75268_Auto_Edited.docx

Effects of nutrients on immunomodulation for patients with severe COVID-19: current knowledge

Severe COVID-19: nutritional profile on immunomodulation

Bruna Teixeira da Costa, Glauber Rocha Lima Araújo, Ronaldo Teixeira da Silva Júnior, Luana Kauany de Sá Santos, Vinícius Lima de Souza Gonçalves, Daniel Bastos Alves Lima, Beatriz Rocha Cuzzuol, Jonathan Santos Apolonio, Lorena Sousa de Carvalho, Hanna Santos Marques, Camilo Santana Silva, Isadora de Souza Barcelos, Márcio Vasconcelos Oliveira, Fabrício Freire de Melo

Abstract

Recent research has demonstrated that critically ill patients with COVID-19 show a significant immune system dysregulation. Due to that, some nutrients that influence immunomodulation have been suggested as a form of treatment against the infection. This review collected information of vitamins' impact on the prognosis of COVID-19, with the intention of facilitating treatment and prevention of the disease risk status in patients. The collected information was obtained using the PubMed electronic database, searching for articles that relate COVID-19 and the mechanisms/effects of the nutrients: proteins, glucose, lipids, vitamin B12, vitamin D, calcium, iron, copper, zinc, and magnesium, including prospective, retrospective and support articles. The findings revealed an optimal response related mainly to omega-3, eicosapentaenoic acid, docosahexaenoic acid, calcium, and iron that might represent benefits in the treatment of critically ill patients. Still, supplementation should be done with caution due to the limited availability of randomized controlled studies.

Core Tip: Immunomodulation has a considerable influence on the response of SARS-CoV-2 infections. Therefore, the medical team must acknowledge different resources to improve the immune system. In the current situation of COVID-19 infections' prevalence, knowing the potential risks and benefits of nutritional supplementation can improve patients' response and avoid severe conditions, facilitating the process of

healing. For that purpose, this article brings which nutrients supplementation might help and those which worsen the immunological regulation and other body functions, pursuing to mitigate the response against the virus.

INTRODUCTION

Among the nutrition studies, some emphasize the importance of vitamins, trace elements and long-chain fatty acids in supporting the immune system, keeping it able to protect against infections such as SARS-CoV-2[1,2,3]. Therefore, the analysis of micronutrient supplementation is necessary to consider the effective optimization of the immune function and its use as adjuvant treatment in some cases[1].

Inadequate and insufficient intake of iron, zinc, vitamin B, C and E can affect the immunological function of the organism and allows the presence of high levels of free radicals favoring oxidative stress[4]. Importantly, according to ESPEN expert statements and practice guidelines for the nutritional management of individuals with SARS-CoV-2 infection[5] oral nutritional supplements (ONS) should be preferred over enteral (EN) and parenteral nutrition (PN), whenever possible to meet the patient's needs. EN should be considered in cases with polymorbid medical inpatients and elderly with reasonable prognosis when ONS is not possible. ONS must provide at least 400 kcal/day, including 30g or more of protein/day, for at least one month[5].

On the other hand, a diet rich in vitamin C and zinc improves neutrophil phagocytosis, monocytic activity, and immune cell locomotion; and vitamin D is related to the mediation of interleukins essential for immune defense, acting in the induction of antimicrobial peptides (AMPs) in macrophages[5]. The strong qualitative T-cell response is crucial against SARS-CoV-2, and lymphopenia is associated with elevated mortality[6,7]. Both CD4+ and CD8+ T-cell responses are present in infection, although the latter is inefficient[6]. Elevated IL-2 associated with decreased IFNγ levels have been observed in these cells, increasing the severity and chronic course of the disease[8].

Successful immune regulation of innate and adaptive immunity is a predictor for avoiding severe responses to SARS-CoV-2 infection[6]. Critically ill infected patients showed increased neutrophil counts, tissue damage, activation of the coagulation cascade, decreased hemoglobin and lymphocyte values[6,9] which, associated with a drop in monocyte HLA-DR expression, demonstrate acquired immunosuppression[6], and nutrition has a role in this management.

Some nutrients such as carbohydrates, proteins, omega 3, vitamin B12, vitamin D, iron, copper, calcium, zinc and magnesium are the focus of this article for being directly linked to the host immune response in COVID-19 cases. Apart from these, conjugated linoleic acid, vitamins A and E regulate cytokine production; proliferation and differentiation of specific leukocyte populations, in addition to acting on immunoglobulin production and lymphocyte differentiation[10-11]. In this review, we summarize the mechanisms of immunomodulation promoted by micro and macronutrients in COVID-19.

METHODS

This methodological review was conducted by two investigators, working independently with the guidance and support of a research advisor. Both prospective, retrospective trials and support articles were identified using The United States National Library of Medicine (PubMed). We surveyed the relevant articles published in English using specific descriptors COVID-19; SARS-CoV-2; immune system; immune response; vitamin B12; cobalamin; macronutrients; micronutrients; carbohydrate; protein; lipid; intensive care; vitamin D; iron; copper; zinc; magnesium and calcium associated with the terms action; severe; nutrition; therapy; critically ill patients; coronavirus; immunomodulation; pro-resolving mediators; inflammation in the current literature between 4 October, 2021 and 15 February, 2022. The descriptors were used isolated and/or combined in the PubMed database. No restriction was made as to the date of publication of the articles, nor was a target age range defined. Articles not written in English and not addressing these topics in the title and/or abstract were

excluded. Original articles describing prospective, retrospective, and cross-sectional studies were included, as well as secondary research, such as systematic and narrative reviews. Guidelines were also included. Commentaries, editor letters, book chapters and manuals were not included. Finally, 3.316 articles were identified, being included 122 in this minireview.

MACRONUTRIENTS INFLUENCE IN COVID-19 SEVERITY

Proteins and glucose

The dietary factor that leads to the weakening of immune functions is the failure of macro and micronutrient intake. In addition, clinical studies have shown that malnutrition, weight imbalance, fragility and dysbiosis of the gut microbiota are the main factors involved in the deterioration of immune functions in infected patients[13].

The use of immunonutrients aimed to increase the production of less potent inflammatory mediators and reduce those highly inflammatory, besides minimizing the production of free radicals and modulating the generalized inflammatory response[14]. For diabetic patients, this formulation is suggested, as it is a supplement already used in the unit. Once a product is removed from the formula, fruit is added to reach the caloric goal and improve palatability. Protein is the most important macronutrient for maintaining immune function and preserving muscle mass[1].

Proteins are types of macromolecules made of amino acid units (AA) that perform various important functions for the body, for example acting as antibodies, enzymes, messengers, transporters, and structural components in the body[15,16]. Some studies indicate that protein supplementation stimulates the immune system, which specifically improves infectious disease surveillance[17].

Studies with hydrolyzed proteins have shown that they are able to reduce the inflammatory state and stimulate IgA function and production. Also, arginine and glutamine are both non-essential amino acids that enhance the action of the immune system. The former is associated with macrophages in the generation of nitric oxide, and the latter provides energy for immune cell utilization[18].

The proteins show antiviral activities against enveloped and non-enveloped viruses. They inhibit virus entry into the cell by adhering to cell receptors[19]. Viruses need some enzymes, including DNA or RNA polymerases, reverse transcriptase, and integrase for replication, and some evidence suggests that proteins can inhibit the activity of these enzymes and eventually prevent virus replication[20, 21].

On the other hand, increased consumption of saturated fats, refined carbohydrates, alcohol, and low levels of fiber, unsaturated fats, micronutrients, and antioxidants significantly impairs adaptive immunity, while increasing innate immunity, which leads to chronic inflammation and severe damages to the host defense against viral pathogens[l]. These dietary patterns might have a detrimental effect on immune responses and are involved in the development of several inflammatory diseases [22]. Excessive macronutrient intake contributes to the propensity to acquire pneumonia, which is the most common high-risk complication of COVID-19 [23].

The high mortality from COVID-19 in obese people points to an important role in nutrition[24]. Food can influence cytokine gene expression levels and thus modulate inflammation and oxidative stress[25]. Cytokines such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-6 when produced excessively have been related to dysregulation of the inflammatory response and stimulation of cytokine storms [26]. Furthermore, increased adipose tissue contributes to greater leptin production, which is related to macrophage activation and proliferation, while adiponectin levels are reduced, decreasing the synthesis of anti-inflammatory compounds. In addition, there is an increase in the release of non-esterified fatty acids into the bloodstream, which also leads to the perpetuation of the chronic inflammatory process[27]. Health-related consequences in populations affected by economic outages, quarantines, and curfews due to SARS-CoV-2 infection include psychological distress[28-30], which is associated with an increase in carbohydrate and lipid intake[31] and a decrease in physical exercise [32], resulting in weight gain and increased rates of overweight and obesity. Adipose tissue, besides to storing energy, is responsible for producing certain substrates that, in

excess, can stimulate a state of constant oxidative stress and contribute to the severity of clinical manifestations during SARS-CoV-2 infection.

Some comorbidities have emerged as risk factors for the severe development of COVID-19, including type 2 diabetes, increased body weight, hypertension, and dyslipidemia. In this sense, increased glucose concentrations may be responsible for the reported poor outcome. A recent study reported that type 2 diabetes was associated with a higher mortality rate due to COVID-19, although the mortality rate was lower with better controlled blood glucose[33]. Furthermore, Diabetes Mellitus (DM) can impair the adaptive inflammatory response by delaying T-cell activation, as well as negatively impact neutrophil chemotaxis and contribute to cytokine storm, leading to a dysregulation of the immune response, susceptibility to infection, and increased chance of severe clinical manifestation during SARS-CoV-2 infection[34]. On the other hand, DM was related to the overexpression of angiotensin-converting enzymes in some organs such as heart, lungs, liver, and pancreas, increasing the severity of the cases and leading to organ failure during infection[35]. Consequently, diabetes was significantly associated with the development of acute respiratory distress syndrome (ARDS), with a hazard ratio of 2.3[36].

The few articles available that mention supportive care in COVID-19 recommend that nutritional status should be assessed in all infected patients on hospital admission[1,5,37] and that patients at nutritional risk should receive nutritional support as early as possible, especially through increasing the protein intake by oral nutritional supplements (ONS)[5,37].

Moreover, studies, including SPEN statements[5], highlight that even patients with COVID-19 who are not at risk of malnutrition should maintain an adequate intake, especially regarding adequate amounts of protein (1.5g/d) and calories (25-30 kcal/d), as well as oral supplementation with whey protein (20g/d) and intravenous solutions of multivitamins, multiminerals and trace elements (goal: satisfaction of recommended dietary intake [RDA] on admission). The choice of whey proteins is based on their

anabolic and antioxidant properties combined with high digestibility[38,39]. Its potential clinical benefits have been highlighted in cancer cachexia[40] and were recently demonstrated in a randomized controlled trial of malnourished patients with advanced cancer [41]. Whey proteins also have immunomodulatory properties[42] and potential antiviral activity[43]. Furthermore, whey protein supplementation has been associated with improved immune recovery in HIV patients during the first 3 mo of antiretroviral treatment (ART)[44].

Figure 1 provides a summary of proteins and other nutrients mechanisms of action in targeting COVID-19 infection.

Lipid profile and omega-3

Decreased or absent lipids in nutritional support can cause essential FA deficiency, especially in preterm infants, and result in insufficient synthesis of omega-3 fatty acids, docosahexaenoic acid (DHA) and omega-6 fatty acid ARA[45]. Lipids also play an important role in the delivery of fat-soluble vitamins such as vitamins A, D, E, and K[46]. Physiological processes such as metabolism, immune response, oxidative stress, blood clotting, organ function, and wound healing have a direct association with FA availability[46,47]. However, this process needs to be well balanced, given that excess lipids can cause undesirable consequences. The excess of linoleic acid (LA) may be associated with exacerbation of inflammation, expressed mainly by increased levels of CRP, although other biomarkers such as IL-6, adiponectin and adhesion molecules have not shown significant change related to higher levels of LA consumption [48]. For this, searches have evaluated the impact of the use of substances able to reduce the expression of cytokines that contribute to the gravity of the infection and the enhancement of the inflammatory state in the infection by the SARS-CoV-2 [49].

Omega-6 polyunsaturated fatty acids (PUFAs) can metabolize linoleic acid (LA) and further desaturate and form arachidonic acid (ARA), the main PUFA in cell membranes involved in inflammation in humans[50]. Omega-6 may influence inflammation due to

the fatty acid composition of the cell membrane phospholipids, which modulates cellular responses and cellular function[50,51]. Membrane phospholipids produce second messengers, such as diacylglycerols, endocannabinoids, and platelet activating factor, that act on biological activity[52]. These second messengers also modulate gene expression and physiological and metabolic responses, affecting the immune and inflammatory response, disease severity, and clinical outcome[53]. Moreover, ARA composes peripheral blood mononuclear cells, such as lymphocytes, neutrophils, and monocytes[52]. ARA also acts as a substrate for the enzymes cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450, constituting eicosanoid mediators such as leukotriene B4 (LTB4) and prostaglandin E2 (PGE2), which induces pro-inflammatory cytokines and interleukin IL-6[53,54]. LTB4 promotes leukocyte chemotaxis, adhesion and degranulation, increases vascular permeability and produces inflammatory mediators, leading to a pro-inflammatory effect[51,54]. ARA metabolism also results in the production of Lipoxin A4 (LXA4) and Lipoxin B4 (LXB4)[55,56]. LXA4 is an anti-inflammatory and pro-resolution mediator that acts by inhibiting inflammatory cell recruitment, cytokine production, and NADPH oxidase function, and restoring normal physiological function in damaged tissue, which leads to decreased inflammation[57,58]. Studies suggest that LXA4 can suppress leukocyte-mediated injury, promote chemotaxis of monocytes, and phagocytosis of apoptotic neutrophils [59]. LXB4 is generated by mucosal tissues in the upper respiratory tract and lower airways, and acts by regulating neutrophil activation[60].

In contrast, polyunsaturated fatty acids (PUFAs), such as Omega-3, are lipid compounds with potent anti-inflammatory activity, responsible for the homeostasis of the organism and regulation of various biological functions. It can be produced in small quantities by the human organism, however, it is possible to obtain this nutrient through foods such as fish, nuts, and soy oil, and the intake of 250 to 2000 mg/d is recommended for adults and 200 to 250 mg/d for children[61]. Lipid and carbohydrate requirements are adapted using the energy ratio of fat and carbohydrates between 30:70 in patients without respiratory impairment and 50:50 in patients on mechanical

ventilation[5]. Linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) correspond to the representatives of this group of essential fatty acids, and their metabolization results in substances such as protectins and resolvins that regulate platelet coagulation and the inflammatory process[62].

EPA and DHA sources has shown potential anti-inflammatory activity, in addition to promoting immune function and improving liver metabolism[63]. Studies have shown that resolvins are able to reduce the inflammatory response by decreasing neutrophil invasion and reducing the synthesis of pro-inflammatory cytokines *via* inhibition of factor nuclear kappa B, in addition to promoting monocyte recruitment, increasing phagocytosis of apoptotic neutrophils, and macrophage clearance[46,64]. Resolvins are specialized pro-resolving lipid mediators (SPMs), endogenous lipid mediators that include protectin, maresins and lipoxins (LXs). SPMs are involved in the pathophysiology of respiratory diseases, such as COVID-19, and play a role in signaling molecules during the inflammatory process[65-67]. Studies have also shown its potential in tissue repair, regression of inflammation - by increasing anti-inflammatory mediators such as IL-10 - and in regulating the adaptive immune response[67,68]. Several studies using animal disease models have shown the potential of SPMs to decrease lung inflammation and tissue damage, and to be able to disrupt the cytokine storm. Furthermore, SPMs do not act as an immunosuppressive agent[65,66,69,70]. Thus, regarding COVID-19, SPMs may in the near future be used to treat inflammation with the active precursors 18-HEPE, 17-HDHA and 14-HDHA[66,69,71]. Furthermore, omega-3 has been linked to reduced expression of Cyclooxygenase 2 and decreased levels of pro-inflammatory cytokines such as IL-6, IL-8, IL-1 beta and free radicals[72].

PUFAs are responsible for altering the composition of cell membranes, modulating cell signaling and influencing immune responses[73,74]. They are present in the cell membrane, taking part in the formation of the phospholipids, assisting in the maintenance of both cell structure and functionality. Thereby, alterations in the composition and homeostasis of these compounds are able to influence celular responses[75]. Thus, studies have shown that, due to their lipophilic capacity, PUFAS can

bind to the cell membrane, altering the permeability of this structure, interfering with the virus' connection with the ECA2 receptor, and to the viral envelope, also interrupting its action as a receptor[76]. In addition, omega-3 could contribute to alterations in the structure of the lipid rafts that carry the angiotensin-converting enzyme 2 (ACE2), being able to modify the ability of the virus to connect to its receptor and reduce replication rates[77]. This lipid could directly regulate and alter the amount, size, and the proteins expressed in the rafts by modulating the binding between the virus and its receptors[78]. Finally, the viral spike protein, which is responsible for interacting with ECA2 and allowing entry into the cell, could be inactivated by PUFAs when they bind, blocking infection [79].

During cases of infection, adequate management of the patient's nutritional status must be performed, since systemic inflammation is capable of increasing the demand for nutrients and propitiating a picture of malnutrition that may worsen the clinical picture generated by COVID-19[80]. As a result of the aforementioned, lipid nutritional support emerges as a possible element in medical nutrition therapy for critically ill patients with COVID-19[73,74]. Studies have shown that there is a possible association between omega-3 Levels in the body and reduced fatal outcomes caused by COVID-19[79]. A cross-sectional study observed a possible relationship between low omega-3 Levels in the body and clinical manifestations of COVID-19. However, there is a need for further research that evaluates a larger population and standardizes the levels of this lipid as a possible predictor of risk in the bloodstream during infection[80]. Of note, patients admitted to intensive care units with respiratory distress syndrome had improved oxygenation and reduced length of stay after administration of this lipid[81]. Similarly, a randomized clinical trial with 101 patients reported that during omega-3 supplementation there was regulation of some laboratory parameters such as normalization of arterial pH, bicarbonate level and base excesses, as well as improvement in renal function[83].

MICRONUTRIENTS INFLUENCE IN COVID-19 SEVERITY

Vitamin B12

Vitamin B12, also known as cobalamin, is a micronutrient obtained mainly through the consumption of animal source foods and absorbed in the gastrointestinal tract through metabolic pathways involving substances such as hydrochloric acid, pepsin, and intrinsic factor. It is a micronutrient with well-established functions in red blood cell synthesis, cell growth, the nervous system, and DNA synthesis. The active forms of cobalamin are hydroxocobalamin and methylcobalamin, which is closely linked to folic acid, and adenosylcobalamin [84,85]. In addition, studies suggest that vitamin B12 plays an important role in the immune system by assisting in balancing the gut microbiota, which is sometimes compromised during SARS-CoV-2 infection[4]. Yet, cobalamin modulates the immune system by exerting influence on T lymphocytes, participating in their differentiation and proliferation and, thus, being important in maintaining the ratio between cytotoxic and helper T cells, in addition to influencing the activity of natural killer cells. By playing a role in cell division, vitamin B12 can may a direct influence on the rapid proliferation of B lymphocytes. Furthermore, vitamin B12 in association with folate is important in the production of antibodies[86]. Considering that Vitamin B12 participates in metabolic reactions involving carbon-1, with interactions occurring with folate metabolism, in individuals with low levels of vitamin B12, 5-methyl-tetrahydrofolate (THF), produced by an irreversible reaction, results in an inactive form of folate. 5-Methyl-THF can result in a secondary folate deficiency, impairing purine and thymidine synthesis. This results in changes in DNA and RNA synthesis and, consequently, in the secretion of immunoglobulins[87].

Thus, insufficiency or deficiency of micronutrients such as vitamin B12 may affect the host immune response against viral infections, inflammatory activity, as well as influence the clinical outcomes of patients with COVID-19 in both immunological, microbiological, and hematological forms[88,89]. A single-center study [90] noted that patients who died from SARS-CoV-2 infection had less vitamin B12 when compared to those hospitalized in intensive care units (ICUs), but no significant differences were observed between them. Another study that evaluated serum micronutrient levels and

disease severity in COVID-19 patients reported that some of these substances, such as cobalamin, were reduced in these individuals[91]. On the other hand, some patients may also have increased B12 Levels, especially those intubated and deceased; with excess vitamin B12 being significantly correlated with worse prognosis, such as ICU admission, intubation, and death[92]. Similarly, this increase was also observed in patients with poor clinical outcomes in another study[92]. The liver is responsible for cobalamin storage and damage to this organ in hospitalized patients and may be the cause for the high levels of this vitamin found in certain individuals. However, despite the high plasma concentration of cobalamin, these patients may have neurological and hematological conditions, which are common in low concentrations of the micronutrient. There are two possible pathways for the occurrence of this paradoxical effect: tissue lysis reduces the intracellular concentration of cobalamin and increases the plasma concentration; thus, the high concentration ends up interfering in the transport of the substance and, consequently, in the intracellular uptake[94].

Given this scenario, studies linked to vitamin B12 supplementation are scarce and show inconclusive results. In this sense, some authors advocate supplementation associated with other micronutrients, making it difficult to analyze their results in isolation. In non-COVID-19 situations, B12 deficiency is classically treated with parenteral injection therapy of 1000 µg for 1 to 2 wk, followed by monthly administration. Intramuscular injections are uncomfortable and painful in children, as well as expensive. Thus, oral preparations are being investigated[95]. In a study, children over six years were treated with a daily pill containing thiamine 250 mg, pyridoxine 250 mg and cyanocobalamin 1000 µg for three months, and under six years old with an ampoule of 1000 µg of B12 vitamin. This treatment was effective for B12 nutritional deficiency[96]. Another study that evaluated 47 individuals aged one month to seventeen years with B12 serum levels less than 200 pg/mL treated for 120 days with 1000 µg of this oral vitamin showed improvement in cobalamin levels. However, despite the high dose, reduced results were achieved in older children, indicating the need for dose adjustment according to weight[97]. Yet, patients with ≥ 6 or ≥ 18 years, that reported gastrointestinal

abnormalities or restricted diet, received 1000 µg of oral B12 or 1000 µg intramuscularly in nine injections for three months and both administrations restored the cobalamin levels of all patients[98].

A study performed joint supplementation of magnesium and vitamins B12 and D3 in individuals aged over 50 years with COVID-19 and observed less need for supplemental oxygen and ICU admission[99]. Therefore, these findings suggest the potential role of B vitamins in limiting disorders and complications related to SARS-CoV-2 infection, and further studies are needed to more reliably establish whether vitamin B12 alone is able to show statistically significant results in these patients[100,101].

Vitamin D

Although the level of vitamin D has been widely studied in patients infected with SARS-CoV-2, other previous studies have evaluated the role of this vitamin in patients with acute respiratory infections (ARI), mainly in the upper airways[102,103].

The role of vitamin D in bone health, through calcium and phosphorus maintenance, is well established[104], but its role in respiratory infections appears to be related to the production of antimicrobial peptides in the respiratory epithelium and in the response of the inflammatory cascade against the virus[105-107]. In addition, vitamin D helps maintain cell junctions and gaps, decreasing the cytokine storm caused by the infection[108], inhibiting type 1 T helper cell response, and T cell induction [109]. Furthermore, vitamin D deficiency causes deprivation in macrophage production and performance, interfering with the innate immune response, favoring the establishment of infection[110]. Thus, although the levels of this macronutrient do not represent a great impact in reducing the risk of contracting the disease, studies show that there is a great impact on the modulation of the innate and adaptive immune response and, consequently, on the severity of the disease[111].

The pathophysiology of SARS-CoV-2 virus infection is favored by high expression of angiotensin-converting enzyme-2 (ACE2), a receptor through which the virus enters cells of the lung epithelium and other organs, triggering activation of the pro-

inflammatory cascade and viral replication[112]. Increased storage of the inactive form of vitamin D (calciferol) increases the risk of virus infection because it stimulates ACE2 production[110]. However, as this is one of the factors for the manifestation of more critical forms of COVID-19, at the experimental level, vitamin D helps to reduce ACE2 expression and viral load by reducing the inflammatory cascade[111,112].

Although the evidence, studies involving this vitamin and the prognosis of patients infected with COVID-19 are inconclusive, most of them are observational or retrospective studies with a small, usually single-center sample. Therefore, the medical recommendation for vitamin D supplementation is based on the observation of increased mortality from COVID-19 in those with low vitamin D levels, even with adjustment for the patient age[111]. Studies indicate that vitamin supplementation is relevant only in patients who are vitamin-deficient or at risk for immune system deficiency, such as patients with chronic diseases[113]. In a study that looked at different doses of vitamin D in patients with COVID-19, the recommendation for people at risk of influenza and/or COVID-19 was supplementation of 10,000 IU/d of vitamin D3 for a few weeks and then 5,000 IU/d, without describing the variation for the patients' age group. The ultimate goal would be to rapidly increase 25(OH)D concentrations and reach concentrations between 40–60 ng/mL (100–150 nmol/L). For the treatment of patients with COVID-19, higher doses, depending on the reference protocol, may be useful[108].

There are studies that have shown lower vitamin D levels in critically ill patients with COVID-19 [78,114] and in addition, a 15% reduction in the number of severe COVID-19 cases with normal vitamin D status was found in a population [115]. However, after removing confounding variables, the results are still inconclusive. Other studies that have found increased mortality from infection in countries with vitamin D deficient populations, such as Italy, point to overlapping risk factors related to old age, obesity and diabetes[104].

Meanwhile, high level supplementation may be recommended for patients at risk. A randomized controlled trial indicates that high doses of vitamin D supplementation are

a successful treatment for high-risk elderly patients, and that this type of treatment would not pose risks to patients. Still, it is clear that further prospective, randomized, controlled, large-scale studies on vitamin D supplementation related to mortality and severity of COVID-19 are needed to conclude[113].

Calcium (Ca)

Hypocalcemia is quite common in viral diseases, which overcomes the fact that studies report its presence in more than 60% of patients hospitalized for SARS-CoV-2 infection[116]. The calcium ion is involved in two important parts of the development of COVID-19. It is of paramount importance for the life cycle of the virus, but it is also related to the inflammatory response and its regulation[117].

Some hypotheses are raised to explain this condition. Among them, we can mention: some degree of malnutrition that causes hypovitaminosis D, hypoalbuminemia in COVID-19, given that the calcium ion is primarily linked to this protein, the high degree of inflammation in the infected patient, as well as a consequence of this, alterations in the receptors and in the hormonal axis of calcium, which causes it to be mobilized from the bones. Furthermore, it is possible to mention the fact that patients with hypocalcemia have fewer lymphocytes and higher levels of D-dimer, justifying the more intense inflammatory response, as well as greater chances of developing coagulopathies. Regarding lymphopenia, it can be justified by mechanisms of bone marrow suppression that may have been caused by the virus and/or by direct destruction of these lymphocytes, due to all the toxic substances that are produced during the SARS-CoV-2 infection, mainly the cytokines[118]. D-dimer is related to the cytokine storm that is caused in more severe cases of infection. This is because this intense immune reaction activates the coagulation cascade, favoring the occurrence of thrombotic events. As D-dimer is involved in blood clotting events, its detection in exams is favorable to attest to a possible state of thrombosis in the patient, which increases the chances of pulmonary complications and thromboembolism[118]. However,

the increase in unsaturated lipids can also contribute to hypocalcemia, due to the link established with the ion[116,119,120].

Given this and studies that have evaluated calcemia in hospitalized patients, it was possible to establish an important risk factor between low calcium levels and increased risk of developing serious diseases, complications in the cardiovascular system, nervous system, as well as muscle and mortality[116,120,127].

In regard to the viral life cycle, much has been studied about the role of calcium. SARS-CoV-2 needs to release its genetic material inside the host cell and to do so it needs to penetrate the host cell membrane and fuse its membrane with the viral membrane[122].

The key and initial point lies with the Spike (S) protein, which is composed of two subunits, S1 and S2, containing a region called fusion peptide (FP) that is crucial in the cell invasion process, along with the help of calcium, which binds to two negatively charged FP residues located in the S2 subunit to allow viral fusion. In this sense, calcium acts directly on the proteins responsible for mediating fusion, playing an activating role and increasing the binding of the spike protein to host cells, favoring viral penetration[122-124]. Importantly, PF interacts with the host cell membrane, changing its structure and allowing membrane fusion[124].

Despite the use of calcium in the process of virus entry into the host cell, what may account for the hypocalcemia is the lack of the viral envelope protein E that alters intracellular calcium metabolism, favoring the increase of IL-1B. This cytokine is responsible for regulating the expression of a calcium-sensitive receptor (CaSR). With the action of the cytokine, the set point of calcium suppression by PTH is reduced. Thus, even though calcium is in lesser amounts, it is able to decrease PTH secretion and corroborate an even greater decrease in serum calcium[117].

Early use of calcium and albumin supplementation is reported to lead to reduced toxicity from free fatty acids, which are then carried by albumin, and to decrease the degree of mitochondrial metabolic dysfunction and organ damage[126]. Also, a meta-analysis of 199,298 patients demonstrated that the use of calcium channel blockers (CCBs) in hypertensive patients reduced mortality rates in hypertensive patients with

COVID-19. This may be explained by the action of BCCs blocking the virus replication cycle through ion-dependent pathways, although the use of BCCs has not been shown to interfere with the severity of disease presentation[126]. Some studies, which used a smaller sample of patients, are against the use of BCCs in the treatment of patients with COVID-19 and found an increased risk of respiratory failure, intubation, and death in patients taking this medication[127].

Further studies are needed, but vitamin D supplementation is hypothesized to prevent hypocalcemia, severe disease and other complications [120,128].

Iron (Fe)

Hemoglobin, Fe and saturated transferrin levels were lower in patients with COVID-19 compared to individuals without the disease, while ferritin levels were higher in SARS-CoV-2 infected patients[129]. Correspondingly, Zhou *et al*[130] reported that serum hepcidin and ferritin levels contribute independently to the severity of COVID-19. Another study points out that the relationship between iron levels and disease severity is U-shaped, considering that patients with mild respiratory failure had significantly lower serum iron levels compared to individuals without respiratory failure, while no significant differences in iron levels were observed between the group without respiratory failure and those with severe respiratory failure[131]. Hippchen *et al*[132] identified an iron concentration < 6 µmol / l as the best cut-off point to predict hospitalization of patients with COVID-19. Furthermore, it has been reported that alterations in iron metabolism can persist for a few months after the initiation of COVID-19 and are associated with pulmonary pathologies[133]. Low serum iron has also been associated with mortality from COVID-19[134].

In order to decrease viral replication, the innate immune system stimulates the reduction of iron bioavailability, so hepcidin levels tend to increase and block ferroportin activity, which results in cellular accumulation of the metal, mainly inside macrophages, hepatocytes and enterocytes[135]. The increase in intracellular iron stimulates the expression of inflammatory cytokines, such as IL-6, IL-8 and TNF-α,

which worsen the accumulation of iron in cells, generating a cycle that contributes to the "cytokine storm" in patients with COVID-19[133].

In general, adequate levels of iron are obtained through diet. However, supplementation of this mineral can be used in patients with challenges in meeting dietary requirements[136]. The usual dosage for therapeutic iron supplementation is 325 mg (equivalent to 65 mg of elemental iron), 3 times a day[137]. Iron supplementation therapy has been considered a more promising approach than transfusion to promote erythropoiesis in pregnant women and cancer patients with anemia and COVID-19[138].

Copper (Cu)

Skalny et al[139] reported that the Cu/Zn ratio, besides being increased in patients with COVID-19 compared to healthy individuals, presents a gradual increase according to the severity of the cases and was considered as a predictor of lower O2 saturation. A cohort of 306 patients with COVID-19 in Wuhan also identified an increase in copper levels in severe cases compared to non-severe patients [140]. On the other hand, Hackler et al[141] reported that patients surviving COVID-19 had higher mean serum Cu levels compared to non-surviving patients. Arrieta et al[142], in turn, carried out a study with patients with severe COVID-19 on parental nutrition and supplemented with zinc, revealing that serum copper concentrations were lower in critically ill participants. However, it should be considered that copper and zinc are competitively absorbed in the small intestine, which may justify the reduction of copper in these patients[143].

SARS-CoV-2 infection involves the induction of an inducible transcription factor (NF-kB), responsible for triggering an inflammatory process. Cu, in turn, acts by preventing inflammatory events, through several mechanisms, such as the generation of reactive oxygen species, which act in the destruction of viral morphology and genomes[143]. Despite a favorable theoretical approach to complementary therapy with copper supplementation, there is still no evidence to support its use in cases of patients with COVID-19[142].

Zinc (Zn)

Zinc plays an important role in modulating the immune system, including roles in antiviral and antibacterial responses[144]. Zinc is essential for the recruitment of neutrophil granulocytes, chemotaxis process and positively influences NK cells, phagocytosis, oxidative burst generation, and CD4+ and CD8+ T cells[145]. It has already been clarified that acute zinc deficiency has the potential to interfere with both innate immunity and T cell-mediated immunity by impairing those defenses, whereas chronic deficiency of that metal is associated with an increase in pro-inflammatory cytokines[146]. In addition, previous studies have already suggested the use of zinc in order to reduce the duration of acute respiratory tract viral infections and to prevent symptoms[147].

A possible therapeutic role of the mineral in respiratory tract infections was the demonstration that Zn gluconate supplementation inhibits the NF-kB-dependent transcription of inflammatory genes, contributing to a reduction of neutrophilic infiltration and TNF-α release in the airways[148]. In that context, it was hypothesized that zinc could inhibit SARS-CoV-2 viral replication since it inhibits RNA-dependent RNA polymerase (RdRp) activity *in vitro* by inhibiting SARS-CoV-2 RdRp elongation and binding of model[149,150]. Furthermore, it is possible that zinc has the potential to restrict SARS-CoV-2 access in host cells by inhibiting ACE2 activity [151,152]. Therefore, during the COVID-19 pandemic, the possibility of reducing infection severity through zinc administration led scientists to research this metal. Among these studies, a non-randomized clinical trial including 113 patients compared the use of combined nitazoxanide, ribavirin, ivermectin, and zinc along with routine supportive treatment and the results showed that the combination effectively cleared SARS-CoV-2 from the nasopharynx faster than supportive therapy; however, patients experienced some side effects such as gastrointestinal disturbances[153]. In contrast, a randomized clinical trial including 214 patients looked at whether high-dose zinc, high-dose ascorbic acid or both substances were able to reduce the severity or duration of symptoms caused by SARS-CoV-2 infection compared to standard care. The results of the study concluded

that there was no significant difference between groups and that treatment with zinc, ascorbic acid or both did not interfere with the symptoms of the disease[157]. In addition, a prospective clinical trial with 134 patients analyzed the zinc serum levels of patients positive for COVID-19 at various severity levels, with and without olfactory alterations, in order to assess the therapeutic potential of zinc supplementation. The authors concluded that there were no significant differences between the subgroups regarding severity, recovery time or the presence or absence of olfactory and taste dysfunction. However, olfactory and taste functions recovered more quickly in patients who underwent zinc therapy ($p < 0.001$)[155]. Zinc supplementation offers numerous benefits for different comorbidities, however its dosage may vary with the patient's age and the specific pathophysiology of the disease[156]. The recommended pharmacological dosage of zinc for adults is greater than 40 mg/d and generally ranges from 220 mg/d to 660 mg/d of zinc chelate, which is equivalent to 50 mg to 150 mg of elemental zinc[157]. Finally, the clinical data obtained to date are not sufficient to support zinc supplementation in outpatients and hospitalized patients with COVID-19 [154,158].

Magnesium (Mg)

Magnesium ion is one of the most relevant elements in the homeostasis of several body systems such as respiratory, neurological, cardiovascular, and digestive. It has anti-oxidant and anti-inflammatory functions and integrates several biochemical and metabolic reactions, such as transport of other ions, activation of vitamin D, and it is involved in energy metabolism[159]; considering the role of magnesium in body homeostasis, this element is involved in the context of the organic disorders caused by COVID-19.

When there is a cytokine storm and an increase in the generalized inflammatory status, there is a functional imbalance between the cells of the immune system and higher energy depletion [160, 161].

A good part of the population already has low serum magnesium levels and, with the infection, food intake reduces in more critical cases. As a result, the organism uses other

means of obtaining this ion which, along with phosphate, is removed from its natural reservoirs mainly, the musculoskeletal system, catabolizing it[159, 161].

Besides the muscle issue involved, which may evolve to kidney injury and rhabdomyolysis, low magnesium levels may favor the development of respiratory complications by integrating membrane proteins involved in energy metabolism. Nevertheless, hypomagnesemia can contribute to endothelial dysfunction, favoring, as the calcium mentioned above, prothrombotic situations[159,160].

To date, little is known about Mg homeostasis during COVID-19, as it is not a commonly assessed parameter, even though many patients have low Mg levels during the disease. However, in addition to all the inflammatory and metabolic issues involved with hypomagnesemia, SARS-CoV-2 has magnesium in its structure. In this sense, the virus would need the ion to remain structurally and functionally active[162,163].

The use of magnesium, vitamin D and vitamin B12 supplementation was positive in the development of COVID-19 in patients over 50 years old, reducing the number of patients who required supplemental oxygen or intensive care unit admission. The doses used were 1000 IU of cholecalciferol, 150 mg of magnesium oxide and 500 µg of methylcobalamin, for a period less than or equal to 14 days[99].

To summarize the influence of nutrients on the immune system, Table 1 brings the macro and micronutrients above cited, relating it to the modulation in cells, cytokines and to clinical outcomes.

CONCLUSION

The relationship between COVID-19 and nutrients is controversial. The expression of pro-inflammatory compounds and the individual's dysregulated immune response are the main causes of modulation in critically ill patients infected with the virus. In view of this, correct modulation is essential to avoid mild or exaggerated responses. The macro and micronutrients mentioned are directly involved in the basic structure of the immune system, participating in the development of cells, cytokines, and antibodies. Some nutrients such as vitamin B12 and copper were contradictory as to the beneficial

effects of their bioavailability, and their overstocking was predictive of a worse prognosis. The lack of studies with this isolated micronutrient requires further analysis to guide medical professionals in prescribing B12 supplementation. Furthermore, supplementation of vitamin D, calcium, iron and magnesium are beneficial, especially in patients with comorbidities, whose risk of developing the most severe forms of the disease is greater. The action of these elements, promoting anti-inflammatory and antioxidant functions, is essential to control the aggressive COVID-19 response. Both vitamin D, calcium and magnesium supplementation are important for patients at risk and with deficiency. In addition, early use of calcium associated with albumin has shown benefits in preventing toxicity and organ damages that can lead to severe cases of COVID-19. Those findings are alied to what is found in ESPEN expert statements and practical guidance for nutritional management of individuals with SARS-CoV-2 infection, which points that vitamins D, B, zinc, iron, and omega-3 polyunsaturated fatty acids should be considered in COVID-19 patients nutritional support. It is also suggested that the daily supply of these micronutrients should be ensured to malnourished patients with SARS-COV-2 infection.

Regarding the high consumption of proteins, carbohydrates, and lipids, there is influence of the excess of these in the diets of prehospital patients, considering the connection with the acquisition of pneumonia. Moreover, these nutrients influence the function of adipose tissue by stimulating the inflammatory response, worsening the patient's condition. Meanwhile, omega 3 is a fatty acid supplementation recommended to improve oxygenation, contributing additionally to the regulation of laboratory tests and renal function. However, further randomized controlled trials are needed to complement and confirm the information on the influence of vitamins and other nutrients on immunomodulation of the COVID-19 response, in order to determine which nutrients are beneficially administered and select the correct doses for the treatment of critically ill patients.

75268_Auto_Edited.docx

ORIGINALITY REPORT

9%

SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	235 words —	3%
2	Klaus W. Lange. "Food science and COVID-19", Food Science and Human Wellness Internet	77 words —	1%
3	www.stitcher.com Internet	55 words —	1%
4	www.nestlehealthscience.com Internet	49 words —	1%
5	www.frontiersin.org Internet	38 words —	1%
6	aymes.com Internet	33 words —	< 1%
7	Www.esp.org Internet	26 words —	< 1%
8	link.springer.com Internet	20 words —	< 1%
9	Silvia Maggini, Eva S. Wintergerst, Stephen Beveridge, Dietrich H. Hornig. "Selected vitamins and trace elements support immune function by strengthening	18 words —	< 1%

epithelial barriers and cellular and humoral immune responses", British Journal of Nutrition, 2007
Crossref

10   Heitor O Santos. "Therapeutic supplementation with zinc in the management of COVID-19– related diarrhea and ageusia/dysgeusia: mechanisms and clues for a personalized dosage regimen", Nutrition Reviews, 2022
Crossref
16 words — < 1%

11   bulletin.mfd.org.mk
Internet
16 words — < 1%

12   Biljana Damnjanović, Đuro Šijan, Ivan Rović, Igor Lazić et al. "Nutritional therapy of COVID-19 disease in Intensive care units", Medicinski glasnik Specijalne bolnice za bolesti štitaste žlezde i bolesti metabolizma, 2021
Crossref
14 words — < 1%

13   f6publishing.blob.core.windows.net
Internet
14 words — < 1%

14   vitamindwiki.com
Internet
14 words — < 1%

15   www.tandfonline.com
Internet
14 words — < 1%

16   pubs.rsc.org
Internet
13 words — < 1%

17   Fatmanur Zehra Zelka, Rümeysa Rabia Kocatürk, Öznur Özge Özcan, Mesut Karahan. "Can Nutritional Supports Beneficial in Other Viral Diseases Be Favorable for COVID-19?", Korean Journal of Family Medicine, 2022
Crossref
12 words — < 1%