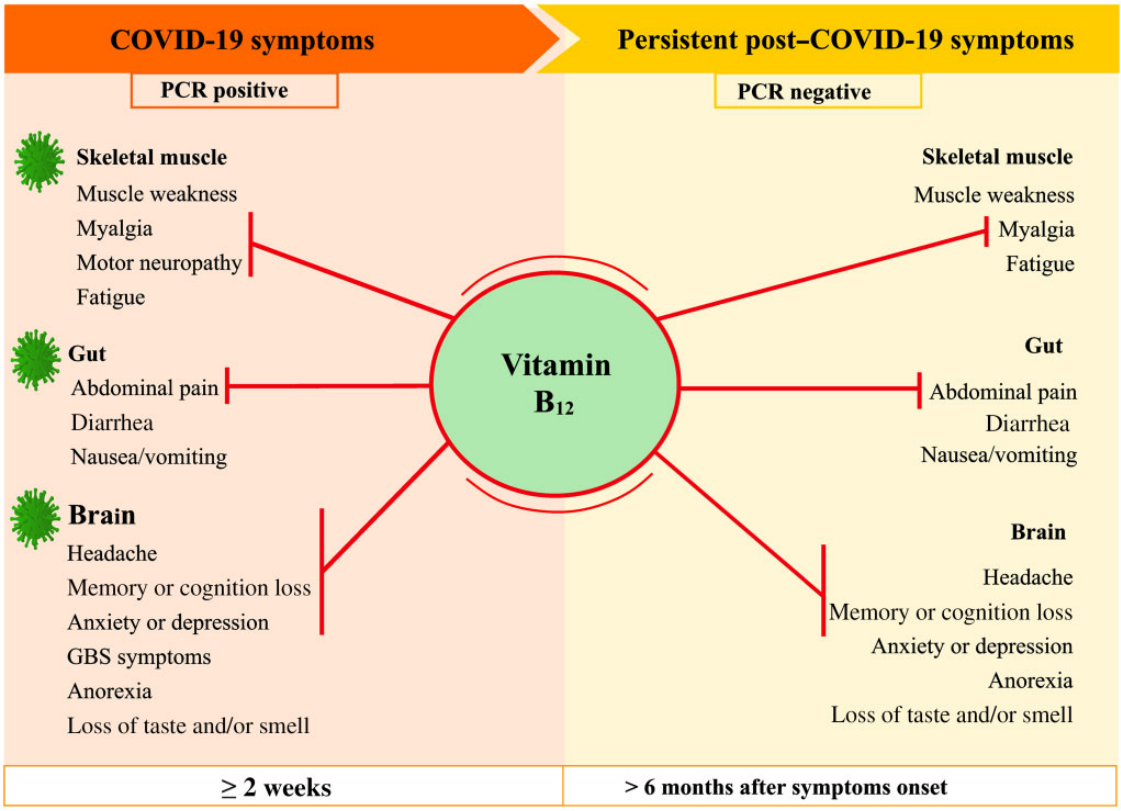

Figure 2 **Vitamin B_{12} action on COVID-19 and post–COVID-19 symptoms**. *Abbreviations*: GBS, Guillain–Barré syndrome; PCR, polymerase chain reaction.

both systemically and in the central nervous system, especially associated with folate; it improves microvascular disease associated with hyperhomocysteinemia[119]; and can alleviate COVID-19 symptoms, thereby improving the prognosis either during or in the post–acute COVID-19 syndrome.

In a cohort study by Tan et al,[17] older patients (≥50 years; n = 43) with COVID-19 received a daily oral combination of 500 µg methylcobalamin, 1000 IU of cholecalciferol (vitamin D_3), and 150 mg of magnesium oxide before the onset of the primary outcome and during 14 days. These patients had lower required need of oxygen therapy during hospitalization than did the control group (which did not receive the combination).

Jang et al[120] evaluated in 6 hospitals in South Korea 80 patients (median age, 63 years) with COVID-19 receiving mechanical ventilatory support, 19 of whom were treated with extracorporeal membrane oxygenation (9.8 days; interquartile range, 7.0–13.7 days). Five patients (31.58%) received vitamin B_{12} therapy and were successfully weaned off extracorporeal membrane oxygenation ($P = 0.013$). However, they did not specify the chemical form of vitamin B_{12} used in hospital treatment. The resolution of lung injury by vitamin B_{12} may be associated with its reduced glutathione-sparing effect to maintain the antioxidant status, as well as its role as a regulator of NFκB levels affecting the expression of genes encoding pro-inflammatory cytokines.[44,48,121]

The daily oral supplementation of vitamin B_{12} (250 µg) in women immunized against influenza A (H1N1) during pregnancy and 3 months postpartum increased the vitamin B_{12} values in plasma, colostrum, and breast milk; increased H1N1-specific immunoglobulin A responses in the plasma and colostrum of mothers, but not in babies; decreased MMA in mothers and children; and reduced the number of babies with high levels of C-reactive protein.[122] The C-reactive protein levels may have an inverse correlation with vitamin B_{12} concentration.[123]

Vitamin B_{12} can block factors that facilitate infection with SARS-CoV-2. Kandeel and Al-Nazawi[27] conducted a virtual screening study of the Food and Drug Administration–approved drugs against COVID-19 main protease 3-C-like protease, which plays a key role in viral replication and transcription.[124] Vitamin B_{12} was in the fourth position of docking scores (relative docking score, 1.99) against COVID-19 main protease 3-C-like protease. The authors suggested combining vitamin B_{12} with nicotinamide (vitamin B_3) or drugs

against COVID-19, such as ribavirin (an potent antiviral drug, especially vs RNA viruses), and telbivudine (used to treat hepatitis B virus) to treat COVID-19.

Methylcobalamin has a significant affinity to bind to the active site of the nsp12 protein of SARS-CoV-2, with a docking score of −8.193, Glide gscore of −8.263, and Glide energy of −75.794 (Schrödinger, New York, NY). Thus, vitamin B_{12} may inhibit the RNA-dependent RNA polymerase activity of nsp12 responsible for the replication of the viral genome.[125] In an in silico study by Narayanan and Nair,[126] the docking score of −10.008, Glide gscore of −10.008, and Glide energy of −86.131 indicated that vitamin B_{12} has an affinity for binding with the active site of nsp14 protein from SARS-CoV-2.

The nsp14 protein has 3′ to 5′ exoribonuclease activity responsible for removing mismatches that arise during genome duplication; this action may impair the inhibitory effect of drugs used to treat COVID-19.[126] Furthermore, Kaur et al[127] proposed that the entry of SARS-CoV-2 facilitated by nsp14 protein in the host cell allows the use of cell *S*-adenosylmethionine for viral RNA capping, culminating in an increase in the Hcy production and angiotensin-converting enzyme-2 activation, which will facilitate greater viral entry into cells.[128,129] It is important to note that the prolonged use of angiotensin-converting enzyme inhibitors to reduce viral infection can reduce vitamin B_{12} levels (<200 pmol/L) in older adult patients aged ≥ 65 years.[57]

In data from 24 262 participants with a mean age of 48.0 (SD, ± 19.0) years, Wolffenbuttel et al[130] found that low serum B_{12} concentrations (<140 pmol/L) were associated with a moderate increase in all causes of death (eg, chronic lower respiratory diseases, Alzheimer's disease, influenza and pneumonia, cerebrovascular diseases, diabetes mellitus) and high serum concentrations of MMA and Hcy; the increase in cardiovascular causes of death was associated with both low (<140 pmol/L) and high (>700 pmol/L) serum B_{12} levels.

On the other hand, excess vitamin B_{12} in the body was also associated with poor outcomes of diseases. Ersöz and Yilmaz[131] reported poor prognostic factors (eg, death of patients in the intensive care unit and intubated) in 310 patients from Turkey with COVID-19 (mean age ± SD, 57.02 ± 18.28 years) with high blood vitamin B_{12} concentration (>911 pg/mL), and low folate, iron, vitamin D, and hemoglobin levels. Some studies indicated an association between higher B_{12} levels (1000–1719 pg/mL) and the death of critically ill adult patients in the intensive care unit (mean age ± SD, range, 53.5 ± 12.0 to 66.7 ± 20.0 years).[84,132,133] Flores-Guerrero et al[134] showed that a plasma vitamin B_{12} concentration >455.41 pg/mL was associated with a higher risk of all-cause mortality in 1394 adults (mean age ± SD, 54.6 ± 11.6 years). Excess vitamin B_{12} is still controversial, and other authors have used distinct values for high plasma B_{12} levels: >950 pg/mL or 701 pmol/L[135]; >771 pg/mL[136]; >203 pg/mL or >601 pmol/L.[137]

Dalbeni et al[138] exposed that 9 of 49 patients with COVID-19–associated pneumonia who did not receive vitamin B_{12} by any route had excess vitamin B_{12} in plasma (median, 1315 ng/mL), low arterial oxygenation (median, 202 partial pressure of oxygen/% inhaled oxygen) and were transferred to the intensive care unit or died.[138] Some factors in this study limit the establishment of the relationship between the high blood levels of vitamin B_{12} and intensive therapy or death resulting from COVID-19: 1) 9 patients (small sample size) were older than the 40 recovered patients by an average of 13 years (83.3 vs 70.2 years old, respectively); 2) the increase in vitamin B_{12} level did not interfere with Hcy values, which were similar between groups (11 μmol/L vs 9 μmol/L) and are within the normal range, 5–15 μmol/L[139]; and 3) the authors did not verify the values of other vitamin B_{12} biomarkers, such as MMA. Aging itself predisposes to lower resistance to viral infections.[140]

The mechanism by which blood excess vitamin B_{12} occurs in patients in the intensive care unit is not clear, but some factors that may partially explain it include: 1) the elevated plasma levels of the cobalamin-carrier proteins, the transcobalamins I and III; 2) elevated release of vitamin B_{12} from liver storage and decreased vitamin B_{12} hepatic clearance; and 3) decreased hepatic production of transcobalamin II with reduction of vitamin B_{12} peripheral tissues uptake, or reduced affinity of carrier proteins for vitamin B_{12}.[44,134,138]

Transcobalamin II gene polymorphisms can decrease tissue distribution of cobalamin, even with high serum cobalamin levels.[141] The 776GG homozygous variant of 776C>G polymorphism encodes a transcobalamin 2 with a lower binding affinity to vitamin B_{12}, whereas polymorphisms in the FUT 6 gene (ie, rs708686, rs78060698, rs3760775, and rs7788053) elevate vitamin B_{12} status.[142] It is interesting to note that polymorphisms in the ATP-binding cassette subfamily D member 4 protein will affect the transporting vitamin B_{12} out of lysosomes, and thus intracellular processing of vitamin B_{12}, which may increase serum levels of vitamin B_{12}.[142]

Several conditions may also result in higher vitamin B_{12} concentrations in critically ill patients, especially in older adults, such as preexisting diseases (eg, renal failure, hepatic diseases, cancer, Alzheimer's disease), nutritional status, and inflammatory status (eg,

sepsis).[44,135] Acute uncontrolled systemic inflammation in severe COVID-19 induces sepsis and multiple organ failure, and can lead to an elevation of vitamin B_{12} level due to higher levels of transcobalamins I and II, their receptors, and unsaturated B_{12} binding capacity in the blood.[141,143] The inactive form of vitamin B_{12} bound to transcobalamin I is the main factor that elevates blood vitamin B_{12} levels.[84] However, the mechanisms by which excess vitamin B_{12} is associated with sepsis remain poorly elucidated and, in these cases, supplementation with vitamin B_{12} should be individually evaluated considering the aforementioned metabolic and genetic factors.

Patients with severe COVID-19 have elevated levels of high mobility group box 1,[144] a potential biomarker of sepsis that is modulated by NFκB.[141] The active form of this vitamin in patients with functional transcobalamin II and normal B_{12} cell metabolism can inhibit production of this biomarker by indirect mechanisms, that is, through downregulation of NFκB levels and increased acetylcholine synthesis, which positively modulates the neuro-immune cholinergic anti-inflammatory pathway.[141]

Some interventional clinical trials on the effects of vitamin B_{12} supplementation in combination with other micronutrients and/or medications in cases of COVID-19 are currently being recorded in the International Clinical Trials Registry Platform and ClinicalTrials.gov databases registration numbers NCT04395768 (500 μg methylcobalamin orally, daily, for 14 days), NCT04751669 (9.6 mg cyanocobalamin orally, once a day, for 14 days), and NCT04828538 (1 mg daily oral B_{12} supplementation for up to 60 days). In addition, 2 registered observational clinical studies will investigate the B_{12} levels in patients positive for COVID-19 (age range, 21–60 years; Clinical Trials Registry India identification number CTRI/2021/02/030946) and in pregnant women positive for COVID-19 (ClinicalTrials.gov registration number NCT04407572).

However, more RCTs of vitamin B_{12} supplementation and analysis of various markers (eg, total B_{12}, holotranscobalamin, total Hcy and MMA, total folic acid, and if possible, polymorphism and/or methylation of genes) are needed to precisely identify the status of this micronutrient (before and after) in people with or without COVID-19 and thus facilitate the proper choice of vitamin B_{12} form to be administered in treatment.

CONCLUSIONS

The evaluation of parameters that determine the deficiency or subclinical levels of vitamin B_{12} deficiency can be an ally in treating patients affected by COVID-19 or in persistent symptoms of the disease, given the important functions of this vitamin in the skeletal muscle–gut–brain axis.

Vitamin B_{12} plays an important role in viral infections. The consumption of a healthy diet containing vitamin B_{12} sources, and especially supplementation with methylcobalamin and cyanocobalamin, are promising alternatives as adjuvants in the treatment of COVID-19, especially in patients with B_{12} deficiency or deficiency risk. However, establishing doses, intervention times, and mechanisms of action of vitamin B_{12} against COVID-19 can be a great challenge.

Researchers are encouraged to identify whether the subclinical deficiency or deficiency itself of this vitamin is a risk factor for COVID-19 complications, and it is necessary to carry out intervention studies with vitamin B_{12} supplementation in both the adjuvant treatment of mild, moderate, and severe COVID-19 and post–COVID-19, with a focus on minimizing symptoms related to the muscle–gut–brain axis.

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REFERENCES

1. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. *Acta Biomed.* 2020;91:157–160.
2. Wong SH, Lui RN, Sung JJ. COVID-19 and the digestive system. *J Gastroenterol Hepatol.* 2020;35:744–748.
3. Zheng YY, Ma YT, Zhang JY, et al. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* 2020;17:259–260.
4. Berger JR. COVID-19 and the nervous system. *J Neurovirol.* 2020;26:143–148.
5. Ali AM, Kunugi H. Skeletal muscle damage in COVID-19: a call for action. *Medicina (Kaunas).* 2021;57:372.
6. Bahouth S, Chuang K, Olson L, et al. COVID-19 related muscle denervation atrophy. *Skeletal Radiol.* 2021;50:1717–1721.

7. Callard F, Perego E. How and why patients made long COVID. *Soc Sci Med*. 2021;268:113426.
8. Fernández-de-Las-Peñas C, Palacios-Ceña D, Gómez-Mayordomo V, et al. Defining post-COVID symptoms (post-acute COVID, long COVID, persistent post-COVID): an integrative classification. *Int J Environ Res Public Health*. 2021;18:2621.
9. Greenhalgh T, Knight M, A'Court C, et al. Management of post-acute COVID-19 in primary care. *BMJ*. 2020;370:m3026.
10. Wu Y, Zhang L, Li S, et al. Associations of dietary vitamin B1, vitamin B2, vitamin B6, and vitamin B12 with the risk of depression: a systematic review and meta-analysis. *Nutr Rev*. 2021;29:nuab014. doi:10.1093/nutrit/nuab014.
11. Gille D, Schmid A. Vitamin B12 in meat and dairy products. *Nutr Rev*. 2015;73:106–115.
12. Cryan JF, O'Riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. *Physiol Rev*. 2019;99:1877–2013.
13. Przewłócka K, Folwarski M, Kaźmierczak-Siedlecka K, et al. Gut-muscle axis exists and may affect skeletal muscle adaptation to training. *Nutrients*. 2020;12:1451.
14. Schlegel P, Novotny M, Klimova B, et al. "Muscle-gut-brain axis": can physical activity help patients with Alzheimer's disease due to microbiome modulation? *J Alzheimers Dis*. 2019;71:861–878.
15. Dao TL, Hoang VT, Gautret P. Recurrence of SARS-CoV-2 viral RNA in recovered COVID-19 patients: a narrative review. *Eur J Clin Microbiol Infect Dis*. 2021;40:13–25.
16. De Araújo Morais AH, Aquino JS, Da Silva-Maia JK, et al. Nutritional status, diet and viral respiratory infections: perspectives for severe acute respiratory syndrome coronavirus 2. *Br J Nutr*. 2021;125:851–862.
17. Tan CW, Ho LP, Kalimuddin S, et al. Cohort study to evaluate effect of vitamin D, magnesium, and vitamin B12 in combination on severe outcome progression in older patients with coronavirus (COVID-19). *Nutrition*. 2020;79-80:111017.
18. Moscatelli F, Sessa F, Valenzano A, et al. COVID-19: role of nutrition and supplementation. *Nutrients*. 2021;13:976.
19. World Health Organization. Vitamin B12. In: *Vitamin and Mineral Requirements in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation*. 2nd ed. Bangkok, Thailand: World Health Organization; 1998:279–288. Available at: https://apps.who.int/iris/handle/10665/42716. Accessed August 20, 2021.
20. Tamura J, Kubota K, Murakami H, et al. Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. *Clin Exp Immunol*. 1999;116:28–32.
21. Yoshii K, Hosomi K, Sawane K, et al. Metabolism of dietary and microbial vitamin B family in the regulation of host immunity. *Front Nutr*. 2019;6:48.
22. Wolffenbuttel BHR, Wouters HJCM, Heiner-Fokkema MR, et al. The many faces of cobalamin (vitamin B12) deficiency. *Mayo Clin Proc Innov Qual Outcomes*. 2019;3:200–214.
23. Wang H, Li L, Quin LL, et al. Oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency. *Cochrane Database Syst Rev*. 2018;2018:CD004655.
24. Tee LY, Alhamid SM, Tan JL, et al. COVID-19 and undiagnosed pre-diabetes or diabetes mellitus among international migrant workers in Singapore. *Front Public Health*. 2020;8:584249.
25. Kulkarni R, Rajput U, Dawre R, et al. Severe malnutrition and anemia are associated with severe COVID in infants. *J Trop Pediatr*. 2021;67:fmaa084.
26. Degnan PH, Taga ME, Goodman AL. Vitamin B12 as a modulator of gut microbial ecology. *Cell Metab*. 2014;20:769–778.
27. Kandeel M, Al-Nazawi M. Virtual screening and repurposing of FDA approved drugs against COVID-19 main protease. *Life Sci*. 2020;251:117627.
28. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. *Annu Rev Nutr*. 2004;24:299–326.
29. Shipton MJ, Thachil J. Vitamin B12 deficiency - a 21st century perspective. *Clin Med*. 2015;15:145–150.
30. Allen LH. How common is vitamin B-12 deficiency? *Am J Clin Nutr*. 2009;89:693S–696S.
31. Allen LH, Miller JW, De Groot L, et al. Biomarkers of Nutrition for Development (BOND): vitamin B-12 review. *J Nutr*. 2018;148:1995S–2027S.
32. Rizzo G, Laganà AS, Rapisarda AMC, et al. Vitamin B12 among vegetarians: status, assessment and supplementation. *Nutrients*. 2016;8:767.
33. O'Leary F, Samman S. Vitamin B12 in health and disease. *Nutrients*. 2010;2:299–316.
34. Wee AKH. COVID-19's toll on the elderly and those with diabetes mellitus – is vitamin B12 deficiency an accomplice? *Med Hypotheses*. 2021;146:110374.
35. Young V, Garza C. Institute of Medicine (US). Vitamin B12. In: Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academies Press (US); 1998:306–356. Available at: https://www.ncbi.nlm.nih.gov/books/NBK114302/. Accessed August 20, 2021.
36. Antony AC. Vegetarianism and vitamin B-12 (cobalamin) deficiency. *Am J Clin Nutr*. 2003;78:3–6.
37. Bito T, Tanioka Y, Watanabe F. Characterization of vitamin B12 compounds from marine foods. *Fish Sci*. 2018;84:747–755.
38. Madry E, Lisowska A, Grebowiec P, et al. The impact of vegan diet on B-12 status in healthy omnivores: five-year prospective study. *Acta Sci Pol Technol Aliment*. 2012;11:209–212.
39. Watanabe F, Yabuta Y, Tanioka Y, et al. Biologically active vitamin B12 compounds in foods for preventing deficiency among vegetarians and elderly subjects. *J Agric Food Chem*. 2013;61:6769–6775.
40. Watanabe F, Yabuta Y, Bito T, et al. Vitamin B12-containing plant food sources for vegetarians. *Nutrients*. 2014;6:1861–1873.
41. Bito T, Okumura E, Fujishima M, et al. Potential of *Chlorella* as a dietary supplement to promote human health. *Nutrients*. 2020;12:2524.
42. Madhubalaji CK, Rashmi V, Chauhan VS, et al. Improvement of vitamin B12 status with *Spirulina* supplementation in Wistar rats validated through functional and circulatory markers. *J Food Biochem*. 2019;43:e13038.
43. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11:506–514.
44. Romain M, Sviri S, Linton DM, et al. The role of vitamin B12 in the critically ill - a review. *Anaesth Intensive Care*. 2016;44:447–452.
45. Rizzo G, Laganà AS. A review of vitamin B12. In: Patel VB, ed. Molecular Nutrition: Vitamins. London, United Kingdom: Academic Press; 2019:105–129. doi:10.1016/B978-0-12-811907-5.00005-1.
46. Green R. Vitamin B12 deficiency from the perspective of a practicing hematologist. *Blood*. 2017;129:2603–2611.
47. Lurz E. Vitamin B12; absorption, metabolism, and deficiency. In: Kuipers EJ, ed. Encyclopedia of Gastroenterology. 2nd ed. London, United Kingdom: Academic Press; 2020:727–733. doi:10.1016/B978-0-12-801238-3.11357-1
48. Manzanares W, Hardy G. Vitamin B12: the forgotten micronutrient for critical care. *Curr Opin Clin Nutr Metab Care*. 2010;13:662–668.
49. Mikkelsen K, Stojanovska L, Prakash M, et al. The effects of vitamin B on the immune/cytokine network and their involvement in depression. *Maturitas*. 2017;96:58–71.
50. Herrmann W, Obeid R. Cobalamin deficiency. In: Stanger OH, ed. Water Soluble Vitamins. Subcellular Biochemistry. vol 56. Dordrecht, Netherlands: Springer; 2012:301–322.
51. Hughes CF, McNulty H. Assessing biomarker status of vitamin B12 in the laboratory: no simple solution. *Ann Clin Biochem*. 2018;55:188–189.
52. Bates CJ, Schneede J, Mishra G, et al. Relationship between methylmalonic acid, homocysteine, vitamins B12 intake and status and socio-economic indices, in a subset of participants in the British National Diet and Nutrition Survey of people aged 65y and over. *Eur J Clin Nutr*. 2003;57:349–357.
53. Herrmann W. Vitamin B12 deficiency in vegetarians. In: Mariotti F, ed. Vegetarian and Plant-Based Diets in Health and Disease Prevention. London, United Kingdom: Academic Press; 2017:791–808. doi:10.1016/B978-0-12-803968-7.00043-5.
54. Herrmann W, Obeid R, Schorr H, et al. Enhanced bone metabolism in vegetarians - the role of vitamin B12 deficiency. *Clin Chem Lab Med*. 2009;47:1381–1387.
55. Pawlak R, Lester SE, Babatunde T. The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B12: a review of literature. *Eur J Clin Nutr*. 2014;68:541–548.
56. Allegra A, Tonacci A, Pioggia G, et al. Vitamin deficiency as risk factor for SARS-CoV-2 infection: correlation with susceptibility and prognosis. *Eur Rev Med Pharmacol Sci*. 2020;24:972–9738.
57. Tal S, Shavit Y, Stern F, et al. Association between vitamin B12 levels and mortality in hospitalized older adults. *J Am Geriatr Soc*. 2010;58:523–526.
58. Murthy VL, Koupenova M, Shah RV. ACEing COVID-19 a role for angiotensin axis inhibition in SARS-CoV-2 infection? *Circ Res*. 2020;126:1682–1684.
59. Green R, Datta Mitra A. Megaloblastic anemias: nutritional and other causes. *Med Clin North Am*. 2017;101:297–317.
60. Grangé S, Bekri S, Artaud-Macari E, et al. Adult-onset renal thrombotic microangiopathy and pulmonary arterial hypertension in cobalamin C deficiency. *Lancet*. 2015;386:1011–1012.
61. Sabry W, Elemary M, Burnouf T, et al. Vitamin B12 deficiency and metabolism-mediated thrombotic microangiopathy (MM-TMA). *Transfus Apher Sci*. 2020;59:102717.
62. Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*. 2016;164:337–340.
63. Lurz E, Horne RG, Määttänen P, et al. Vitamin B12 deficiency alters the gut microbiota in a murine model of colitis. *Front Nutr*. 2020;7:83.
64. Allen JM, Mailing LJ, Niemiro GM, et al. Exercise alters gut microbiota composition and function in lean and obese humans. *Med Sci Sports Exerc*. 2018;50:747–757.
65. Turnbaugh P, Backhed F, Fulton L, et al. Marked alterations in the distal gut microbiome linked to diet-induced obesity. *Cell Host Microbe*. 2008;3:213–223.
66. Nicholson JK, Holmes E, Kinross J, et al. Host-gut microbiota metabolic interactions. *Science*. 2012;336:1262–1267.
67. Lustgarten MS. The role of the gut microbiome on skeletal muscle mass and physical function: 2019 update. *Front Physiol*. 2019;10:1435.

68. Ticinesi A, Lauretani F, Milani C, et al. Aging gut microbiota at the cross-road between nutrition, physical frailty, and sarcopenia: is there a gut–muscle axis? *Nutrients*. 2017;9:1303.
69. Roth JR, Lawrence JG, Bobik TA. Cobalamin (coenzyme B12): synthesis and biological significance. *Annu Rev Microbiol*. 1996;50:137–181.
70. Troen AM. Folate and vitamin B12: function and importance in cognitive development. *Nestle Nutr Inst Workshop Ser*. 2012;70:161–171.
71. Majumder A, Behera J, Jeremic N, et al. Hypermethylation: causes and consequences in skeletal muscle myopathy. *J Cell Biochem*. 2017;118:2108–2117.
72. Tamaddonfard E, Tamaddonfard S, Cheraghiyan S. Effects of intracerebroventricular injection of vitamin B12 on formalin-induced muscle pain in rats: role of cyclooxygenase pathway and opioid receptors. *Vet Res Forum*. 2018;9:329–335.
73. Kennedy DO. B vitamins and the brain: mechanisms, dose and efficacy—a review. *Nutrients*. 2016;8:68.
74. Alfawaz H, Al-Onazi M, Bukhari SI, et al. The independent and combined effects of omega-3 and vitamin B12 in ameliorating propionic acid induced biochemical features in juvenile rats as rodent model of autism. *J Mol Neurosci*. 2018;66:403–413.
75. Remacha AF, Montagud M, Cadafalch J, et al. Vitamin B12 transport proteins in patients with HIV-1 infection and AIDS. *Haematologica*. 1993;78:84–88.
76. Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ*. 2017;356:i6583.
77. Muscogiuri G, Barrea L, Savastano S, et al. Nutritional recommendations for COVID-19 quarantine. *Eur J Clin Nutr*. 2020;74:850–851.
78. Martineau AR, Jolliffe DA, Greenberg L, et al. Vitamin D supplementation to prevent acute respiratory infections: individual participant data meta-analysis. *Health Technol Assess*. 2019;23:1–44.
79. Iwarson S, Lindberg J. Coenzyme-B12 therapy in acute viral hepatitis. *Scand J Infect Dis*. 1977;9:157–158.
80. Baldewicz TT, Goodkin K, Blaney NT, et al. Cobalamin level is related to self-reported and clinically rated mood and to syndromal depression in bereaved HIV-1+ and HIV-1- homosexual men. *J Psychosom Res*. 2000;48:177–185.
81. Semeere AS, Nakanjako D, Ddungu H, et al. Sub-optimal vitamin B-12 levels among ART-naïve HIV-positive individuals in an urban cohort in Uganda. *PLoS One*. 2012;7:e40072.
82. Rocco A, Compare D, Coccoli P, et al. Vitamin B12 supplementation improves rates of sustained viral response in patients chronically infected with hepatitis C virus. *Gut*. 2013;62:766–773.
83. Balfour L, Spaans JN, Fergusson D, et al. Micronutrient deficiency and treatment adherence in a randomized controlled trial of micronutrient supplementation in ART-naïve persons with HIV. *PLoS One*. 2014;9:e85607.
84. Sugihara T, Koda M, Okamoto T, et al. Falsely elevated serum vitamin B12 levels were associated with the severity and prognosis of chronic viral liver disease. *Yonago Acta Med*. 2017;60:31–39.
85. Tenforde MW, Yadav A, Dowdy DW, et al.; NWCS319 and ACTG 5175 study team. Vitamin A and D deficiencies associated with incident tuberculosis in HIV-infected patients initiating antiretroviral therapy in multinational case-cohort study. *J Acquir Immune Defic Syndr*. 2017;75:e71–e79.
86. Shivakoti R, Ewald ER, Gupte N, et al.; NWCS 319 and ACTG PEARLS Study Team. Effect of baseline micronutrient and inflammation status on CD4 recovery post-cART initiation in the multinational PEARLS trial. *Clin Nutr*. 2019;38:1303–1309.
87. Williams AM, Ladva CN, Leon JS, et al. Changes in micronutrient and inflammation serum biomarker concentrations after a norovirus human challenge. *Am J Clin Nutr*. 2019;110:1456–1464.
88. Jutzeler CR, Bourguignon L, Weis CV, et al. Comorbidities, clinical signs and symptoms, laboratory findings, imaging features, treatment strategies, and outcomes in adult and pediatric patients with COVID-19: a systematic review and meta-analysis. *Travel Med Infect Dis*. 2020;37:101825.
89. Figliozzi S, Masci PG, Ahmadi N, et al. Predictors of adverse prognosis in COVID-19: a systematic review and meta-analysis. *Eur J Clin Invest*. 2020;50:e13362.
90. Mahumud RA, Kamara JK, Renzaho AMN. The epidemiological burden and overall distribution of chronic comorbidities in coronavirus disease-2019 among 202,005 infected patients: evidence from a systematic review and meta-analysis. *Infection*. 2020;48:813–833.
91. Zhang P, He Z, Yu G, et al. The modified NUTRIC score can be used for nutritional risk assessment as well as prognosis prediction in critically ill COVID-19 patients. *Clin Nutr*. 2021;40:534–541.
92. Abu-Rumeileh S, Abdelhak A, Foschi M, et al. Guillain–Barré syndrome spectrum associated with COVID-19: an up-to-date systematic review of 73 cases. *J Neurol*. 2021;268:1133–1170.
93. Andalib S, Biller J, Di Napoli M, et al. Peripheral nervous system manifestations associated with COVID-19. *Curr Neurol Neurosci Rep*. 2021;21:9.
94. Raahimi MM, Kane A, Moore CE, et al. Late onset of Guillain-Barré syndrome following SARS-CoV-2 infection: part of "long COVID-19 syndrome." *BMJ Case Rep*. 2021;14:E240178.
95. Havervall S, Rosell A, Phillipson M, et al. Symptoms and functional impairment assessed 8 months after mild COVID-19 among health care workers. *JAMA*. 2021;325:2015–2016.
96. Kamal M, Abo Omirah M, Hussein A, et al. Assessment and characterisation of post-COVID-19 manifestations. *Int J Clin Pract*. 2021;75:e13746.
97. Carfì A, Bernabei R, Landi F; Gemelli Against COVID-19 Post-Acute Care Study Group. Persistent symptoms in patients after acute COVID-19. *JAMA*. 2020;324:603–605.
98. Ortelli P, Ferrazzoli D, Sebastianelli L, et al. Neuropsychological and neurophysiological correlates of fatigue in post-acute patients with neurological manifestations of COVID-19: insights into a challenging symptom. *J Neurol Sci*. 2021;420:117271.
99. Letizia Mauro G, Martorana U, Cataldo P, et al. Vitamin B12 in low back pain: a randomised, double-blind, placebo-controlled study. *Eur Rev Med Pharmacol Sci*. 2000;4:53–58.
100. Sun Y, Lai M-S, Lu C-J. Effectiveness of vitamin B12 on diabetic neuropathy: systematic review of clinical controlled trials. *Acta Neurol Taiwan*. 2005;14:48–54.
101. Vidal-Alaball J, Butler C, Cannings-John R, et al. Oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency. *Cochrane Database Syst Rev*. 2005;CD004655.
102. Butler CC, Vidal-Alaball J, Cannings-John R, et al. Oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency: a systematic review of randomized controlled trials. *Fam Pract*. 2006;23:279–285.
103. Talaei A, Siavash M, Majidi H, et al. Vitamin B12 may be more effective than nortriptyline in improving painful diabetic neuropathy. *Int J Food Sci Nutr*. 2009;60:71–76.
104. Volkov I, Rudoy I, Freud T, et al. Effectiveness of vitamin B12 in treating recurrent aphthous stomatitis: a randomized, double-blind, placebo-controlled trial. *J Am Board Fam Med*. 2009;22:9–16.
105. Syed EU, Wasay M, Awan S. Vitamin B12 supplementation in treating major depressive disorder: a randomized controlled trial. *Open Neurol J*. 2013;7:44–48.
106. Taneja S, Strand TA, Kumar T, et al. Folic acid and vitamin B-12 supplementation and common infections in 6-30-mo-old children in India: a randomized placebo-controlled trial. *Am J Clin Nutr*. 2013;98:731–737.
107. Almeida OP, Ford AH, Flicker L. Systematic review and meta-analysis of randomized placebo-controlled trials of folate and vitamin B12 for depression. *Int Psychogeriatr*. 2015;27:727–737.
108. Scholten AM, Vermeulen E, Dhonukshe-Rutten RAM, et al. Surplus vitamin B12 use does not reduce fatigue in patients with irritable bowel syndrome or inflammatory bowel disease: a randomized double-blind placebo-controlled trial. *Clin Nutr ESPEN*. 2018;23:48–53.
109. Wang JY, Wu YH, Liu SJ, et al. Vitamin B12 for herpetic neuralgia: a meta-analysis of randomised controlled trials. *Complement Ther Med*. 2018;41:277–282.
110. Didangelos T, Karlafti E, Kotzakioulafi E, et al. Vitamin B12 supplementation in diabetic neuropathy: a 1-year, randomized, double-blind, placebo-controlled trial. *Nutrients*. 2021;13:395.
111. Markun S, Gravestock I, Jäger L, et al. Effects of vitamin B12 supplementation on cognitive function, depressive symptoms, and fatigue: a systematic review, meta-analysis, and meta-regression. *Nutrients*. 2021;13:923.
112. Stein J, Geisel J, Obeid R. Association between neuropathy and B-vitamins: a systematic review and meta-analysis. *Eur J Neurol*. 2021;28:2054–2064.
113. Paul C, Brady DM. Comparative bioavailability and utilization of particular forms of B12 supplements with potential to mitigate B12-related genetic polymorphisms. *Integr Med (Encinitas)*. 2017;16:42–49.
114. Obeid R, Fedosov SN, Nexo E. Cobalamin coenzyme forms are not likely to be superior to cyano- and hydroxyl-cobalamin in prevention or treatment of cobalamin deficiency. *Mol Nutr Food Res*. 2015;59:1364–1372.
115. Huemer M, Diodato D, Schwahn B, et al. Guidelines for diagnosis and management of the cobalamin-related remethylation disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR deficiency. *J Inherit Metab Dis*. 2017;40:21–48.
116. Fettelschoss V, Burda P, Sagné C, et al. Clinical or ATPase domain mutations in ABCD4 disrupt the interaction between the vitamin B12-trafficking proteins ABCD4 and LMBD1. *J Biol Chem*. 2017;292:11980–11991.
117. Ponti G, Maccaferri M, Ruini C, et al. Biomarkers associated with COVID-19 disease progression. *Crit Rev Clin Lab Sci*. 2020;57:389–399.
118. Naseri M, Sarvari G-R, Esmaeeli M, et al. High doses of oral folate and sublingual vitamin B12 in dialysis patients with hyperhomocysteinemia. *J Renal Inj Prev*. 2016;5:134–139.
119. Guest J, Bilgin A, Hokin B, et al. Novel relationships between B12, folate and markers of inflammation, oxidative stress and NAD(H) levels, systemically and in the CNS of a healthy human cohort. *Nutr Neurosci*. 2015;18:355–364.
120. Jang WS, Kim J, Baek J, et al. Clinical course of COVID-19 patients treated with ECMO: a multicenter study in Daegu, South Korea. *Hear Lung*. 2021;50:21–27.
121. van de Lagemaat EE, de Groot L, van den Heuvel EGHM. Vitamin B12 in relation to oxidative stress: a systematic review. *Nutrients*. 2019;11:482.
122. Siddiqua TJ, Ahmad SM, Ahsan KB, et al. Vitamin B12 supplementation during pregnancy and postpartum improves B12 status of both mothers and infants but vaccine response in mothers only: a randomized clinical trial in Bangladesh. *Eur J Nutr*. 2016;55:281–293.
123. McMillan DC, Maguire D, Talwar D. Relationship between nutritional status and the systemic inflammatory response: micronutrients. *Proc Nutr Soc*. 2019;78:56–67.

124. Dai W, Zhang B, Jiang XM, et al. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. *Science*. 2020;368: 1331–1335.
125. Narayanan N, Nair DT. Vitamin B12 may inhibit RNA-dependent-RNA polymerase activity of nsp12 from the SARS-CoV-2 virus. *IUBMB Life*. 2020;72:2112–2120.
126. Narayanan N, Nair DT. Ritonavir may inhibit exoribonuclease activity of nsp14 from the SARS-CoV-2 virus and potentiate the activity of chain terminating drugs. *Int J Biol Macromol*. 2021;168:272–278.
127. Kaur H, Sarma P, Bhattacharyya A, et al. Folic acid as placebo in controlled clinical trials of hydroxychloroquine prophylaxis in COVID-19: is it scientifically justifiable? *Med Hypotheses*. 2021;149:110539.
128. Li T, Yu B, Liu Z, et al. Homocysteine directly interacts and activates the angiotensin II type I receptor to aggravate vascular injury. *Nat Commun*. 2018;9:11.
129. Singh Y, Gupta G, Kazmi I, et al. SARS CoV-2 aggravates cellular metabolism mediated complications in COVID-19 infection. *Dermatol Ther*. 2020;33:11–13.
130. Wolffenbuttel BHR, Heiner-Fokkema MR, Green R, et al. Relationship between serum B12 concentrations and mortality: experience in NHANES. *BMC Med*. 2020;18:307.
131. Ersöz A, Yılmaz TE. The association between micronutrient and hemogram values and prognostic factors in COVID-19 patients: a single-center experience from Turkey. *Int J Clin Pract*. 2021;75:e14078.
132. Sviri S, Khalaila R, Daher S, et al. Increased vitamin B12 levels are associated with mortality in critically ill medical patients. *Clin Nutr*. 2012;31:53–59.
133. Cappello S, Cereda E, Rondanelli M, et al. Elevated plasma vitamin B12 concentrations are independent predictors of in-hospital mortality in adult patients at nutritional risk. *Nutrients*. 2016;9:1.
134. Flores-Guerrero JL, Minović I, Groothof D, et al. Association of plasma concentration of vitamin B12 with all-cause mortality in the general population in the Netherlands. *JAMA Netw Open*. 2020;3:e1919274.
135. Andrès E, Serraj K, Zhu J, et al. The pathophysiology of elevated vitamin B12 in clinical practice. *QJM*. 2013;106:505–515.
136. Hinkel J, Schmitt J, Wurm M, et al. Elevated plasma vitamin B12 in patients with hepatic glycogen storage diseases. *J Clin Med*. 2020;9:2326.
137. Arendt JFH, Sørensen HT, Horsfall LJ, et al. Elevated vitamin B12 levels and cancer risk in UK primary care: a thin database cohort study. *Cancer Epidemiol Biomarkers Prev*. 2019;28:814–821.
138. Dalbeni A, Bevilacqua M, Teani I, et al. Excessive vitamin B12 and poor outcome in COVID-19 pneumonia. *Nutr Metab Cardiovasc Dis*. 2021;31:774–775.
139. Tinelli CD, Pino A, Ficulle E, et al. Hyperhomocysteinemia as a risk factor and potential nutraceutical target for certain pathologies. *Front Nutr*. 2019;6:1–13.
140. Nehme J, Borghesan M, Mackedenski S, et al. Cellular senescence as a potential mediator of COVID-19 severity in the elderly. *Aging Cell*. 2020;19:e13237.
141. Wheatley C. A scarlet pimpernel for the resolution of inflammation? The role of supra-therapeutic doses of cobalamin, in the treatment of systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic or traumatic shock. *Med Hypotheses*. 2006;67:124–142.
142. Surendran S, Adaikalakoteswari A, Saravanan P, et al. An update on vitamin B12-related gene polymorphisms and B12 status. *Genes Nutr*. 2018;13:2.
143. Zafer MM, El-Mahallawy HA, Ashour HM. Severe COVID-19 and sepsis: immune pathogenesis and laboratory markers. *Microorganisms*. 2021;9:159.
144. Chen R, Huang Y, Quan J, et al. HMGB1 as a potential biomarker and therapeutic target for severe COVID-19. *Heliyon* 2020;6:e05672.